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# Eurosurveillance

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# Editorials

## Collaborative efforts are needed to improve use of influenza immunisation in Europe

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This week's special issue of Eurosurveillance highlights various aspects and challenges related to the prevention of influenza by vaccination. Influenza is among the infectious diseases with the highest incidence and associated serious morbidity and mortality that can be prevented by vaccination. In the article of the Vaccine European New Integrated Collaboration Effort (VENICE), investigators report details of vaccine coverage among different segments of the target population in the European Union (EU) and European Economic Area (EEA) Member States. Among elderly persons, only the Netherlands succeeded in reaching vaccine uptake levels above 75%, the 2010 target of the World Health Organization (WHO); twelve countries reported 50% to 75% coverage, nine countries were even below the 2006 target of 50% and seven countries could not report any data. Importantly, vaccine uptake among clinical risk groups and health care workers was even lower. In a detailed report from France, F. Rance *et al.* reported that only 17% of asthmatic children were vaccinated against influenza. Furthermore, on behalf of the European Vaccine Manufacturers, M. Rodriquez de Azero *et al.* showed that the vaccine doses per capita only marginally increased from 17% to 20% in the years 2003 to 2006. So how can we be more successful in the prevention of influenza?

In the United States (US), it has been estimated that on average 51,000 persons die from influenza during epidemics each year [1]. Based mostly on figures from the US, most of the influenza burden is among persons with risk-elevating medical conditions such as chronic respiratory, cardio- or cerebrovascular or renal disease, diabetes and immunodeficiency, and among infants, older adults and residents of long-term health care settings [2]. Similar epidemiological studies in Europe could be of use to convince local politicians about the need to reduce the burden among these vulnerable groups. The Health Council of the Netherlands, for example, decided to lower the age threshold for vaccination from 65 to 60 years in 2007 based on the large excess in the number of primary care visits, hospitalisations and mortality among the healthy aged 60 to 64 years during epidemics [3]. Indeed, the lack of data on influenza burden at the more severe end of the clinical spectrum in many European countries probably contributes to the large variations in vaccine uptake reported to the VENICE investigators.

The success of vaccination is largely determined by its impact on disease burden in the target group when applied in practice. Recently, the effects of influenza vaccination on the incidence of pneumonia and mortality from all causes among the elderly have been debated. In the US, the influenza-associated mortality among elderly persons has not declined over the last decades despite increase in vaccine uptake, whereas in the Netherlands a clear reduction in mortality seems to have taken place after the national influenza vaccination campaign [4,5]. These contrasting findings have led to much discussion mainly about the potential for confounding in non-randomised observational studies, which may have had an impact on the validity of reported effect estimates so far.

**The success of vaccination is largely determined by its impact on disease burden in the target group**

An important feature of randomisation is that it removes all kinds of biases; hence randomised controlled trials (RCTs) are considered the paradigm to study vaccine effects. Many RCTs have been conducted among healthy adults showing that vaccination prevented a considerable part of proven influenza infections [6]. Also, a landmark trial among elderly persons demonstrated a 50% reduction in influenza illness [7]. However, such trials with death as an outcome are unlikely to be carried out in Europe. Influenza vaccines are currently recommended for a wide variety of patients, and serious outcomes such as deaths due to infection are infrequent. Thus the design of an RCT would require very large representative study samples. Also the vaccines can only be effective when patients are actually exposed to the virus and the vaccine matches circulating strains neither of which can be predicted. Finally, placebo-controlled influenza vaccine trials in the elderly and most high-risk groups are usually considered unethical in Europe, since as the VENICE survey found vaccinating these persons is recommended in immunisation guidelines in most countries.

Non-randomised case-control or cohort vaccine effectiveness studies are feasible alternatives to RCTs. They have the advantages of applicability in different patient populations, timeliness, reduction of costs, and increased feasibility. However, in observational studies the selection of patients for vaccination is influenced by their risk profile, which may lead to 'confounding by indication'. Typically, the vaccinated group comprises patients with more severe disease or higher risk than the unvaccinated group. Crude, uncontrolled, estimates of the association between vaccination and outcome in such studies, therefore, lead to an underestimation of vaccine effectiveness. Conversely, if refusal of vaccination is typically associated with low functional health status, the unvaccinated group may comprise persons with a worse prognosis than the control group.

This so-called 'healthy user bias' will lead to an overestimation of the true vaccine effectiveness. Both types of biases can be present in influenza vaccine studies and it is therefore a challenge to the investigator to prevent and adjust for the confounding in the design of data collection and analysis, and, if possible, to quantify its potential magnitude [8-10].

The report by M. Valenciano *et al.* provides the reader with a very complete overview of the observational studies that were conducted in the EU Member States and the potential for confounding bias. The authors suggested that in designing studies aimed at measuring accurately and in a timely manner the vaccine effectiveness in Member States, based on an extensive literature review and expert meetings, case-control and cohort studies should be set up, and in the case-control study the main outcome should be laboratory-confirmed influenza. In the same paper much attention has been given to measure as many potential confounding factors as possible. To quantify potential unmeasured bias it was suggested to also conduct cohort studies during pre- and post-influenza seasons. However, pre-influenza seasons are invalid reference seasons because influenza can still be present. Also, terminal patients may be included in cohorts evaluating the pre-influenza season, which can also induce selection bias such that vaccine effects are overestimated, because these patients may refrain from vaccination. These limitations notwithstanding and although more methods are available to quantify the potential impact of unmeasured confounding, the proposed studies are essential attempts to maintain confidence in the benefit of the vaccine programme.

Furthermore, country-specific data on influenza burden and European estimates of the effectiveness of vaccination are needed to estimate the cost-effectiveness of the vaccination programmes. Based on data from the Dutch PRISMA nested case-control study [11] and the abovementioned excess study [3], it was estimated that the vaccination programme in the Netherlands certainly resulted in saving money and concluded that it was cost-effective to vaccinate all adults aged between 60 and 64 years [12]. Consequently, the Dutch ministry of health decided to extend the vaccination programme to the lower age limit of 60 years. However, since the use of resources is different from country to country, such analysis should be initiated in each country or undertaken at an EU level to support the actual implementation of the vaccination programme.

Alarming reports of sudden cardiac failure after influenza vaccination in Israel [13] and the Netherlands [14] during the 2007 influenza season had a negative impact on vaccine acceptance, even though national surveillance data indicated that these few fatal cases could be explained by chance alone and no causative relationship was found. Undoubtedly, more potent adjuvanted vaccines will replace current conventional vaccines in the next few years and many countries are currently considering stockpiling (pre)pandemic vaccines for use on a large scale in a pandemic. For these reasons, a carefully developed risk management plan is necessary to be able to prevent potential harm during mass vaccination campaigns [15].

Finally, it needs to be acknowledged that the development of immunisation recommendations even when supported by, preferably local, evidence does not necessarily lead to acceptance of the vaccine by the public. Various factors determine the uptake of vaccination and educational programmes should be based on evidence from surveys that attempt to predict vaccine acceptance according to health behavioural and implementation models [16-19]. National commitment by government and professionals is crucial and this partly explains the successful performance of countries with better vaccination coverage. Such commitment is now needed at an EU level so that all countries can achieve such results. To conclude, collaborative action involving experts from the fields of public health, clinical epidemiology, psychology and health economy is needed to set up a European-wide infrastructure for studies on the epidemiology, (cost-)effectiveness, risk management and acceptance to further improve confidence and coverage in the influenza immunisation programmes. Reports published in this issue of Eurosurveillance provide useful guidance how to proceed.

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# Research articles

## National Seasonal Influenza Vaccination Survey in Europe, 2008

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A cross-sectional survey was undertaken with the European Union (EU) Member States and Norway and Iceland to describe seasonal influenza immunisation in the 2006-7 season, in particular to identify country-specific recommendations for risk groups, obtain vaccine uptake information and allow comparison with global recommendations. A standardised questionnaire was completed electronically by each country's project gatekeeper. Of the 29 countries surveyed, 28 recommended seasonal influenza vaccination for older age groups (22 for those aged > 65 years), and in one country vaccine was recommended for all age groups. All countries recommended vaccinating patients with chronic pulmonary and cardiovascular diseases and most countries advised to immunise patients with haematologic or metabolic disorders (n=28), immunologic disorders (n=27) and renal disease (n=27), as well as residents of long-term care facilities (n=24). Most countries recommended vaccination for staff in hospitals (n=25), long-term care facilities (n=25) and outpatient clinics (n=23), and one-third had such recommendations for workers in essential (n=10), military (n=10) and veterinary services (n=10) and poultry industry (n=13). Eight countries recommended vaccine for pregnant women; and five advised to vaccinate children (with age limits ranging from 6 months to 5 years). Twenty countries measured influenza vaccine uptake among those aged > 65 years (range 1.8%-82.1%), seven reported uptake in healthcare workers (range 14%-48%) and seven assessed coverage in persons with underlying medical conditions (range 27.6%-75.2%). The data provided by this study can assist EU member states to assess and compare their influenza vaccination programme performance with other countries. The information provides a comprehensive overview of policies and programmes and their outcomes and can be used to inform joint discussions on how the national policies in the EU might be standardised in the future to achieve optimal coverage. Annual surveys could be used to monitor changes in these national policies.

### Background

Although immunisation against influenza is believed to benefit the elderly, measuring precise effectiveness of vaccine against morbidity and mortality in this group is difficult. Several recent studies and reviews have calculated widely varying levels of effectiveness and have described methodological hurdles for making accurate measurements [1,2]. One randomised study among older adults found that vaccine efficacy was 57% for preventing laboratory-confirmed influenza infection among adults aged 60-69 years and 23% among a small number of persons aged 70 years and older [3].

In May 2003, the World Health Assembly (WHA) recommended vaccination for all people at high risk, which it defined as the elderly and persons with underlying diseases. The participating countries, including all European Union (EU) Member States, also committed to the goal of attaining vaccination coverage of the elderly population of at least 50% by 2006 and 75% by 2010 [4].

The Vaccine European New Integrated Collaboration Effort (VENICE, http://venice.cineca.org/) project was launched in January 2006. It is funded by the European Commission Directorate General for Health and Consumer Protection (DG SANCO) within the framework of the EU Public Health Programme and supported by the European Centre for Disease Prevention and Control (ECDC). Currently 27 EU Member States and two EEA countries (Norway and Iceland) participate in the project whose aim is to establish a European network of experts who work with national immunisation programmes. Immunisation programmes and vaccination policies in Europe differ from country to country, partially reflecting the differences in healthcare delivery systems [5]. Prior to this work there had only been one European wide survey published in 2003 and there was no information routinely available to policy makers on the current status of influenza programmes and how they were implemented and monitored [6]. There is a need to improve knowledge on which population groups are targeted for vaccination, how immunisation programmes are resourced and which indicators are (or could be) used for monitoring vaccine uptake.

We conducted a web-based survey to describe the policies and practices of seasonal influenza immunisation programmes in the European Union and two countries of the European Economic Area (EEA), Norway and Iceland, for the 2006-7 influenza season.

This survey may establish the basis for conducting annual surveys of influenza vaccination policies and practices.

More information on the project and detailed results are presented in the "Final Report. National Seasonal Influenza Vaccination Survey in Europe, 2007" (henceforth referred to as: "Final report"), available from: http://venice.cineca.org/Influenza_Study_Report_v1.0.pdf

## Methods

The survey was a collaborative study between the ECDC, VENICE project and EU and EEA countries. Each country had previously identified and enrolled gatekeepers responsible for conducting all VENICE surveys inside their countries.

A standardised questionnaire was developed predominantly using close-ended questions. Information was sought to describe seasonal influenza vaccination policies during the 2006-7 influenza season; to identify influenza recommendations for different risk groups and the general population; to determine data sources, capacity and feasibility for routine seasonal influenza vaccination coverage monitoring; and to obtain the most recent vaccination coverage results for the general population and for the risk groups targeted by the recommendations. As vaccination coverage is estimated through a variety of means, we asked for information on the methodology used by each country to make these estimates: administrative methodologies (using some kind of information from those who are responsible for delivering vaccination to calculate the numerator and denominator); survey methodologies (using a sample of those targeted for vaccination); or by using pharmaceutical distribution or sales data. In addition information was collected on the form of payment for the costs of vaccine and its administration. The questionnaire is available in the "Final report", Appendix 2.

The questionnaire was piloted by three VENICE project-leading partners: Italian Istituto Superiore di Sanitá (ISS), the French Institut de Veille Sanitare (INVS) and the Irish Health Protection Surveillance Centre (HPSC). After the pilot, the questionnaire was reviewed and amended. The questionnaire was deployed as a cross-sectional web-based survey in January 2008 and was available for all participating countries on VENICE website. Gatekeepers in each participating country entered data directly on-line. The data were analysed using the computer-based Epi Info (version 3.3.2) software. Gatekeepers in each country were asked to validate the results.

Not all countries were able to provide data on influenza vaccine uptake in our survey, but information on some countries was available from a study undertaken by the University of Zurich for the 2006-7 season [7]. In this study a population-based computer-assisted telephone survey was carried out in eleven countries. These data were used for vaccine coverage comparisons in our study and are presented here.

## Results

### Response rate and results of data validation

The response rate to the survey was 100% (29/29). Response rate to data validation was 83% (24/29) as of 2 April 2008.

### Recommendations for specific target groups

All countries reported having recommendations on influenza immunisation for specific target groups in the population.

#### *Age groups*

The elderly were included in vaccination recommendations in all 29 countries (100%). Twenty-two countries reported specific recommendations for those aged 65 years or older, in six countries immunisation was recommended from the age of 50 (Poland), 55 (Malta) or 60 years (Germany, Greece, Hungary and Iceland). Austria was the only country in the survey which recommended influenza vaccination for all age groups. Besides Austria, five countries (Estonia, Finland, Latvia, Slovakia and Slovenia) recommended routine immunisation of children (with the age limits varying from six months to five years). Detailed information regarding vaccination recommendations for various age groups is presented in "Final report" Table 1.

#### *People with chronic medical conditions*

Seasonal influenza vaccine for patients with chronic pulmonary and cardiovascular diseases was recommended by all countries (100%). Nearly all countries recommended vaccinating individuals with haematological or metabolic disorders (n= 28, 97%), those with immunologic disorders (with or without HIV/AIDS) (n=27, 93%) and those with renal diseases (n= 27, 93%). Eight countries (28%) recommended vaccine for pregnant women (Table 1).

#### *Other groups*

Twenty-four participating countries (83%) recommended vaccination for residents of long-term care facilities. Fourteen countries (48%) advised to vaccinate household contacts of persons for whom vaccination was recommended.

#### *Occupational groups*

Most countries indicated that influenza immunisation was recommended for healthcare staff working in occupational settings such as hospitals (n=25, 86%), long-term care facilities (n=25, 86%) and outpatient care clinics (n=23, 79%). Some countries recommended vaccination for poultry industry workers (n=13, 45%) and essential, military and veterinary services (each n=10, 34%) (Figure 1). Three countries, Denmark, Finland and Sweden (10%) did not have recommendations for vaccination in any occupational setting.

**Table 1**

**Influenza immunisation recommendations for persons with chronic medical conditions (without regard to age) or pregnancy. National seasonal influenza vaccination survey in Europe, January 2008 (n=29)**

| Condition | Number of countries (%) |
|---|---|
| Pulmonary diseases | 29 (100) |
| Cardiovascular diseases | 29 (100) |
| Haematologic or metabolic diseases | 28 (97) |
| Renal diseases | 27 (93) |
| Diseases of the immune system | 27 (93) |
| HIV/AIDS | 26 (90) |
| Children on aspirin | 17 (59) |
| Hepatic diseases | 14 (48) |
| Any condition that can compromise respiratory function | 11 (38) |
| Pregnancy | 8 (28) |

**Monitoring vaccine coverage**

All countries except one have mechanisms to monitor influenza vaccination coverage. Most (n=14) measure uptake in both the general population and selected target groups, some (n=7) only in target groups, some (n=7) only in the general population. One country does not have any means of monitoring influenza vaccine coverage.

Concerning monitoring vaccination coverage in specific risk groups targeted by vaccine recommendations, only one country, the United Kingdom reported having mechanisms to monitor influenza vaccination coverage in each of the recommended target groups by actively collecting immunisation data. Further 20 countries had mechanisms for monitoring coverage in some selected risk groups, including 18 countries that monitored uptake in the elderly. Norway reported monitoring influenza vaccination coverage in a combined group including those aged ≥65 years and persons with underlying clinical conditions.

Monitoring coverage in groups other than the elderly was uncommon. Aside from the UK only the Netherlands, Hungary and Iceland reported having mechanisms for monitoring uptake among clinical risk groups and Hungary, Portugal and Iceland had mechanisms to monitor vaccine coverage among staff working in occupational settings. Seven countries reported they had no mechanisms to monitor influenza vaccine coverage in risk groups: Austria, Bulgaria, Czech Republic, Greece, Latvia, Spain and Poland. However, with the exception of Greece these countries monitored coverage rates in the general population. ("Final report", Table 5).

**Methods of monitoring coverage**

The mechanisms used to measure vaccination coverage vary by country and include health record data, surveys or pharmaceutical data.

Eight countries reported using only administrative methods (number of vaccines administered, payment reimbursement claims) to monitor vaccination coverage. Fourteen countries combined administrative with other methods (surveys or pharmaceutical data), one country combined survey and pharmaceutical data, one used only survey, and four only pharmaceutical data. Only one country does not have any method and does not monitor vaccine coverage.

Twenty-seven (93%) countries reported using one or several methods to measure the numerator (number of people vaccinated) for assessing influenza vaccine coverage in recent years (2004-2007). Sources used most frequently were health record data (medical documentation and/or computerised medical records and/or immunisation registries/data) which were used in 20 countries. Other countries used pharmaceutical data, surveys or other administrative methods.

Eleven countries (39%) collected data for numerator assessment annually and ten (36%) collected this data once at the end of season (Table 2). Only six countries attempted to monitor coverage during the season.

Eight countries used administrative methodology to estimate the denominator (number of people who should be vaccinated) for the occupational target groups and the group comprising persons with underlying clinical conditions, and ten countries have some information on other group categories. ("Final report" Table 9)

Seven countries used survey methods to estimate vaccination coverage, including household surveys (Germany), telephone surveys (Germany, Ireland, Portugal, Sweden, and France), mail surveys (Cyprus, Sweden) or individual interviews (Belgium).

Pharmaceutical distribution or sales data were formally collected in sixteen countries ("Final report", Table 6)

**Vaccination coverage results**

***The elderly***

Influenza vaccination coverage among those aged > 65 years age group was measured in nineteen countries (65%). The range of uptake in this age group varied from 1.8% to 82.1%. In addition, Norway provided combined vaccine uptake for those aged > 65 years and clinical risk groups (50%) (Figure 2). Generally,

**Figure 1**

**Vaccination recommendations for occupational groups. National seasonal influenza vaccination survey in Europe, January 2008 (n=29)**

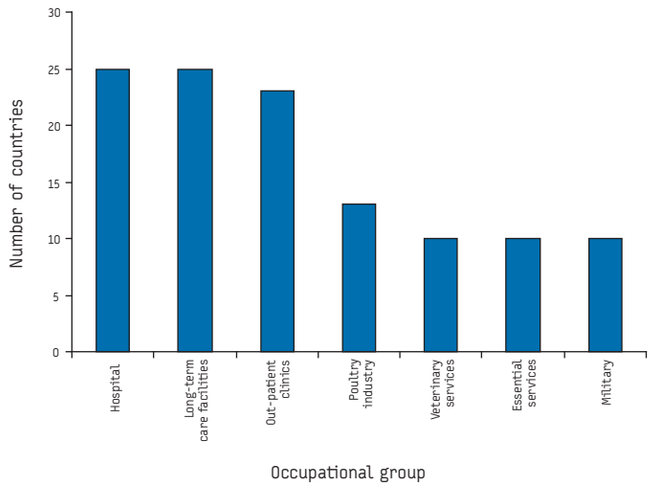



**Table 2**

**Frequency and time of collecting numerator data for assessing influenza vaccine coverage. National seasonal influenza vaccination survey in Europe, January 2008 (n=28)**

| Frequency of numerator assessment | Countries |
|---|---|
| Monthly | Latvia, Lithuania, United Kingdom |
| Every two months | Ireland |
| Every three months | Estonia, Poland |
| Once, at the end of influenza season | Cyprus, Czech Republic, Hungary, Germany, Luxemburg, Norway, Romania, Slovenia, Malta, Portugal |
| Annually (specify date/time) | Austria (spring)<br>Bulgaria (April )<br>Denmark (first quarter of the year)<br>Finland (April)<br>Iceland (December)<br>Italy (late spring)<br>Netherlands<br>Slovakia (May)<br>Spain (first quarter of the following year)<br>Sweden (end of summer, before next season)<br>France (September) |
| Never | Greece |

members of EU-15 had better coverage than the 12 countries which joined the EU more recently. In the former, vaccine coverage in the elderly ranged from 32.1% to 82.1%, while in the latter coverage ranged from 1.8% to 34.1% (Figure 2).

### *Healthcare workers and clinical risk groups*

Nine countries were able to report vaccination coverage for either healthcare workers or persons with underlying clinical conditions. Five countries reported coverage for both risk groups, two countries reported coverage of healthcare workers only and two countries had data on clinical risk group coverage only. Coverage in these groups ranged from 14% to 48% for healthcare workers and 27.6% to 75.2% for clinical risk groups (Figure 3).

### Payment for vaccination

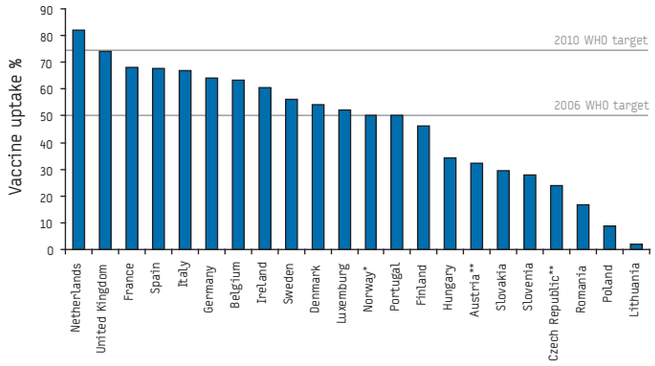
People aged > 65 years received the influenza vaccine free of charge in 13 (45%) countries, eight of which had achieved coverage > 50%. In three countries the elderly paid the full cost of vaccine and administration. In 12 countries the vaccine was free of charge for some people in the older age groups or there were partial subsidies for this age group, whereas in one (Sweden) the form of payment varied by county. (Figure 4)

Most countries offered free or partially refunded vaccination for other target groups:

In 12 countries vaccination was free for all and in five for some of the patients with underlying chronic illness. Nine countries offered free vaccination to all recommended occupational groups, 12 countries to some recipients in these groups. All children received the vaccine free of charge in three countries and some

**Figure 2**

**Vaccination coverage in those aged ≥ 65 years. National seasonal influenza vaccination survey in Europe, January 2008 (n=22)**




* Vaccination coverage in combined group of those aged ≥ 65 years and those with underlying clinical conditions
**Vaccination coverage estimated through telephone surveys; source: University of Zurich [7]
Note: Data on vaccination coverage in season 2006-7, except for Germany and Poland (season 2005-6) and Belgium (season 2003-4)

**Figure 3**

**Vaccination coverage in clinical risk groups and healthcare workers. National seasonal influenza vaccination survey in Europe, January 2008 (n=9)**

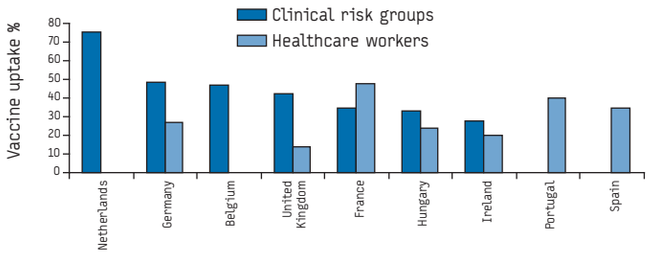
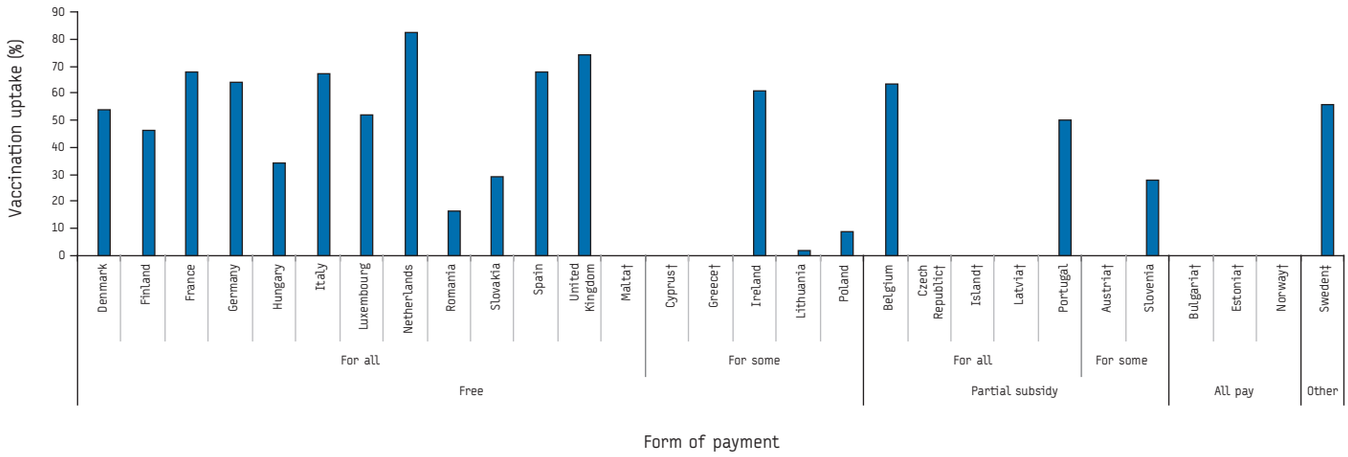



**Figure 4**

**Costs of vaccination and vaccination coverage for persons aged ≥ 65 years, by country. National seasonal influenza vaccination survey in Europe, January 2008 (n=29)**




Note: Data on vaccination uptake in season 2006-7, except for Germany and Poland (season 2005-6) and Belgium (season 2003-4)
† Countries unable to provide data on vaccination coverage in persons aged > 65 years
‡ In Sweden subsidies vary by county, approximately two-third of counties give free vaccination to this age groups

children in 10 countries. (Figure 5, more details in "Final report" Table 13).

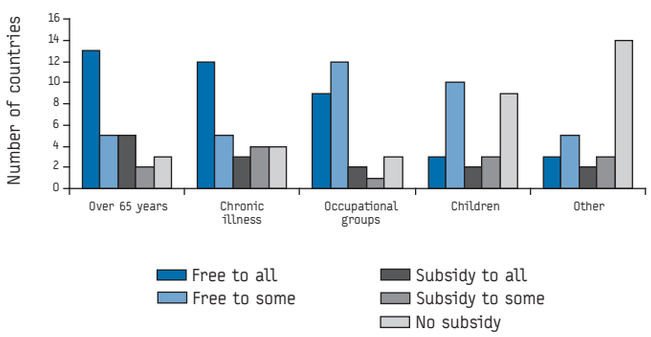
## Discussion

This is the second published study which investigates influenza vaccination policy across all EU Member States, Norway and Iceland simultaneously, and reflects most up to date information available from each country [6]. ECDC undertook a smaller study with its Advisory Forum members in 2006 that served as a model to develop this study. Validation for the results is afforded by comparison with a recent population-based computer-assisted telephone survey carried out in eleven European countries for 2006-7 influenza season [7]. The coverage results were similar except for two countries. The VENICE approach involving gatekeepers already engaged in immunisation services would seem to be successful although validation and 'sign-off' by the authorities themselves was more difficult. It was impressive that this survey, despite its complexity, was completed in a short time (12 weeks from start to finish). A strong conclusion would be that ECDC and VENICE could make this an annual survey. Annual completion would become easier as it would simply be a matter of updating the previous years' results and noting differences. The standardised information that this could provide would enable the EU Member States, ECDC, other EU institutions and WHO to assess their progress towards achieving implementation of internationally accepted recommendations on influenza prevention and control.

Our study highlights the challenges facing those authorities in Europe that have to implement the 2003 WHA resolution. The health systems in Europe are quite different. Some countries have different policies regarding influenza vaccination between different regions/counties within national borders. Vaccine coverage is measured by different methods (medical records, surveys, data from the pharmaceutical industry) in different countries making direct comparisons difficult. However this survey shows that all EU countries, Norway and Iceland have adopted the 2003 WHA recommendation that vaccine should be offered to the elderly. All countries offer influenza vaccine for those aged 65 years and older, with a few countries lowering the age limit to 50, 55 or 60 years. What countries are finding hard is to monitor and achieve performance when compared against the WHA targets (coverage in the elderly of 50% by 2006 and 75% by 2010).

**Figure 5**

**Costs of vaccination by group. National seasonal influenza vaccination survey in Europe, January 2008 (n=29)**




In the 2003 survey of 26 European countries, 18 countries reported having mechanism for monitoring vaccination coverage, and 14 could monitor coverage in the elderly with an uptake ranging from 25% to 81% [6]. In our survey, 19 of the 29 countries monitored uptake specifically in the elderly obtaining a range of 2% to 82%. Norway measured it combined with risk groups. Comparison of the results for the nine countries that participated in both surveys is encouraging, with all countries improving coverage, some dramatically, which suggests that an ability to monitor uptake results in improvement ("Final report" Table 10). The fact that more countries were able to provide vaccine coverage data this year is also encouraging and suggests that countries are striving to obtain this data. However extrapolation of the trend data from the telephone surveys conducted by the University of Zurich [7] suggest that unless there is a radical improvement in the next two seasons only two or three of the 29 EU/EEA countries are likely to achieve the 2010 WHA target. Coverage in the elderly and those with chronic illnesses will become ever more important in the EU. Population projections for the 25 EU countries indicate that the proportion of the elderly population that was 17% in 2003 will rise to 29% by 2050 [8].

Currently only five countries recommend vaccination for young children and one country recommends vaccination for all age groups (Austria). Increasingly, children are seen as a group that bears substantial morbidity from influenza and plays a role in transmitting influenza to vulnerable contacts. Vaccination of this age group is already recommended in some countries outside Europe [9,10]. The limited effectiveness of the currently available vaccines in young children may have been an impediment for many countries to embark on such a strategy. However an ECDC convened panel noted few data from Europe itself and that information is now urgently needed [11].

The European situation regarding vaccination coverage among other groups for whom vaccination is recommended (occupational groups, people with underlying medical conditions, residents of long stay care facilities, household contacts of persons to whom vaccination is recommended etc.) is highly variable. Influenza vaccine is recommended for these groups in many countries but monitoring and data for vaccine coverage was available for less than one-third of the countries. It seems a major challenge for monitoring vaccine coverage are the difficulties in obtaining information on the denominator, i.e. the size of these risk groups, which can be inaccurate due to the lack of registries for target groups, population movement, or inaccurate census.

Another issue regarding influenza vaccine coverage is assessment of the numerator, i.e. the number of those who are vaccinated. All, except two, countries assess some numerator, but have to use different methods to obtain this information: health records, pharmaceutical distribution and sales data or surveys (telephone, mail, household). As different methods are used it is challenging to harmonise vaccine coverage monitoring and to compare vaccine coverage rates between countries. Numerators can be underestimated due to underreporting, incomplete reporting and failure to include information from all relevant sources. The numbers can also be overestimated, such as when vaccine sales data are used, as these data may not necessarily reflect the number of actually administered doses. Because of the demonstrated diversity of European immunisation delivery and monitoring systems it may be worth to consider obtaining comparable influenza vaccine

coverage data through the utilisation of a standard sampling methodology across all countries.

Vaccination of healthcare workers was recommended in the WHA in 2003 [12]. This is based on a number of reasons. There is good evidence that vaccination of staff provides indirect protection to vulnerable elderly patients in care homes, a group at high risk of the severe effects of influenza [13,14]. Vaccination of healthcare workers also has direct benefit for this occupational group as it provides individual protection and reduces absenteeism from work, and fewer working days are lost [15,16]. Our study found that most of the countries recommended vaccination for staff working in healthcare facilities but vaccine coverage was known only in one–third of the countries and, as seen in other studies, the uptake was very low [7].

Thirteen of the 20 countries which were able to estimate vaccine uptake among those aged 65 years or older achieved the 2006 WHO target. One country (the Netherlands) has already achieved the 2010 WHO target uptake in this group. The fact that nine EU/EEA countries still in early 2008 did not have any system in place with which they could estimate uptake in this high risk group is worrying and suggests that Europe will struggle to achieve the WHA target for 2010 or even to produce good statistics. Therefore a strong conclusion of this study is the need for all European authorities to have information systems in place that can monitor influenza vaccine coverage.

Previous studies have shown that subsidising the cost of vaccination increases the uptake rate [7]. Costs associated with vaccination can be a deterrent to the uptake, particularly if borne by the individual. This survey reports that half of the countries have adopted a policy of provision of free vaccine, in total or in part, predominantly for the elderly, individuals with chronic disease, occupational groups. Only three countries reported that the costs of vaccine and its administration are borne fully by recipients >65 years and these countries had noticeably low uptake.

Increasingly, European states are appreciating the need to have in place systems and processes to deal with the emergence of a pandemic strain including use of specific vaccines when these become available [17]. All countries should have the ability to deliver and monitor influenza vaccination programmes in the non-emergency setting (seasonal influenza programme) to be able to build on these well established, tested systems to prepare for the potential pandemic.

## Conclusion

The limitations of our results predominantly relate to factors which make comparison of data between countries difficult but which are beyond the control of this study. As demonstrated, there is substantial variation in health systems, delivery of immunisation programmes and immunisation recommendations between and, sometimes, within countries. Various methodologies are used to measure immunisation coverage, and even when similar methodologies are described, it is possible that the accuracy of such estimates may vary between countries depending on the strength of information systems (ability to calculate numerator and denominator, target population groups etc.). However, having identified these differences, European countries are now better informed to identify how they can standardise their approach and in future provide more easily comparable data. The importance of high quality and comprehensive information systems in identifying populations targeted for influenza vaccine and in then monitoring uptake in these groups has been highlighted in this study. Countries can benefit by learning from each other; how some countries achieved high uptake, whether related to additional immunisation resources, social mobilisation, or incentives.

This is one of the first EU-wide surveys on influenza vaccination programmes and shows variability between countries with regard to recommendations for vaccine usage and uptake rates. Our survey revealed that there is substantial gap between recommendations and real vaccine uptake. Vaccine uptake in most countries needs to be improved.

However the data provided by this study can assist in standardising national and EU-level policies and recommendations and monitoring influenza immunisation programmes in future years. Survey results have shown that achieving high vaccine coverage for those who are at risk remains a serious public health challenge. We believe that European countries can use the results of our study to assess their own progress towards achieving WHO goals of influenza vaccine uptake and identify local obstacles that must be overcome if these goals are to be met. Policies and resources of countries that perform best can provide insight to guide other states struggling to achieve high uptake rates.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# Research articles

## Trends in influenza vaccination coverage rates in the United Kingdom over six seasons from 2001-2 to 2006-7

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In order to understand motivations and barriers to vaccination, and to identify people's intentions to get vaccinated for season 2007-8, influenza vaccination coverage was assessed in the United Kingdom (UK) from 2001 to 2007. Between 2001 and 2007 representative household surveys were performed by telephone interview with 12,143 individuals aged 16 or older. The overall influenza vaccination coverage rate dropped non-significantly from 25.9% in 2005-6 to 25.0% in 2006-7 (p=0.510). In the elderly (≥65 years) the rate decreased from 78.1% to 65.3% (p=0.001), and the odds ratio of being vaccinated compared to those not belonging to any of the risk groups targeted by vaccination decreased from 36.6 to 19.9. Healthcare workers and chronically ill persons had odds ratios of 2.0 and 15.5, respectively. The most important reason for getting vaccinated was a recommendation by the family doctor or nurse, and this was also perceived as the major encouraging factor for vaccination. No recommendation from the family doctor was the main reason for not getting vaccinated. A total of 38.4% of the respondents intended to get immunised against influenza in 2007-8. From 2001 to 2006 a slightly increasing trend (p for trend across seasons <0.0001) in vaccination coverage was observed in the UK, but in 2006-7 the rates returned to the level of 2004-5. Less media attention to the threat of avian influenza after 2005 may have contributed to the recent decrease of vaccination rates.

### Introduction

Experts at the World Health Organization and elsewhere agree that the world is now closer to another influenza pandemic than at any time since the 1968 pandemic which was the last of the three influenza pandemics that occurred in the twentieth century [1]. This underlines the importance of achieving sufficiently high immunisation coverage in the general population and above all in sub-populations at high risk of influenza complications.

There is ample evidence in the medical literature that vaccination is an efficacious and safe preventive measure against seasonal influenza [2-4]. It not only provides substantial health benefits, but may also be associated with significant economic benefits [5,6], particularly among the elderly, healthy working adults and children. In the United Kingdom (UK), where complications of influenza cause 3,000 to 4,000 deaths every year, the government policy [7] is to vaccinate: i) all people aged 65 years and over (age-related policy introduced in 2000–1), ii) individuals aged 6 months and over who fall into a clinically defined risk group (chronic respiratory disease, including asthma, chronic heart disease, chronic renal disease, diabetes and immunosuppression), iii) individuals living in long-stay, residential-care institutions, iv) health and social-care professionals involved in direct care. Despite the relatively high influenza vaccination coverage of the target groups in UK, continuing efforts by physicians, the National Health Service and policy makers, are needed to contain the burden of the disease.

Earlier publications based on cross-sectional data have reported influenza vaccination rates in the UK [8-10]. However, the availability of a consistent dataset for six consecutive seasons permits us to expand the usual cross-sectional approach for the analysis of vaccination rates.

In this study we analyse influenza vaccination coverage and related trends in the UK over six consecutive vaccination seasons, with special regard to high-risk group coverage. Further objectives are to elucidate the motivations for being or not being vaccinated, and to reveal the intentions to get vaccinated for the season 2007-8.

### Methods

The present survey is part of an ongoing international assessment of influenza immunisation uptake in five European countries, France, Germany, Italy, Spain and UK [11-14]. During six influenza seasons, from 2001-2 to 2006-7, a population–based telephone survey addressing different topics was carried out in December and January among UK households. Computer Assisted Telephone Interviews (CATI) were conducted, and the interviewees' consent was obtained at the beginning of each call. There was no study intervention. Using quotas and weights based on data from official national sources guaranteed that the reported sample of the survey (completed interviews) was representative of the non-institutionalised UK population aged 16 years or older [15]. The weighting was applied in terms of sex, age, profession, geographic region and town size.

Four target groups based on national recommendations were specified [7]:

1. Individuals aged 65 years or older
2. Individuals who suffer from a chronic illness
3. Individuals who work in the medical field
4. Individuals belonging to one or more of the above groups 1, 2 and 3 (composite target group)

The non-target group comprised individuals belonging to neither of groups 1, 2 and 3. The survey questionnaire has been published before [15]. The questions covered vaccination uptake, reasons for and against vaccination, as well as the intention to get vaccinated the next season. In order to assess the gap between actual and intended vaccination rates, the ratios between the actual coverage level in a given season and the intended level in the same or the next season were calculated. Since 2003-4, supplementary information on the chronic illness status of the interviewees was collected. Data comparing target groups with the non-target group were obtained from season 2003-4 to 2006-7. Starting with season 2005-6, the questionnaire also included questions on pandemic and avian influenza.

Sample weights were applied, and the annual datasets were pooled to correct for small deviations from the age and sex quotas requested. SPSS® version 14 for Windows was used for the statistical evaluation. The chi-square test was used to assess bivariate associations of categorical variables and the chi-square test for trends was used for assessing time trends of categorical variables. For all statistical tests two-sided $p \leq 0.05$ was set as the level of statistical significance. If available, exact p-values were displayed. Ninety-five percent confidence intervals (CI) were reported where appropriate. Expected predictor variables were considered candidates for multivariate analysis, and logistic regression was used to identify independent correlates of the outcome of interest, i.e. vaccination coverage. The following variables were regarded as potential predictors of vaccination coverage: sex, age, chronic illness, working in the medical field, educational level, and income. Multivariate logistic regression analysis was used to assess the independent explanatory value of these covariates. A full model (containing all covariates) was first fitted from the 2006-7 data. Non-significant predictors ($p > 0.05$) were subsequently removed on a stepwise basis. The regression models for all other seasons were based on the remaining set of influential covariates identified from the 2006-7 dataset. Due to the descriptive nature of this data, no correction for multiple testing was made.

## Results

### Response rate

In the 2006-7 coverage study 2,037 individuals completed the interview (6.0% of responses). A total of 12,143 persons were interviewed since 2001. An overview of the samples is shown in Table 1. The samples were composed similarly over the years and are representative of the population aged 16 or older [15,16].

### Vaccination coverage rate

Figure 1 shows the actual as well as the intended influenza vaccination rates over time. Overall vaccination coverage rates declined non-significantly from 25.9% (95%CI: 23.9;27.9) in season 2005-6 to 25.0% (95%CI: 23.0;27.0) in season 2006-7 (p=0.510). With regard to the coming season of 2007-8, 38.4% (95% CI: 36.8-40.1) of the interviewees intended to get immunised against influenza (Figure 1). The ratio of actual and intended vaccination rates ranged between 0.58 and 0.69 over the years. Throughout, the intention to get vaccinated was much higher than the actual rate in the current or in the previous season (Figure 1).

In 2006-7, the proportion of vaccinated persons who had also been vaccinated in the past (22.4%) was very similar as in the previous season (22.6%), but significantly higher than in the seasons before 2005-6 (19.8% to 20.4%). At the same time, the proportion of individuals who had been vaccinated in the past, but not in the current season, decreased from 17.6% in 2005-6 to 16.9% in 2006-7 (not statistically significant), possibly a fluctuation of an increasing vaccination trend since season 2001-2. In 2006-7, the proportion of respondents who were vaccinated

**Table 1**

**Overview of samples included in the influenza vaccination coverage surveys, United Kingdom, from 2001-2 to 2006-7 (n = 12,143)**

| | 2001-2 | 2002-3 | 2003-4 | 2004-5 | 2005-6 | 2006-7 |
|---|---|---|---|---|---|---|
| **Total sample size** (N) | 2,023 | 2,028 | 2,026 | 2,005 | 2,024 | 2,037 |
| **Mean age** (years) | 44.5 | 45 | 44.9 | 45.2 | 44.8 | 45 |
| (95% CI) | (43.7- 45.4) | (44.2- 45.8) | (44.1- 45.7) | (44.4- 46.0) | (44.0- 45.6) | (44.1- 45.8) |
| **Male** | 48.8% | 48.8% | 48.9% | 48.8% | 48.9% | 48.6% |
| (95% CI) | (48.3%- 49.1%) | (48.3%- 49.1%) | (48.4%- 49.4%) | (48.3%- 49.1%) | (48.4%- 49.4%) | (46.7%- 50.5%) |
| N | 987 | 989 | 991 | 978 | 990 | 990 |
| **Age ≥ 65 years** | 18.1% | 18.7% | 19.2% | 18.7% | 19.0% | 18.9% |
| (95% CI) | (17.9%- 18.3%) | (18.5%- 18.9%) | (19.0%- 19.4%) | (18.5%- 18.9%) | (18.8%- 18.9%) | (18.7%- 19.1%) |
| N | 366 | 380 | 389 | 375 | 384 | 384 |
| **Work in the medical field** | 6.8% | 8.2% | 7.1% | 6.8% | 6.6% | 6.4% |
| (95% CI) | (4.9%- 8.7%) | (6.3%- 10.1%) | (5.2%- 9.0%) | (4.9%- 8.7%) | (4.7%- 8.5%) | (4.5%- 8.3%) |
| N | 138 | 167 | 144 | 136 | 133 | 130 |
| **Chronic illness** | - | - | 12.0% | 14.0% | 14.2% | 14.4% |
| (95% CI) | | | (10.1%- 13.9%) | (12.1%- 15.9%) | (12.3%- 16.1%) | (12.6%- 16.3%) |
| N | | | 243 | 281 | 288 | 294 |
| **Combined target group*** | - | - | 33.0% | 33.2% | 33.1% | 33.2% |
| (95% CI) | | | (31.0%- 35.1%) | (31.1%- 35.2%) | (31.1%- 35.2%) | (31.1%- 35.2%) |
| N | | | 669 | 665 | 671 | 676 |

*Includes people aged 65 years and over, suffering from chronic illnesses or working in medical field

for the first time (2.6%) was one fifth lower than in the preceding season (3.3%), whereas the proportion of those who had never been vaccinated increased from 57.1% to 58.1%. In spite of this small increase, there is no statistical evidence for a reversal of the decreasing trend in the long-term (p for trend across seasons <0.0001).

Vaccination coverage in target groups

For the target groups, changes in vaccination coverage over time are shown in Figure 2. In the group aged 65 years or older, coverage peaked in season 2005-6 at 78.1% (CI: 73.1%;83.1%), and then returned to 65.3% (95% CI: 60.3-71.3) in 2006-7 (p=0.001). Statistical significance in this case indicates that there may be a long-term upwards trend despite a substantial degree of yearly variation. In every season, coverage in this group was at a significantly higher level than in the non-target group (p<0.0001).

**Figure 1**

**Actual vaccination rate and intended vaccination rate; influenza vaccination coverage surveys, United Kingdom, from 2001-2 to 2007-8**

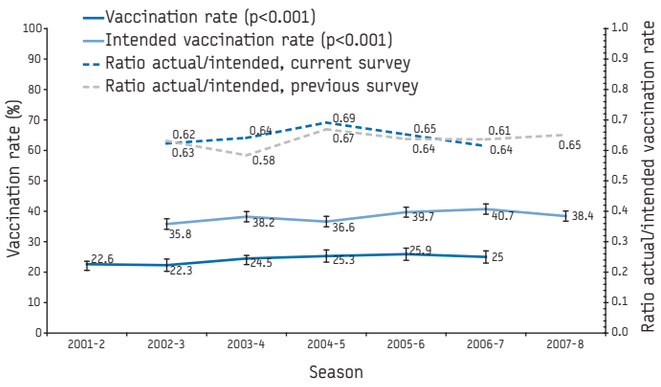
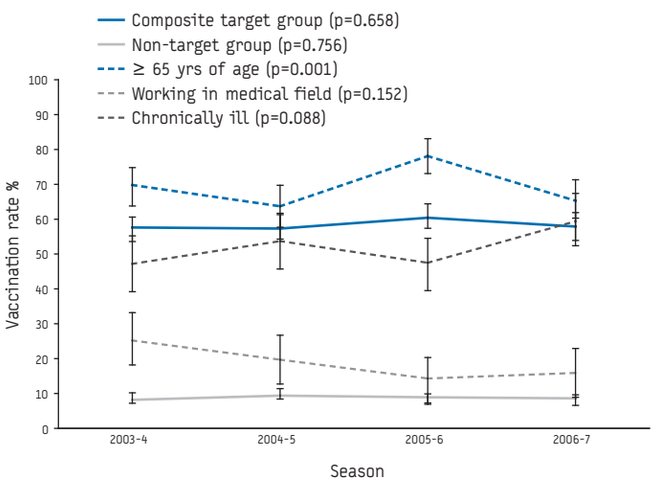



**Figure 2**

**Trend curves of actual vaccination rates in high-risk target groups and in the non-target group; influenza vaccination surveys, United Kingdom, from 2003-4 to 2006-7 (p-values for trends across seasons)**




Age-related differences in vaccination coverage over time were shown in Figure 3. Being elderly (≥65 years) was associated with the highest coverage (Figure 3). The lowest values were seen in the 16-39 years old.

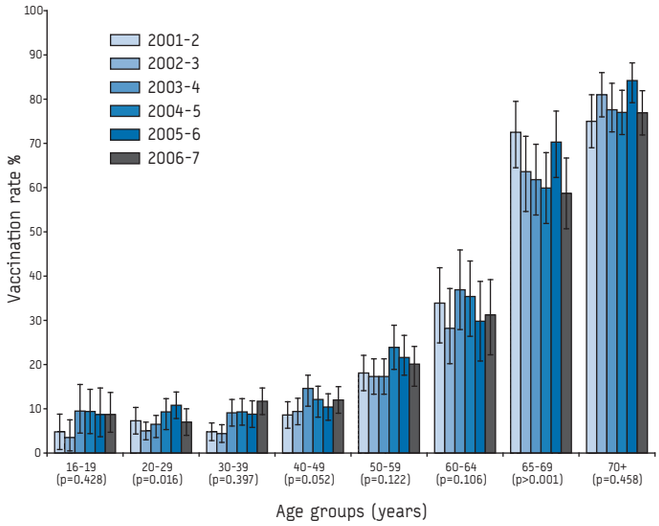
A question exploring the prevalence of chronic illness was included in the questionnaire from season 2003-4 onwards. Over the four observed seasons, significantly higher vaccination rates were found among the chronically ill, compared to the non-target group. In season 2006-7, an increase to 59.4% (95% CI: 52.4-67.4) was seen in this group, contrasting with values of 47.2% (95% CI: 39.2-55.2) in 2003-4 and 47.5 (95% CI: 39.5-54.5) in season 2005-6. Vaccination coverage in the group of healthcare professionals tended to decline over the years (p for trend = 0.152) but after the lowest value of 14.3 (95% CI: 7.3-20.3) in season 2005-6 rose to 15.9% (95% CI: 8.9-22.9) in season 2006-7. Even though the coverage in this group is the lowest among target groups, it still is about twice as high as in the non-target group (8.6%; 95% CI: 6.6-9.6). In the composite target group, vaccination coverage was essentially stable in the period from 2003-4 to 2006-7, with three seasons in the range from 57.3% to 57.9% and a peak of 60.4% (95% CI: 57.4-64.4) in season 2005-6.

### Factors influencing vaccination coverage

Multivariate analysis of immunisation coverage accounted for membership in one or several target groups covering age, sex, educational level, and income. Since target group membership was the only covariate that showed a statistically significant effect in season 2006-7, the other potential influences (some of which suffered from considerable numbers of missing values) were excluded from the final logistic regression models (Table 2). A sex difference was apparent over time, with men being moderately less likely than women to be vaccinated (unadjusted odds ratio for women in season 2006-7: 1.2; CI 1.0; 1.5; p=0.062). However, no sex difference was present after adjusting for age, chronic illness, healthcare work and income. In 2006-7, the percentage of men

**Figure 3**

**Vaccinated population by age groups and influenza seasons; influenza vaccination coverage surveys, United Kingdom, from 2001-2 to 2006-7 (p-values for trends across seasons)**



in the non-target group was 52%, in the elderly it was 39.6%, in the chronically ill 48.5% and in the healthcare workers 30.8%.

Age 65 years or older was a significant predictor of vaccination (adjusted OR in 2006-7, compared to the non-target group: 19.9; odds ratios (ORs) ranging from 16.8 in season 2004-5 to 36.6 in season 2005-6). Individuals in the chronically ill target group had an odds ratio of 15.5 in season 2006-7, which was higher than in the previous three seasons (OR ranging from 9.3 to 11.2). Being aged 65 years or older and chronically ill raised the prediction of getting vaccinated distinctively in all seasons, with a maximum of 76.4 in season 2004-5. The likelihood of vaccination of health-care professionals was in the range of 1.8 and 3.8, and was 2.0 in season 2006-7.

The probability of vaccination for the composite target group (at least one of age ≥65 years, chronic illness, health-care worker) was 14.6 (CI: 11.5; 18.7) in season 2006-7, which was equal to the average of the four seasons from 2003-4 (data not shown). The highest probability of getting vaccinated was seen in season 2005-6 (OR 15.8; CI: 12.4; 20.1), and there was no trend over the four seasons covered (p for trend = 0.658, data not shown).

**Motivations and barriers to vaccination**

Table 3 shows reasons for getting or not getting vaccinated and how frequently they were named. In all seasons between 2001-2 and 2006-7 the reasons most frequently stated by those who had been vaccinated were "My family doctor/nurse advised me to do it" and "Because the flu is a serious illness and I did not want to get it". The media coverage of avian influenza and influenza pandemics had influenced the decision of 6.7% of the vaccinated respondents in 2006-7. This subgroup was not statistically different from the other vaccinated in terms of age, sex and belonging to a target group.

In season 2006-7 the most common reason for having never been vaccinated was "My family doctor did not recommend it to me" (38%, Table 3). Individuals previously vaccinated, but not in the current season (2006-7), most frequently said "I didn't think about it, I forgot it" (27.8%, previous season 28.1%), followed by "I do not feel concerned" (23.8%, same as in previous season).

There was little change in the knowledge about influenza vaccination in season 2006-7 compared to the previous seasons. Three-quarters of the surveyed were aware that it is possible to catch influenza even if vaccinated, and about two-thirds knew that

**Table 2**

**Adjusted odds ratios of vaccination coverage in target groups vs. the non-target group (adjusted for age ≥ 65 years, chronic illness, working in the medical field); influenza vaccination coverage surveys, United Kingdom, from 2003-4 to 2006-7 (n = 8,048)**

| Target group | 2003-4 n=2,013* | 2004-5 n=1,994* | 2005-6 n=2,015* | 2006-7 n=2,026* |
|---|---|---|---|---|
| **Age ≥ 65 years** | | | | |
| OR | 25.9 | 16.8 | 36.6 | 19.9 |
| (95% CI) | (18.8; 35.6) | (12.3; 23.0) | (25.9; 51.7) | (14.6; 27.3) |
| p-value | <0.001 | <0.001 | <0.001 | <0.001 |
| N | 266 | 282 | 248 | 258 |
| **Chronic illness** | | | | |
| OR | 10.0 | 11.2 | 9.3 | 15.5 |
| (95% CI) | (6.8; 14.6) | (7.8; 16.1) | (6.5; 13.4) | (10.8; 22.2) |
| p-value | <0.001 | <0.001 | <0.001 | <0.001 |
| N | 144 | 169 | 179 | 186 |
| **Chronic illness and age ≥ 65 years** | | | | |
| OR | 42.8 | 76.4 | 46.2 | 51.9 |
| (95% CI) | (24.3; 75.3) | (40.3; 144.7) | (27.1; 78.5) | (30.1; 89.6) |
| p-value | <0.001 | <0.001 | <0.001 | <0.001 |
| N | 83 | 103 | 105 | 119 |
| **Work in medical field** | | | | |
| OR | 3.8 | 2.4 | 1.8 | 2.0 |
| (95% CI) | (2.4; 6.0) | (1.5; 3.9) | (1.0; 3.1) | (1.1; 3.4) |
| p-value | <0.001 | <0.001 | 0.055 | 0.017 |
| N | 134 | 123 | 117 | 118 |
| **Work in medical field or chronic illness or age ≥ 65 years** | | | | |
| OR | 18.0 | 13.0 | 12.6 | 3.9 |
| (95% CI) | (17.1; 45.4) | (4.4; 38.6) | (5.5; 28.8) | (1.4; 10.9) |
| p-value | <0.001 | <0.001 | <0.001 | 0.01 |
| N | 20 | 14 | 24 | 17 |

* n< total sample for the season due to missing covariate values
Reference category: non-target group (persons who do not belong to any target group)

the infection is then less severe. A third of the interviewed persons agreed with the statement that the influenza vaccine would protect them against avian influenza, whereas a weak majority disagreed (52.5%).

The survey also showed that most people would be encouraged to get vaccinated in the future:

- "If my family doctor/nurse recommended it to me" (rank 1, 72%),
- "If I had more information on the vaccine regarding efficacy and/ or tolerance" (rank 2, 46%),
- "If my pharmacist recommended it to me" (rank 3, 35%),
- "If I knew more about the disease" (rank 4, 37%),
- "If there were other ways of administering the vaccine (orally, injection without needle)" (rank 5, 34%).

## Discussion and conclusion

Telephone interviews have been used on a number of occasions to study vaccination coverage in the UK [9]. The random drawing of telephone numbers has been shown to be a good basis for a high quality selection process [17].

Despite correct sampling non-response is the major potential reason for selection bias. Comparisons of telephone, mail and face-to–face surveys on health-related issues, however, revealed only minor differences between modes of administration and modest non-response effects with respect to prevalence estimates [16,18]. In comparison with mailed surveys, non-response was found to be less content-oriented in telephone surveys [19]. Furthermore, bias due to dissimilar sociodemographic characteristics of individuals not reachable by telephone only slightly affected reporting of illness and related use of medical services, as long as the general population was addressed, and telephone coverage exceeded 90% [19,20]. These reports support the validity of our approach, even though we had no ways to independently confirm self-reported vaccination status. An earlier publication has described the limitations of the present data collection in greater detail [15]. The use of wireless telephones is a growing problem. In the United States (US) persons with landlines were shown to have higher odds of being vaccinated than those with exclusive access to wireless telephones (OR 1.27) [21]. If the same is true in the UK where mobile phones are even more common than in the US [22-24], our reported vaccination rates may have been slightly over-estimated.

The decrease in overall vaccination coverage in the UK in season 2006-7, compared to seasons 2004-5 and 2005-6, was not statistically significant. Coverage was still higher than in the seasons before 2004-5 and there is no strong evidence for a long-term change of trend. In 2006-7, 38.4% of the respondents expressed the intention to get vaccinated in season 2007-8. Thus, in the UK there may be a potential to increase future vaccination coverage provided that those who intend to get vaccinated but in the end do not are better targeted. Additionally, the increasing trend of those who had been vaccinated in the past but not in the current season could be explained by a failure of vaccine campaigns to maintain their trust in vaccination. A decreasing trend, however, was apparent in the age group of 65 to 69 years old respondents whose coverage dropped to the lowest level since season 2001-2 (Figure 3). As vaccinations are offered free of charge by the UK National Health Service, and as it is government policy to vaccinate all people aged 65 years and older we have no explanation for the decreasing trend in this particular age group. No trend over all seasons was apparent in the age group of 70 years or older, although the vaccination coverage in this group also reached the peak level in season 2005-6 and in 2006-7 returned to similar value as in season 2004-5.

In the two years before season 2006-7, the UK media have frequently reported on avian influenza and a potential shortage of antiviral agents. This may have increased the primary care providers' awareness of the risk of influenza pandemic and, by consequence, may have positively affected vaccination coverage in one of the high risk groups, namely the elderly, in season 2005-6. However, after season 2005-6 avian influenza lost the focus of the media [25], which may be a possible cause of the coincident decline in vaccination rates in 2006-7 to levels observed before the 2005-6 season. However, only less than 7% of respondents

**Table 3**

**Ranking of reasons for and against vaccination; influenza vaccination coverage surveys, United Kingdom, from 2001-2 to 2006-7 (n = 10,252)**

| Motivations to get vaccinated (among those vaccinated in the current season) | 2001-2 n=458 Rank (%) | 2002-3 n=451 Rank (%) | 2003-4 n=497 Rank (%) | 2004-5 n=507 Rank (%) | 2005-6 n=524 Rank (%) | 2006-7 n=509 Rank (%) |
|---|---|---|---|---|---|---|
| My family doctor/nurse advised me to do it | 2 (70) | 2 (75) | 1 (49) | 1 (60) | 1 (51) | 1 (60) |
| Because flu is a serious illness and I did not want to get it | 1 (73) | 1 (82) | 2 (47) | 2 (46) | 2 (42) | 2 (50) |
| Because of my age | 3 (59) | 4 (56) | 3 (41) | 3 (39) | 3 (40) | 3 (42) |
| Because I am not in a very good health | 6 (34) | 6 (33) | 5 (25) | 4 (30) | 5 (25) | 4 (32) |
| So I do not pass the flu bug to my family and friends | 4 (56) | 3 (57) | 4 (28) | 5 (28) | 4 (27) | 5 (32) |
| Because the social security system pays for it | 5 (40) | 5 (36) | 6 (25) | 6 (26) | 6 (24) | 6 (29) |
| **Reasons for not getting vaccinated (among those never vaccinated)** | **2001-2 n=1,281 Rank (%)** | **2002-3 n=1,274 Rank (%)** | **2003-4 n=1,228 Rank (%)** | **2004-5 n=1,185 Rank (%)** | **2005-6 n=1,155 Rank (%)** | **2006-7 n=1,183 Rank (%)** |
| My family doctor did not recommend it to me | 1 (56) | 1 (54) | 2 (33) | 1 (37) | 2 (37) | 1 (38) |
| I have never considered it before | 2 (56) | 2 (51) | 3 (33) | 2 (34) | 1 (37) | 2 (35) |
| I do not think I am very likely to catch the flu | 4 (32) | 3 (41) | 1 (34) | 3 (33) | 3 (30) | 3 (33) |
| I am too young to be vaccinated | 3 (34) | 4 (37) | 4 (29) | 4 (31) | 4 (29) | 4 (32) |
| My pharmacist did not recommend it to me | - | 5 (34) | 5 (17) | 5 (18) | 5 (19) | 5 (21) |

listed the media as one of the factors influencing the decision to get vaccinated. Also, it neither explains the transitory decline in vaccination coverage in the chronically ill group in season 2005-6, nor the more long-term decrease in the healthcare professionals' vaccination rates. Working as helth professional in the UK did not distinctly encourage vaccination as the adjusted odds were several magnitudes lower than those of the other defined target groups. Furthermore, the rate of vaccinated healthcare workers seemed to be decreasing (although statistically non-significant). Previous publications on influenza vaccination coverage [26-31] found low coverage rates in healthcare workers in Germany, ranging from 8% to 26% [27]. In comparison, in the UK surveys from 2001-2 to 2006-7 we obtained vaccination coverage in healthcare workers ranging between 14.3% and 25.2%, whereas the rates in non-target group never exceeded 9.4%.

Our observations on immunisation uptake in the UK population are largely consistent with findings from studies performed in the UK using a representative general practice database [32]. One notable difference regarding coverage trends is that in contrast with our findings the study by Coupland *et al.* found invariably increasing vaccination rates from every season to the next in all risk groups. The reason for these divergent results may primarily lie in the different approaches to collecting data; while Coupland's data were sampled from a subset of the population that visited a general practitioner (QRESEARCH database [33,34]), our data were sampled from the entire population accessible by telephone, irrespective of whether the respondents visited a physician or not.

Vaccination rates of children and young people under 16 years of age were not covered by our article. However, high vaccination coverage in children will be difficult to achieve at least in some countries, as paediatric recommendations for influenza vaccination in healthy children in most countries are nonexistent. This is why reaching high vaccination levels in the risk populations is even more important.

The overall vaccination rate in eleven European countries was 20.2% in season 2006-7 [our survey series, unpublished data]. Thus, in season 2006-7 the vaccination rate in the UK (25.0%) was higher than the European average. In previous seasons, the UK rates were above or slightly below the average of five European countries [13,19,35].

Regarding individual motivations for vaccination, our data confirm that the recommendation from the family doctor or nurse is the most important encouraging factor. Other publications support this finding [15,26,31,36-38]. A better understanding of the disease and administration of the vaccine without needle would generally encourage a third of the surveyed, and more information on the vaccine would encourage two-fifths of the respondents to get vaccinated in the future.

In order to achieve higher vaccination coverage, dealing with barriers to vaccination and enhancing positive motivations remain an important undertaking in the UK. This challenge should be accepted not only by the patients' key motivators, the primary care professionals, but also by government agencies, health professional organisations and independent media, which could all contribute to bridging the knowledge gap.

According to the WHO, the influenza pandemic risk remains on a high level [1]. Efforts should be made at national and international levels to raise coverage as set out in the WHO objectives (i.e. 50% vaccination coverage in the elderly to be reached in 2006 and 75% in 2010 [39]). As in the years before, the UK exceeded the 2006 goal with the 2006-7 vaccination rate reaching 65.3% in those aged 65 years and older. To arrive at the WHO objectives for 2010, though, additional efforts are required, and this remains a challenge for health organisations, primary healthcare providers, government, and the media.


## Competing interests

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# Research articles

## Trends in seasonal influenza vaccine distribution in the European Union: 2003-4 to 2007-8

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Seasonal influenza is widely regarded as a continuing threat to public health, with vaccination remaining the principal measure of prophylaxis. In 2003, the World Health Organization issued targets for influenza vaccine coverage in the elderly of at least 50% by 2006 and 75% by 2010, endorsed by the European Parliament in two resolutions in 2005 and 2006. However, a number of European public health systems lack mechanisms to assess progress in influenza vaccine uptake. The European Vaccine Manufacturers group (EVM) undertook a Europe-wide survey of vaccine distribution over the last five seasons (between 2003 and 2008) to provide baseline data from which vaccination trends may be extrapolated. The survey data showed that the dose distribution level *per capita* in the 27 EU countries increased from 17% in 2003-4 to 20% in 2006-7; this growth was not maintained in the season 2007-8. Even without information on which age or risk groups received the vaccine, an immunisation rate of approximately 20% of the whole population falls short of the public health goal by more than half: an estimated 49% of the total population fall into risk groups recommended to receive the influenza vaccine in Europe. These data provide the only systematic review of vaccine dose distribution across Europe from a uniform source. Although they represent an important baseline parameter, age- and risk-group related vaccine uptake data with sufficient detail are needed to assist public health policy decision making, immunisation planning and monitoring. In light of this situation, and to support the improvement of immunisation rates across the EU, EVM aims to provide dose distribution data for each influenza season to assist Member States in the implementation of local immunisation policies.

### Introduction

Annual influenza epidemics continue to pose a substantial threat to public health. In Europe, estimates suggest that influenza is responsible for between 40,000 excess deaths in a moderate season and up to 220,000 during a severe epidemic [1]. Despite the recent focus on pandemics and accompanying extensive coverage in the media, seasonal influenza is responsible for many more deaths than those caused by influenza pandemics [1]. Consequently, the World Health Organization (WHO) and the Member States of the European Union (EU) recommend annual influenza vaccination for those at high risk of complications. A survey conducted in 2006 by the European Centre for Disease Prevention and Control (ECDC) among EU and European Economic Area (EEA) countries found that in the 23 Member States that responded, immunisation was recommended for the two largest groups targeted by WHO: the elderly above a nationally defined age limit (often 65 years but in some cases 60 or 50 years) and those over the age of six months with chronic illnesses such as heart or lung disease [1,2].

In 2003, the 56th World Health Assembly (WHA) recognised that influenza epidemics "cause fatal complications in up to one million people each year" and "that many of these deaths could be prevented through increased use, particularly in people at high risk, of existing vaccines, which are safe and highly effective". The WHA urged its member states to increase immunisation against seasonal influenza, and set a coverage target of at least 50% of the elderly by 2006, rising to 75% by 2010 [3]. In October 2005 and June 2006 the European Parliament adopted resolutions calling on the Member States to increase influenza vaccination in line with the WHO recommendations [4,5]. With these guidelines in place, Ryan et al. estimated that risk groups recommended for vaccination against influenza every year accounted for up to 49% of the population of the 25 EU countries in 2006, or 223 million people [6].

Despite these guidelines and targets, no Europe-wide systematic data are available to monitor vaccine uptake. Monitoring is conducted in only some Member States. Furthermore, there is no system to allow performance comparisons across the EU. Consequently, following a request from the European Commission, the European Vaccine Manufacturers group (EVM) surveyed suppliers in the region to provide baseline data on influenza vaccine distribution in the EU. These data represent a valuable indirect measure of vaccine use, and as such can be utilised by public health policy makers in conjunction with information on local vaccination recommendations, implementation measures and reimbursement criteria to assess gaps in provision and improve coverage where necessary.

### Methods

In 2008, EVM issued a standardised, retrospective survey to its member companies (Baxter, Crucell, GlaxoSmithKline Biologicals, Novartis Vaccines, Sanofi Pasteur, Sanofi Pasteur MSD, Solvay, and Wyeth) and the Australian company CSL Biotherapies, who collectively supply nearly all of the influenza vaccines distributed in Europe, with the exception of Hungary and Romania, which each have a national producer. The survey was designed to assess the total number of doses supplied to each of the 27 EU Member States during the last five influenza seasons. The supply period was defined by influenza seasons rather than calendar years to reflect immunisation practise: in temperate zones influenza occurs in

epidemics during the winter and each influenza season consequently straddles the end of one calendar year and the beginning of the next. In the United Kingdom (UK), the study utilised data provided by the local national vaccine industry group (UVIG), which were collected by a similar methodology.

Data were collected covering the five influenza seasons from 2003-4 to 2007-8, which represent the period since the establishment of the WHA targets for influenza vaccine coverage in the elderly. To ensure full compliance with competition law, the manufacturers submitted dose distribution information to a single, independent collection point, where the data were aggregated and anonymised before further analysis was undertaken by the survey group. To determine the level of dose distribution per unit of population, the Eurostat database was accessed to ascertain the number of inhabitants of each of the 27 EU countries on the first of January each year during the survey period (1/1/2003; 1/1/2004; 1/1/2005; 1/1/2006; and 1/1/2007).

## Results

The five-year dataset generated by the survey provides a comprehensive picture of influenza vaccine distribution and supply trends in the EU.

### Dose distribution: macro-analysis

Throughout the study period, the total number of doses distributed across the region showed a general growth trend, rising from 81.1 million at the lowest point in 2004-5 to a peak of 98.6 million in 2006-7 (Figure 1). However, this overall trend was non-uniform with a slight drop in supply between the first and second year of the surveyed period, and a similar decrease in the last two years of the study (2006-7 to 2007-8).

When comparing these data against the number of doses that would be required to cover the proportion of the population at risk that is recommended for vaccination in Europe (up to 49.1% for the EU25; ranging from 41.6% in Cyprus to 56.4% in the UK [6]) it became clear that neither the EU Member States collectively (Figure 2) nor any individual country (Figure 3) sustained this level. Throughout the survey period, sufficient doses to immunise the entire at-risk population were available only during one season in a single country: during a substantial increase in supply in Malta in the 2005-6 season (Figure 3). Across the EU as a whole, the dose distribution level *per capita* reached above 20% in a single season during the study period; the level of supply required to immunise all those in at-risk groups was not reached in any of the years (Figure 2).

### Dose distribution: by country

The country data show wide variations in distribution between different EU Member States. Not surprisingly, the five largest countries (France, Germany, Italy, Spain and the UK) accounted for the majority of doses distributed in the EU region. Regarding the number of distributed doses, these countries received a consistent share of the total, amounting to approximately 75% in each season (ranging from 74.7% in 2004-5 to 76.6% in 2005-6). Based on the population of these countries, this corresponds to a greater than representative proportion as their aggregate population remained steady during the period, with just over 62% of the total inhabitants of the 27 EU countries.

Disproportionate vaccine distribution is even more evident when the data are analysed in conjunction with the Eurostat database; the supply per unit of population shows wide variance between countries, in some Member States from year to year (Figure 3). Of particular note is the dramatic, albeit unsustained, increase in dose distribution in Malta in 2005-6, during a season of robust policy support targeting at-risk groups. Also noteworthy is the trend towards higher distribution in the EU15 countries versus the newer Member States. However, this is confounded to some degree by the relatively lower supply in some of the Nordic countries.

## Discussion

This study provides for the first time a systematic view, drawn from a uniform source, of seasonal influenza vaccine distribution across Europe. While a number of methodological limitations

**Figure 1**

**Total number of seasonal influenza vaccine doses distributed in the 27 EU countries, seasons 2003-4 to 2007-8 (n= 450.069 million)**

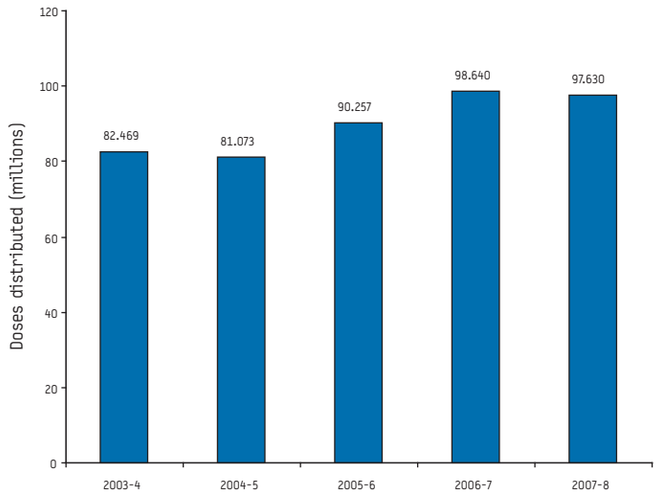
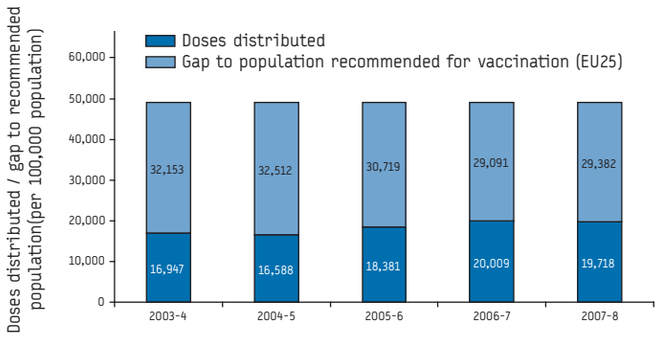



**Figure 2**

**Influenza vaccine doses distributed per 100,000 inhabitants of the EU27 countries versus proportion of the population recommended for vaccination (EU25)*, seasons 2003-4 to 2007-8**




* [6]

necessarily exist, these data nonetheless represent an important baseline against which the implementation of immunisation guidelines in the Member States may be assessed.

The survey group performed a number of quality audits, including an assessment against information compiled by national vaccine industry groups. However, a number of methodological limitations remain:

- Although vaccine supply and coverage are likely to be inextricably linked, the survey data represent the total number of doses distributed per country rather than the direct uptake in specific groups recommended for immunisation.
- The data necessarily provide an overestimation of vaccine coverage as a small percentage of doses remain unused and/or are returned each year. This proportion varies from year to year and by country (ranging from approximately 0 to 10%) and can be determined accurately only for those territories with centralised purchasing systems.
- The data for Hungary and Romania include the doses supplied by EVM survey participants, but not those supplied by local Hungarian and Romanian manufacturers.

Notwithstanding, the study reveals a clear variance in vaccine distribution between the 27 EU countries, with a trend towards greater provision in the EU15 Member States. However, it is noteworthy that these generally higher levels of supply *per capita* are not consistent, and whilst detailed economic analysis is beyond the scope of this paper, distribution levels do not appear to follow a simple direct correlation with economic development status. For instance, during the last three years of the study, Malta consistently achieved the highest levels of supply *per capita* throughout the EU27, while Cyprus and, in the latter years of the survey, Romania had a performance similar to those of several EU15 countries. Similarly, distribution levels in Denmark and Sweden were below those in many other EU15 Member States, including the five largest (France, Germany, Italy, Spain and the UK). These confounders suggest that a more subtle blend of factors relating to immunisation policy implementation influence overall vaccine supply, rather than a simple linear correlation with income.

At the macro level, the data collected over the 2003-4 to 2007-8 influenza seasons describe a modest growth in the dose distribution level *per capita* (from approximately 17% to just under 20%). However, while this trend is encouraging, the distribution rates both at the European and Member State level remain substantially below the rates required to immunise the estimated 49% of the population recommended for seasonal influenza vaccination [6].

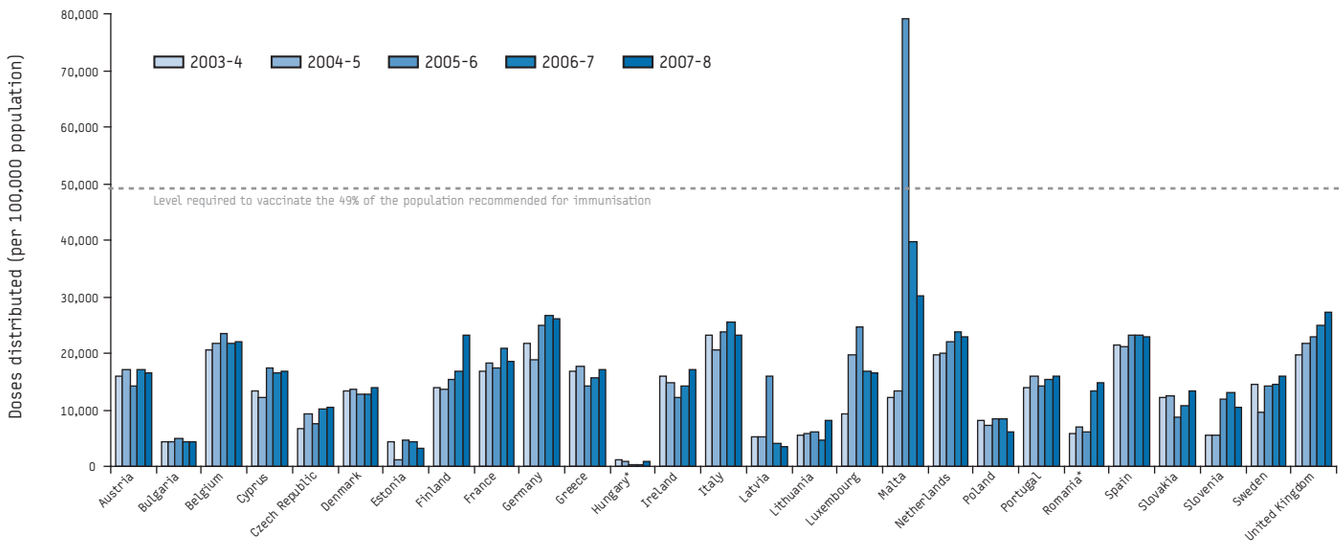
Given the serious and ongoing threat posed by annual influenza epidemics, improving immunisation coverage remains an important policy objective for WHO and the EU. Achieving this will require concerted efforts at the national level to ensure the effective implementation of existing guidelines. In some countries the current recommendations will need to be adapted to encompass all those who are at risk.

Previous research conducted in 11 European countries identified a number of key factors that would motivate at-risk populations to seek influenza vaccination [7]. Most important was the proactive recommendation by a healthcare professional, followed by information on the disease and the vaccine, and adequate funding to reimburse patients for vaccination or to make it cheaper. Notably, the three countries in which authorities provided low or no funding for seasonal influenza vaccination achieved the lowest coverage levels [7].

Based on a recognition at the national level of the need for robust vaccination policies, combined with long-term commitment to their effective implementation, vaccine production and distribution capacity can expand to meet the challenge of improving coverage

**Figure 3**

**Distribution of seasonal influenza vaccine doses per 100,000 inhabitants in EU27 countries, seasons 2003-4 to 2007-8 (compared with the level required to immunise those in recommended groups in the EU25 assuming no wastage or return of doses)**




*The numbers for Hungary and Romania do not take into account the doses distributed from the national vaccine manufacturers in these countries

rates. While increasing capacities is a long-term process requiring significant investment, it is clear from historical data that vaccine distribution can increase dramatically to meet demand: notably, during the period 1994-2003, global distribution of influenza vaccines more than doubled [8].

## Conclusion

With influenza continuing to pose a public health challenge, the introduction of robust vaccine monitoring systems represents an important step to assess progress in reaching immunisation goals in Europe, and to inform public health decision making for improving protection across the region. This survey provides for the first time a unique view of vaccine supply throughout the EU. The data demonstrate significant differences in vaccine distribution between European countries. With some variation, the results indicate that immunisation in many countries often does not even reach half of those who are considered by national authorities to be at high risk of complications from influenza infection. However, complementary surveys with age- and risk-group specific information will be needed to focus national health interventions on the specific drivers and hurdles for influenza immunisation. Analysis of the study data in conjunction with local recommendations, reimbursement processes and public health communication campaigns should provide valuable insights into the efficacy of vaccination policies in the Member States. EVM aims to provide systematic supply data on a regular basis which will complement national efforts to assist policy makers in determining the most effective approaches to improving vaccination levels amongst those at risk from seasonal influenza.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# Research articles

## Low influenza vaccination coverage in asthmatic children in France in 2006-7

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In France, annual seasonal influenza vaccination has been recommended since 2000 for patients suffering from chronic respiratory diseases, including asthma. Since 1988, each year from September to December, a free influenza vaccination voucher is sent by the French Public Health Insurance authorities to patients with chronic respiratory disease, including severe asthma. In November 2006, this measure was extended to all asthmatic patients, irrespective of asthma severity. The present paper examines the 2006-7 influenza vaccination coverage rate (VCR) in 433 asthmatic children aged 6 to 17 years (mean age: 9.5 years; male: 61%) who consulted a paediatric pulmonologist between March and September 2007 in eight hospitals throughout France. The influenza VCR was 15.7% for the 2006-7 season (13.9% for the 2005-6 season and 10.9% for the 2004-5 season). General practitioners vaccinated 72.1% of the children. "Lack of information" (42%) was the most frequently reported reason for non-vaccination. Vouchers (received by 39.6% of the children) significantly increased the VCR (31% versus 5.9%; $p<0.001$). In France, in 2006-7, the influenza VCR in asthmatic children was far below the national public health objective (at least 75% for the year 2008). Concerted action is needed to improve the influenza VCR in asthmatic children.

### Introduction

Influenza can be a serious infection for patients with asthma because it may trigger or exacerbate asthma symptoms. About 80% of acute exacerbations in asthmatic children are triggered by a respiratory virus infection, and influenza virus is one of the most common viruses affecting the respiratory tract together with rhinovirus, respiratory syncytial virus and coronavirus, particularly during winter epidemics [1,2]. For the 2003-4 season, Bhat *et al.* reported that 12 of 132 (9%) US children over six months of age with fatal influenza disease had asthma without other pulmonary disease [3].

Influenza vaccines are safe and effective in children [4,5], and recommended by the World Health Organization (WHO) for all children aged over six months with chronic conditions such as pulmonary and cardiovascular illness [6]. Most European countries have introduced annual influenza vaccination recommendations for high-risk patients from six months of age [7,8].

In France, annual seasonal influenza vaccination has been recommended since 2000 for patients of any age from six months old suffering from diverse chronic diseases including respiratory disorders such as asthma [9]. One of the French national public health objectives for 2008 is to achieve a 75% influenza vaccination coverage rate (VCR) for all high-risk patients, including asthmatic patients [10]. In France, asthma prevalence reaches 10% in children and 5% in adults [11]; in 1998, 3.5 million people were suffering from asthma [12].

Since 1988 [13], each year from September to December a free influenza vaccination voucher is sent by the French Public Health Insurance authorities to patients with chronic respiratory disease, including severe asthma. In November 2006, this measure was extended to all asthmatic patients, irrespective of asthma severity [14].

There is only very limited specific data available on influenza vaccination coverage in French asthmatic children [15]. To the best of our knowledge, the present study is the first to specifically assess influenza VCR in French asthmatic children. The primary objective was to estimate the VCR for the 2006-7 season in French asthmatic children. The secondary objectives were to examine factors influencing vaccination uptake and reasons for non-vaccination for the 2006-7 season and to estimate the VCR for the previous two influenza seasons.

### Methods

#### Study design

This descriptive observational study was performed in France from March to September 2007 (i.e. after the 2006-7 influenza season). The study was initially submitted to 11 investigators (paediatric pulmonologists) from nine French academic hospitals.

A total of nine paediatric pulmonologists from eight academic hospitals agreed to participate in the study. The eight academic hospitals were located throughout France (Bordeaux, Clermont-Ferrand, Grenoble, Lille, Marseille, Paris, Strasbourg and Toulouse) and represented two thirds (N=8/12) of the French hospitals with a paediatric pulmonology unit.

Being an observational study which did not change the patient's usual medical management, the study protocol was not submitted to an ethics committee for approval, in line with current French legislation. Patients and their parents (or guardians) were provided by the paediatric pulmonologist with oral and written information, and oral consent was obtained from the parents before children were included in the study. Only patients whose parents gave their oral consent were included in the study. They were informed of their rights under the French information protection law. All questionnaire data were rendered anonymous using the Mapi-Naxis procedure validated by the *Commission Nationale de l'Informatique et des Libertés* (French Information Protection Commission) [16].

### Study population

Each paediatric pulmonologist participating in the study consecutively included all children (girls and boys) who met the following inclusion criteria:

- aged ≥6 and ≤17 years in September 2006,
- seen at hospital by a paediatric pulmonologist,
- suffering from asthma diagnosed for at least six months,
- having a vaccination card enabling influenza vaccination status to be checked.

Diagnosis of asthma in children aged five and younger is considered to be difficult and unreliable according to the Global Initiative for Asthma (GINA) report, therefore only children ≥6 years were included in the present study [17,18].

### Data collection

The paediatric pulmonologist filled in an anonymous questionnaire for each child included. The following demographic and clinical data were collected: birth date, sex, date of asthma diagnosis and asthma severity evaluated at inclusion according to the GINA classification [18] (i.e., intermittent, mild persistent, moderate persistent and severe persistent asthma). Parents were asked for the 2006-7 influenza season vaccination status of their children (yes or no) and receipt of a voucher for free influenza vaccination from the National Public Health Insurance authority for the 2006-7 season (yes or no). For vaccinated patients, the parents were asked for the identity of the vaccinator (i.e. health care professional who had administered the vaccine). The influenza vaccination date was recorded from the vaccination card. For children who had not been vaccinated parents were asked for the reasons for non-vaccination. Parents could give one or more than one reason for non-vaccination. The following reasons were specified in the questionnaire: lack of information, vaccine useless (disease considered as benign), forgotten or lack of time, vaccine considered as ineffective, vaccine considered as dangerous, allergy to egg, other allergy, vaccine contraindication, concomitant disease, current asthma exacerbation, afraid of injection. Parents could spontaneously report reasons for non-vaccination via the item "other reasons". Status and date of vaccination were recorded from the vaccination card for the 2004-5 and 2005-6 seasons.

### Statistical Methods

All data were analysed by Mapi-Naxis (Lyon, France). Statistical analyses were performed on SPSS 14.0 software. A descriptive analysis for all the variables of the questionnaire was performed. For each variable, percentages were calculated using available data (missing data ignored). The influenza VCR value was given with its 95% confidence interval (95% CI). The chi-square test was used for comparison of VCRs in asthmatic children with and without free vouchers; the significance threshold was set at 0.05.

## Results

### Study population characteristics

Paediatric pulmonologists collected data for 435 asthmatic children. Data for two children were excluded from the analysis because they had been vaccinated against influenza before the official availability of the 2006-7 influenza season vaccine in France on 12 October 2006 [19]). Finally, data from 433 children were analysed.

In September 2006, at the beginning of the 2006-7 influenza season, the mean age of the analysed study population (N=433) was 9.5 ± 2.9 years (mean ± standard deviation). The distribution according to age groups was as follows: 6-9 years of age, 56.4% (N=244); 10-13 years of age, 30.9% (N=134); 14-17 years of age, 12.7% (N=55). The children were mainly boys (61%). There were more boys than girls in the 6-9 year age group (N=145 versus 90, respectively) and in the 10-13 year age group (N=88 versus 42, respectively) and fewer in the 14-17 year age group (N=22 versus 31, respectively) (Table 1).

**TABLE 1**

**Study population characteristics**

| Demographic and clinical characteristics | All (N = 433) | Male (N = 255) | Female (N = 163) |
|---|---|---|---|
| Male: N (%) | 255 (61.0) | - | - |
| Age (years): Mean ± SD | 9.5 ± 2.9 | - | - |
| Asthma duration (years): Mean ± SD | 6.1 ± 3.5 | - | - |
| Age at diagnosis (years): Mean ± SD | 3.6 ± 2.6 | - | - |
| **Asthma severity, Global Initiative For Asthma (GINA) classification: N (%)[a,b]** | | | |
| 6-9 year old patients | 244 (56.4) | 145 | 90 |
| Intermittent<br>Mild persistent | 64 (26.2)<br>110 (45.1) | 38 (26.2)<br>65 (44.8) | 22 (24.4)<br>40 (44.4) |
| Moderate persistent | 60 (24.6) | 38 (26.2) | 22 (24.4) |
| Severe persistent | 10 (4.1) | 4 (2.8) | 6 (6.7) |
| 10-13 year old patients | 134 (30.9) | 88 | 42 |
| Intermittent<br>Mild persistent | 33 (24.8)<br>43 (32.3) | 26 (29.9)<br>27 (31.0) | 7 (16.7)<br>13 (31.0) |
| Moderate persistent | 41 (30.8) | 23 (26.4) | 17 (40.5) |
| Severe persistent | 16 (12.0) | 11 (12.6) | 5 (11.9) |
| 14-17 year old patients | 55 (12.7) | 22 | 31 |
| Intermittent<br>Mild persistent | 14 (25.5)<br>17 (30.9) | 8 (36.4)<br>8 (36.4) | 6 (19.4)<br>9 (29.0) |
| Moderate persistent | 20 (36.4) | 5 (22.7) | 13 (41.9) |
| Severe persistent | 4 (7.3) | 1 (4.5) | 3 (9.7) |

Missing data:
[a]For 15 patients data on sex missing
[b]For 1 patient asthma severity data missing

In September 2006, the mean duration of asthma in the analysed study population was 6.1±3.5 years. Mean age at diagnosis was 3.6±2.6 years. The severity of asthma according to the GINA classification [18] was known for all but one patient: 111 (25.7%) had intermittent, 170 (39.4%) mild persistent, 121 (28.0%) moderate persistent and 30 (6.9%) severe persistent asthma. In boys the highest proportion of intermittent asthma was found in 14-17 year-olds, mild persistent asthma in 6-9 year-olds and severe asthma in 10-13 year-olds. In girls the proportion of patients with severe persistent asthma increased with age and was highest in the age groups 10-13 and 14-17 years (Table 1).

**Influenza VCRs for the 2006-7, 2005-6 and 2004-5 seasons**

Of the 433 children analysed, 68 were vaccinated against influenza during the 2006-7 season. The global 2006-7 VCR was 15.7% (CI 95%: 12.6%-19.3%). The VCRs for the previous two seasons (2005-6 and 2004-5) were 13.9% (CI 95%: 10.9%-17.3%) (60 vaccinated children) and 10.9% (CI 95%: 8,2%-14.0%) (47 vaccinated children), respectively.

**Influenza VCR for the 2006-7 season according to age, sex and severity of asthma**

A total of 29/244, 24/134, and 15/55 children from the 6-9, 10-13, and 14-17 age groups, respectively, were vaccinated during the 2006-7 season. The influenza VCRs increased with age: 11.9% in the 6-9, 17.9% in the 10-13, and 27.3% in the 14-17 age group (Table 2).

Girls aged 6-9 years were less frequently vaccinated than boys in the same age group (7.8% versus 13.8%), whereas girls aged 10-13 years and 14-17 years were more frequently vaccinated than boys in the same age groups (21.4% and 35.5% versus 15.9% and 18.2%, respectively) (Table 2).

A total of 7/111 patients with intermittent asthma, 25/170 patients with mild persistent asthma, 25/121 patients with moderate persistent asthma and 10/30 patients with severe persistent asthma were vaccinated for the 2006-7 season. The influenza VCR increased with asthma severity, from 6.3% in children with intermittent asthma to 33.3% in those with severe persistent asthma (chi-square test: p<0.001) (Figure 1 and Table 2).

**Influenza VCR for the 2006-7 season according to free vaccination voucher reception**

Data regarding the receipt of a free vaccination voucher for the 2006-7 season were available for 424 children (nine missing

TABLE 2

**Vaccination coverage against influenza among asthmatic children according to age, sex, and asthma severity, France, influenza season 2006-7**

| Groups | Number | Vaccinated | % | CI 95% |
|---|---|---|---|---|
| Total | 433 | 68 | 15.7 | (12.6; 19.3) |
| **Age** | | | | |
| 6-9 years | 244 | 29 | 11,9% | (7,8% - 16,0%) |
| 10-13 years | 134 | 24 | 17,9% | (11,4% - 24,5%) |
| 14-17 years | 55 | 15 | 27,3% | (16,1% - 41,0%) |
| **Sex: female** | | | | |
| 6-9 years | 90 | 7 | 7,8% | (3,1% - 15,4%) |
| 10-13 years | 42 | 9 | 21,4% | (10,3% - 36,9%) |
| 14-17 years | 31 | 11 | 35,5% | (19,2% - 54,7%) |
| **Sex: male** | | | | |
| 6-9 years | 145 | 20 | 13,8% | (8,1% - 19,5%) |
| 10-13 years | 88 | 14 | 15,9% | (8,9% - 25,3%) |
| 14-17 years | 22 | 4 | 18,2% | (5,1% - 40,3%) |
| **Asthma severity** | | | | |
| Intermittent | 111 | 7 | 6,3% | (1,7% - 10,9%) |
| Mild persistent | 170 | 25 | 14,7% | (9,3% - 20,1%) |
| Moderate persistent | 121 | 25 | 20,7% | (13,4% - 27,9%) |
| Severe persistent | 30 | 10 | 33,3% | (17,2% - 52,9%) |

FIGURE 1

**Vaccinated asthmatic children according to asthma severity (Global Initiative For Asthma - GINA classification), 2006-7 influenza season, France (n=432)**

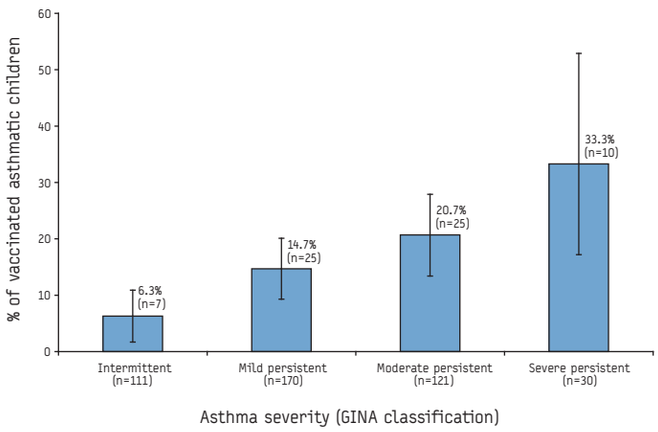
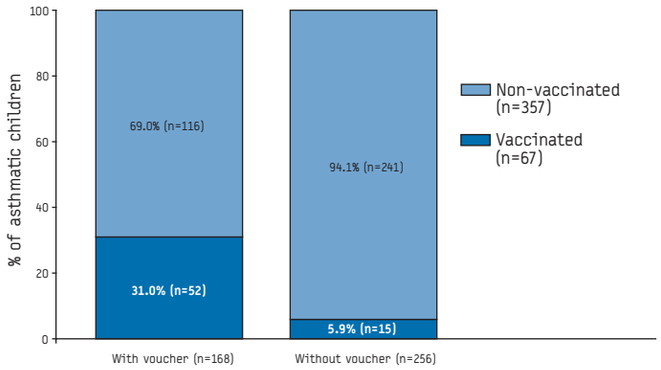



FIGURE 2

**Vaccinated and non-vaccinated asthmatic children according to reception of voucher*, 2006-7 influenza season, France (n=424)**




* A voucher for free influenza vaccination is provided by the French Public Health Insurance authorities to all asthmatic patients, irrespective of asthma severity

values). According to the information provided by parents, 168 (39.6%) children had received a voucher.

A total of 52 (31.0%) of the 168 children who received a free voucher were vaccinated compared to 15 (5.9%) of the 256 children who did not receive any voucher (Figure 2): Receiving a free vaccination voucher increased vaccination coverage in asthmatic children (31.0% versus 5.9%; chi-square test: p<0.001). A total of 116 of the 168 children who received a free voucher (69.0%) were not vaccinated.

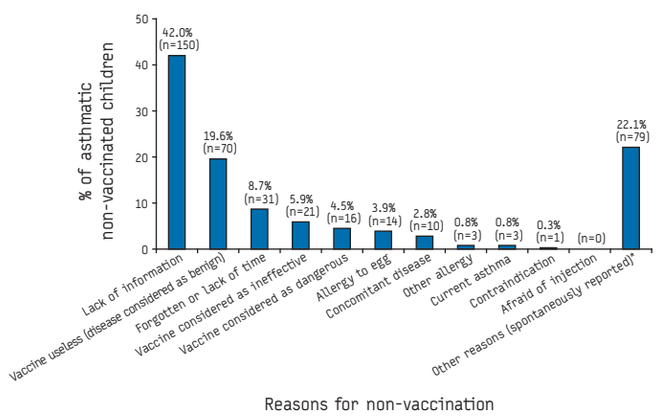
**Vaccinators and reasons for non-vaccination in the 2006-7 season**

The vaccination was mainly performed in private practice: 49 of the 68 children (72.1%) vaccinated for the 2006-7 influenza season were vaccinated by a general practitioner (GP), seven (10.3%) by a family paediatrician, and three (4.4%) by a hospital practitioner. Others were vaccinated by a nurse (N=5; 7.4%), their parents (N=3; 4.4%), or a pharmacist (N=1; 1.5%).

Reasons for non-vaccination were given for 357 of the 365 non-vaccinated children for the 2006-7 season. Among the reasons specified in the questionnaire, "Lack of information" (N=150; 42.0%), "Vaccine useless (disease considered as benign)" (N=70; 19.6%), "Vaccine considered as ineffective or as dangerous" (globally 10.4% of cases: for each item N=21, 5.9% and N=16, 4.5% respectively), and "Forgotten or lack of time" (N=31; 8.7%) were the most frequently reported reasons for non-vaccination (Figure 3). Allergy was a major motive for influenza non-vaccination for 17 children: 14 children (3.9%) declared an "Allergy to egg". Among them, there was one case of egg allergy with clinical signs of anaphylactic shock and 13 with egg allergies but no history of anaphylactic shock. "Other allergy" was reported as reason for non-vaccination for three children (0.8%). No allergy to one of the vaccine components was reported. A case of permanent rhinitis was considered as vaccine contraindication. The most frequent spontaneously reported reasons for non-vaccination were: "Vaccine not proposed" (N=26, 32.9%), "No medical indication" (N=15, 19.0%), and "No favourable opinion of this vaccine by the family practitioner" (N=10, 12.7%).

**FIGURE 3**

**Reasons for non-vaccination of asthmatic children, 2006-7 influenza season, France (n=357, info missing for 8 children, multiple answers possible)**




* The first three spontaneously reported reasons were: vaccine not proposed, no medical indication, no favourable opinion of this vaccine by the family practitioner

## Discussion

Our study provides the first estimates of influenza VCR in France among asthmatic children.

It shows that the influenza VCR in asthmatic children was very low for the 2006-7 influenza season, as it had been over the previous two seasons. Only 15.7% of 433 asthmatic children ≥6 years of age seen in a hospital by a paediatric pulmonologist were vaccinated against influenza for the 2006-7 season, the percentages were even lower for the 2005-6 and 2004-5 seasons: 13.9% and 10.9%, respectively. These results are consistent with previous studies that have shown low VCRs in children with chronic respiratory diseases. In a recent French study conducted in the Parisian Region (seven general paediatric wards) in 239 children with underlying chronic disease, Weil-Olivier *et al.* reported a 12.8% VCR for the 2003-4 influenza season in the subset of 39 children suffering from a chronic respiratory disorder, of whom 33 were asthmatic [15]. In Spain, Lopez de Andres *et al.* observed an influenza VCR of 19.9% in 2003 in 6,869 children suffering from a chronic respiratory disorder [20]. In the United States (US), a 29% influenza VCR in asthmatic children for the 2004-5 season and a 36.2% influenza VCR in asthmatic patients (children and adults) for the 2005-6 season were reported [21,22].

In our study, for the 2006-7 season, the influenza VCR did increase with the severity of asthma; one third of children with severe persistent asthma were vaccinated. In the US, for the 2004-5 season, children with current asthma who experienced an asthma attack or episode in the past 12 months had higher VCRs than those without an attack or episode (35.9% versus 20.0%, respectively); children with current asthma who had ≥10 health-care visits had higher VCRs than children without current asthma (42.0% versus 14.6%, respectively) [21].

Influenza VCR remained far below the French national public health objective of at least 75% for the year 2008. Our study took place the year after the sending of a free influenza vaccination voucher to all asthmatic patients, irrespective of the severity of asthma, was implemented. Provision of a free voucher has already been shown to significantly improve VCR in children with cystic fibrosis in France [23]. According to the parents, only two in five asthmatic children have received a voucher for free vaccination and as the receipt of a voucher significantly improved VCR in asthmatic children, the reasons for non-receipt need to be analysed. Possible reasons are: asthma not declared to the Public Health Insurance authorities, lack of update of the database by the Public Health Insurance authorities, parents not remembering they had received the voucher, etc. After a period of adjustment, including provision of information about the voucher for free vaccination to the asthmatic children and their parents and updating of the database by the Public Health Insurance authorities, the decision to deliver a voucher to all asthmatic patients promises to help improve influenza VCR in asthmatic children in the near future. However, this measure, although necessary, will probably not be sufficient to reach the stated national objective, because receipt of a voucher during the 2006-7 season was not followed by influenza vaccination in as many as 69% of children.

The most frequently reported reasons given for non-vaccination were "Lack of information" (42.0%), "Vaccine useless (disease considered as benign)" (19.6%), "Vaccine considered as ineffective or dangerous" (in 10.4% of cases: 5.9% and 4.5% respectively), and "Forgotten or lack of time" (8.7%). These findings emphasise

the need for parents of asthmatic children to receive targeted information on the potential seriousness of influenza in asthmatic patients and on the tolerance and efficacy of influenza inactivated vaccine in children [4]. In conjunction with the voucher sent to all asthmatic patients, this information should also improve influenza VCR. Indeed, Schoeffer *et al*. [24] found that the clinical impact of influenza was underestimated or insufficiently well known to young people. These results were obtained from a study involving 2,131 German patients over 18 years of age, seen at a specialised medical centre for chronic respiratory disorders (asthma or chronic obstructive respiratory disease).

Anaphylactic hypersensitivity reaction to eggs or to one of the vaccine components is the only absolute contraindication to vaccination with trivalent inactivated influenza vaccine [25]. In the present study, 14 children (3.9%) declared an "allergy to egg" as reason for non-vaccination against influenza. However, only one of these 14 children had ever presented clinical signs of anaphylactic shock subsequent to exposure to egg, a contraindication to inactivated influenza vaccine, suggesting that the other 13 may have been eligible for influenza vaccination. This illustrates that some children may have failed to be vaccinated because the specific contraindications for inactivated influenza vaccine are not well known.

The vaccinator was a GP in around two in three children and a family paediatrician for around one in ten. This result should be interpreted with caution taking into account the fact that included children were ≥6 years old, an age which requires fewer visits to the paediatricians. Information on the potential seriousness of influenza in asthmatic patients and on the tolerance and efficacy of inactivated influenza vaccines in children should be provided by health care professionals during GP/paediatric consultations and/or in the waiting room via posters, leaflets, etc. especially during the last trimester of the year.

One limitation of our study could refer to the nature of the asthmatic children enrolled. Investigators were strictly limited to paediatric pulmonologists to ensure the accurate recruitment of children with asthma. Since the study included only children seen in a hospital, it could have been possible that there were more severe persistent asthma cases in this population than in general practice; nevertheless 7% of asthma cases were severe persistent in the present study compared with 10% in asthmatic general population [12].

## Conclusions

In France, the 2006-7 influenza VCR in asthmatic children was substantially lower than the national target of at least 75% by 2008. The recent decision (November 2006) to deliver a free influenza vaccination voucher to all asthmatic patients, irrespective of asthma severity, has shown to improve the VCR in our study. To reach the national objective, however, this promising measure needs to be accompanied by timely information on the potential seriousness of influenza in asthmatic patients and by information about the tolerance and efficacy of inactivated influenza vaccines in children. Such information should be provided to, and by, health care professionals to parents of asthmatic children.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## Research articles

# First steps in the design of a system to monitor vaccine effectiveness during seasonal and pandemic influenza in EU/EEA Member States

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Estimating influenza vaccine effectiveness (IVE) early in the season helps measuring the consequences of a mismatch between the vaccine and the circulating strain and guiding alternative or complementary interventions. The European Centre for Disease Prevention and Control is funding a project to develop pilot studies to monitor IVE in the Member States (MS) of the European Union and European Economic Area (EU/EEA) during seasonal and pandemic influenza. To identify key methodological and practical issues in developing protocols for pilot studies, we conducted a survey among EU/EEA MS, a literature review on IVE methods, and consultations of experts. The survey and literature review highlighted the variety of the data sources used to estimate IVE and the difficulty to interpret data on IVE, which varies with age, risk group, outcome specificity and virus-vaccine mismatch. We also found that negative and positive confounding can bias IVE. The experts consultations lead to the following recommendations: to measure IVE in the same population in various seasons; to control for positive/negative confounding (including pre- and post-influenza season IVE estimates); and to include laboratory confirmation as outcome in various study designs. In the 2008-9 influenza season, two cohort studies using general practitioners' databases and six case control studies will be piloted in EU/EEA MS and will adhere to the above recommendations. The pilot studies will be the basis for the development of robust methods to monitor IVE in EU/EEA MS.

### Background

Because influenza viruses are constantly changing and vaccines are reformulated every year, the influenza vaccine effectiveness (IVE) estimates from previous years cannot be used to estimate IVE in the subsequent years. Having annual IVE estimates at European level available as soon as possible after the start of a seasonal influenza epidemic or pandemic and monitoring it along the course of the epidemic/pandemic is essential in order to:

- decide on recommendations for the use of the vaccine by specific age and risk groups,
- target complementary or alternative public health measures (e.g. antivirals) to population segments in which the vaccine is less effective,
- estimate more precisely the impact of current vaccination strategies on the burden of disease with a view to supporting vaccination campaigns,
- provide some quantification to the current virological system of comparing antigenic matches of vaccine and circulating viruses,
- trigger further investigations on seasonal and pandemic vaccines (improving their composition, use of adjuvants, need for booster doses),
- better manage and respond to eports of vaccine failures (especially during a pandemic),
- counterbalance the reports of adverse events following immunisation by providing a basis for adequate risk management and cost-effectiveness analysis.

In addition, in order to be able to measure IVE for the pandemic vaccine it is necessary to develop already now a robust method that provides early estimates of IVE.

As the vaccine is recommended for risk groups, clinical trials to estimate IVE in Europe would not be ethical. Only observational studies can be considered when trying to obtain IVE estimates early in the season [1]. It is therefore necessary to define which observational study designs can be adopted in the Member States (MS) of the European Union and European Economic Area (EU/EEA) that would provide IVE estimates during an ongoing influenza season and allow monitoring it through consecutive seasons. These methods need to take into account the specific situation of each MS in terms of resources and available data.

The European Centre for Disease Prevention and Control (ECDC) is funding the development and piloting of study protocols for monitoring IVE in EU/EEA MS in the context of seasonal and pandemic influenza. A consortium of 18 European public health institutes coordinated by EpiConcept is carrying out this project. The first phase (January-July 2008) consisted of the development of protocols for the pilot studies. To identify key methodological and practical issues to be considered in the study protocols, we conducted a survey among EU/EEA MS, a literature review on methods used to estimate IVE, and three consultations of experts. These three approaches are described in the following sections of this article.

## Survey

### Survey methods

We carried out a survey among EU/EEA Member States to identify, in each MS, observational IVE studies and available data sources that could be used for real-time IVE studies.

We contacted 29 experts from 29 EU/EEA MS involved in influenza surveillance. The experts were the representatives of the institutions included in the consortium and, for MS not participating in the consortium, the epidemiologist focal point of the European Influenza Surveillance Scheme (EISS) or the gatekeeper of the Vaccine European New Integrated Collaboration Effort (VENICE). The experts were given the options either to provide information through a self-completed questionnaire or during a telephone or face to face interview. In addition, we reviewed available reports from EISS and VENICE, web pages from European institutions involved in influenza surveillance and articles on IVE studies conducted in EU/EEA MS.

We collected data on IVE studies conducted in the MS, available data sources for case identification (identification of influenza cases, death registries, hospital registries, general practitioners' (GP) databases, other) and for documenting influenza vaccination status, as well as potential interest in conducting a pilot study during the season 2008-9.

### Survey results

Among the 29 MS we contacted, 24 (83%) accepted to participate in the survey. In four MS, we interviewed the experts face to face, in 12 by telephone and in eight MS, the experts self-completed the questionnaire we sent them.

Of the participating 24 MS, ten had conducted IVE studies in the past. We identified 43 published articles reporting results of case control studies (12 articles), of cohort studies (28 articles) and of studies using a screening method (three articles). Additional details on the studies including data sources and study outcomes are reported in the Table. A complete survey report is also planned to be published on the ECDC website.

In most of these studies, the study population and data sources had been identified through health delivery services. In the Czech Republic, Italy and Portugal, other data sources had been used for IVE studies as reported in the Table.

#### *Computerised databases*

Malta, Norway and Sweden have population registries including an individual unique identifier which allows linking existing databases (e.g. death registers, in-patient registers, vaccination registers if available). The linkage of the various databases is not immediate and an ethical or a personal protection approval is needed.

In Finland, France, Ireland, the Netherlands, Norway, and the United Kingdom (UK), various GP networks have computerised databases. Computerised GP databases are also available in some regions in Spain and in some counties in Sweden.

Computerised GP databases allow evaluating various outcomes: influenza-like illness (ILI)/acute respiratory infections (ARI), death, hospitalisation, vaccine status and some confounding factors (e.g. co-morbidities). However, certain issues need to be considered when using computerised databases for IVE studies, such as the representativeness, completeness, timeliness and quality of the data. For some of the databases, *ad hoc* studies may be necessary to further evaluate data quality.

Computerised databases have been used in the Netherlands, Spain, Sweden and the UK to conduct IVE cohort studies. They can provide rapid estimates for some outcomes (e.g. ARI/ILI) and more solid estimates at the end of the season (e.g. estimates adjusted for confounding factors, estimates for severe clinical outcomes).

#### *Sentinel surveillance*

In all 24 responding MS, the main source to identify clinical cases of influenza on a real-time basis was the virological or epidemiological sentinel influenza surveillance system. Case definitions vary from MS to MS but most sentinel networks report cases of ILI symptoms or ARI [44].

Laboratory confirmation of influenza cases is usually done in a subset of patients consulting the sentinel practitioners. In most MS, the decision of which patients to collect laboratory specimens from is based on clinical criteria. Thus, patients with laboratory tests are not a representative sample of all patients consulting a GP because of influenza symptoms [45]. In Denmark and France, the patients to be sampled are selected in a systematic way. Following EISS recommendations, laboratory request forms include the patients´ vaccination status.

Sentinel surveillance systems have been used to conduct case control studies of IVE in Denmark, France, Germany, the Netherlands, and the UK (Table).

#### *Hospitalisation discharge databases*

In most MS, cases with severe clinical influenza outcome (hospitalisations, deaths) are not identified in real time. Hospitalisation discharge databases are available with delays varying from three months to two years. In France, hospitals report on a daily basis to the Institut de Veille Sanitaire individual data from in-patients and out-patients consulting emergency rooms.

Various MS have developed or are developing real-time mortality monitoring [46]. Mortality has not yet been used in Europe to estimate real-time IVE.

#### *Influenza vaccination status*

Sources to document influenza vaccination status include medical records, computerised medical records, immunisation registries, surveys, and pharmaceutical data [47]. Vaccination registries allowing the extraction of real-time vaccination status are currently available at regional level in Finland, in some counties in Sweden and in some regions in Spain. In 2008-9, Spain plans to estimate real-time vaccination coverage using vaccination coverage reported by the sentinel practitioners.

## Literature review

In addition to the survey described above, a literature review was conducted to identify the key elements to be considered in the design of the pilot studies. In particular, we focused on factors affecting IVE estimates and on methods described to control them. In the following paragraphs, we summarise factors that will have an influence on the choices made when developing the pilot study protocols: outcomes and confounding factors.

**Table**

**Influenza vaccine effectiveness studies conducted in EU/EEA Member States, by study design and country**

| Country | Reference | Data source | Outcome |
|---|---|---|---|
| **Cohort Studies** | | | |
| **Czech Republic** | Chlíbek 2002 [2] | Mail questionnaire to volunteers | Influenza-like illness |
| | Berran 2003 [3] | Medical records employees Skoda Auto factory | Influenza-like illness |
| **Italy** | Comeri 1995 [4] | Questionnaire to a sample of the elderly population in one city | Clinical influenza |
| | Consonni 2004 [5] | Phone interviews, ambulatory patients | Influenza-like illness, acute respiratory infection |
| | Pregliasco 2002 [6] | Interviews, medical records geriatric units | Acute respiratory infection, hospitalisation |
| | Rizutto 2006 [7] | Interviews volunteer participants from Ministry of Health | Influenza-like illness |
| | Landi 2003 [8], Landi 2006 [9] | Minimum data Set for home care, Italian 'Silver Network' home careproject | Death (2003), hospitalisation (2006) |
| **The Netherlands** | Smits 2002 [10] | Computerised primary care practices | Low respiratory tract infection, otitis media |
| | Tacken 2004 [11] | GP database | Primary care contact rate during influenza epidemics |
| | Voordow 2003 [12], 2006 [13] | GP database | Influenza, pneumonia, death, low respiratory tract infection, hospitalisation for pneumonia |
| **Portugal** | 2006-7, 2007-8 (unpublished data) | Pharmacies, voluntary recruiters | Laboratory-confirmed influenza |
| **Spain** | Castilla, 2006 [14] | Sentinel GPs | Clinical influenza |
| | Gené Badía 1991 [15] | Records from five health centres, hospital, death register | Death, all hospitalisations, hospitalisations for respiratory diseases |
| | López Hernández 1994 [16] | Records from one health centre, hospital records, death register | Hospitalisation, death |
| | Salleras, 2006 [17] | Questionnaires in clinics | Acute febrile illness, influenza-like illness, laboratory-confirmed influenza |
| | Vila-Córcoles 2007 [18] | GP electronic files, demographic database, death registry | Death |
| **Sweden** | Christenson 2001 [19], Christenson 2004 [20], Orktvist 2007 [21] | Population register, vaccination database, discharge diagnosis database | Influenza hospitalisation, hospitalisation for pneumonia |
| **UK** | Fleming 1995 [22] | GP database | Death, death or severe respiratory illness, death or any respiratory illness without further specification |
| | Armstrong 2004 [23] | GPs, Office for National Statistics | Death attributable to influenza |
| | Mangtani 2004 [24] | General Practice Research Database | Hospitalisation for respiratory disease, death from respiratory disease |
| **Cohort studies during outbreak investigations** | | | |
| **France** | Aymard 1979 [25] | Geriatric hospital | Disease, death |
| **Italy** | Caminiti 1994 [26] | Medical charts, hospital records, death certificates | Influenza-like illness, hospitalisation for influenza-like illness, hospitalisation for all respiratory illness, death from respiratory illness |
| **UK** | Arroyo 1984 [27] | One nursing home | Influenza-like illness, pneumonia, death from respiratory disease |
| | Mukerjee 1994 [28] | 14 nursing homes | Upper respiratory tract infection |
| | Nicholls 2004 [29] | | Influenza-like illness |
| **Case control studies** | | | |
| **Denmark** | Mazick 2006 [30] | GP surveillance network | Influenza-like illness laboratory-confirmed |
| **France** | Carrat 1998 [31] | GP practices | Acute respiratory infection, influenza-like illness laboratory-confirmed |
| | Lavallée 2002 [32] | Medical records of hospitalised cases, interviews | Hospitalisation for acute respiratory infection and hospitalisation for brain infarction |
| **Germany** | Grau 2005 [33] | Hospital records, patient interviews | Hospitalisation for ischaemic or haemorrhagic stroke / transient ischaemic attack |
| | Uphoff 2006 [34] | Sentinel GPs<br>cases: influenza-like illness influenza-positive<br>controls: influenza-like illness influenza-negative | Influenza-like illness laboratory-confirmed |
| **Italy** | Crocetti 2001 [35] | Discharge diagnoses, mailed questionnaire, telephone interviews | Hospitalisation for pneumonia or influenza |
| **The Netherlands** | Hak 2002 [36] | Administrative and medical databases from a health plan | GP visit and hospitalisations for acute respiratory disease and cardiovascular disease |
| | RIVM 2006-7<br>(unpublished data) | Sentinel GPs<br>cases: influenza-like illness influenza-positive<br>controls: influenza-like illness influenza-negative | Influenza-like illness laboratory-confirmed |
| **Spain** | Puig-Barberá 1997 [37], 2004 [38], 2007 [39] | Hospital emergency logs and records | Hospitalisation for acute coronary syndrome, hospitalisation for cerebrovascular accident, hospitalisation for pneumonia |
| **UK** | Ahmed 1995 [40] | Death certificates, GP records | Certified influenza death |
| | Jordan 2007 [41] | GP practice registries and hospital discharge registries | Hospitalisation for acute respiratory infection |
| **UK (Scotland)** | Health Protection Scotland, 2005-6 and 2006-7<br>(unpublished data) | Sentinel GPs<br>cases: influenza-like illness influenza-positive<br>controls: influenza-like illness influenza-negative | Influenza-like illness laboratory-confirmed |
| **Screening** | | | |
| **France** | Carrat 1998 [42] | Cases: sentinel GPs;<br>vaccine coverage: national health survey | Influenza-like illness |
| | Legrand 2006[43] | Cases: sentinel GPs;<br>vaccine coverage: national health survey | Influenza-like illness |
| **Germany** | Uphoff 2006[34] | Cases: sentinel GPs;<br>vaccine coverage: national health survey | Influenza-like illness laboratory-confirmed |
| **Spain** | Instituto de Salud Carlos III<br>(unpublished data) | Cases: sentinel GPs;<br>vaccine coverage: national health survey | Influenza-like illness |

GP: General Practitioner

**Literature review methods**

To identify relevant papers, we searched the Cochrane database and consulted Cochrane reviews on influenza vaccine effectiveness [48,49]. Additionally, we reviewed the Health Technology Assessment report "Systematic review and economic decision modelling for the prevention and treatment of influenza A and B" [50]. We also included a recent Sanofi Pasteur-MSD review [51]. Finally, we also reviewed references from each of the selected articles.

We selected studies providing IVE estimates. We also included studies addressing methodological aspects of IVE estimates and certain studies addressing the methodology of VE measurements for infectious diseases.

**Literature review results**

Overall, we reviewed 284 scientific articles and of them selected 93 descriptive observational studies (34 cohort studies, 26 outbreak investigations, 31 case control studies and two studies using the screening method). In addition we consulted 23 articles focusing on methodological issues.

***Clinical outcome***

The main clinical outcomes reported in the literature were hospitalisations for all or specific causes (e.g. pneumonia and influenza), deaths from all or specific causes (e.g. pneumonia and influenza), ILI, ARI and laboratory-confirmed cases of influenza.

IVE studies using non-specific clinical outcomes will include as cases individuals with clinical symptoms unrelated to influenza, leading to an underestimation of the IVE [52,53]. The influenza case definition combined with laboratory confirmation results has the highest specificity for influenza, and laboratory confirmation is therefore essential to estimate the true IVE [54]. Due to the costs involved, some authors have suggested to perform laboratory tests only in a small proportion of the study participants (validation set) [55].

***Confounding factors***

Comparing the crude IVE estimates and the IVE estimates adjusted for confounding factors reported in the literature provides an overview of the magnitude of confounding in IVE studies. We found a difference in percentage between crude and adjusted IVE in case control studies (Figure 1) and cohort studies (Figure 2) that ranged from -220% to 21%.

The list of potential confounding factors reported in the literature is very long (Box).

The main confounding factors discussed in the literature are factors resulting either in an underestimation of the IVE (negative confounding) or in an overestimation of the IVE (positive confounding factors). Negative confounding is the result of 'confounding by indication': Individuals that are at high risk of influenza are more likely to be vaccinated than individuals that are at low risk, and consequently, IVE is underestimated. Positive confounding is the consequence of healthier individuals being more conscious about their health, more motivated to accept vaccination and therefore more likely to be vaccinated than unhealthier individuals. An alternative explanation for positive confounding is the fact that critically ill patients are not offered (or refuse) to be vaccinated. Therefore, vaccinated individuals have a better baseline health status than the unvaccinated group leading to an overestimation of the IVE ('healthy vaccinee' effect).

Different alternatives have been proposed to adjust for the 'healthy vaccinee' and 'confounding by indication' effects. Some authors restricted the study population to groups that were more homogeneous with regard to the potential confounding factor. Others stratified the results according to risk groups. A majority of the studies reviewed included the potential confounders as covariates in a regression model. Some authors controlled for confounding using propensity scores, the conditional probability of being vaccinated given observed covariates [11,18,39,56-58]. They are used to group individuals at levels of the propensity score or as a covariate in the regression model.

***Comparison with non-influenza season data***

Some authors considered those adjustment methods insufficient to adjust for the 'healthy vaccinee' effect and suggest that residual confounding may persist. They proposed to compare the IVE estimates in the influenza season with estimates from periods with

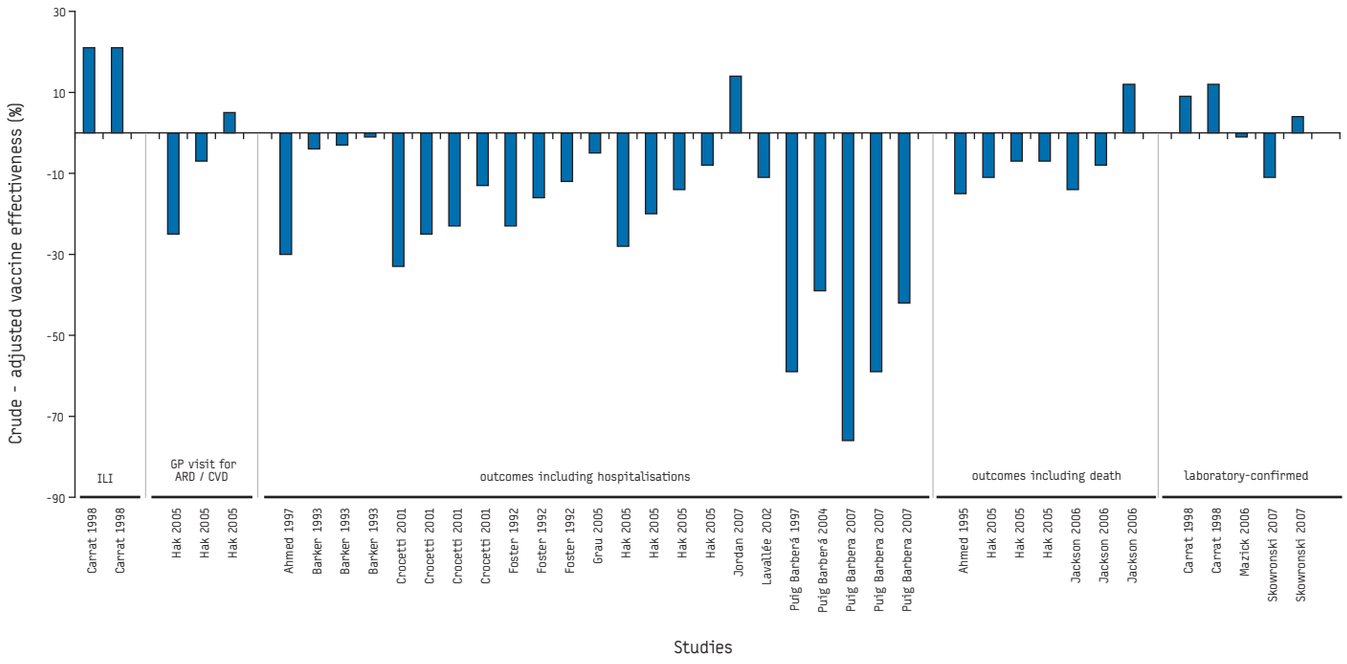
**Box**

**List of potential confounding factors in influenza vaccine effectiveness studies reported in the literature**

- Age
- Allergy to egg protein
- Asthma
- Diabetes mellitus and other endocrine diseases
- Disease severity
- Education level
- Functional status
- Former Influenza vaccination
- Former Pneumococcal vaccination
- Health medical organisation
- Health-related behaviours
- Heart diseases
- House heating
- Immunosuppression including haematopoietic malignant diseases and steroid and immunosuppressive treatment
- Index case in the family
- Length of hospital stay
- Level of social interaction
- Lifestyle factors
- Living together with grandchildren
- Malignant disorders
- Marital status
- Medication prescribed and number of repeat prescriptions
- Musculoskeletal and connective tissue diseases
- Neurological diseases (including dementia, Parkinson's disease and cerebrovascular diseases)
- Number of co-habitants
- Number of hospital admissions and out-patient visits
- Other pulmonary diseases
- Physical activity
- Place of residence: nursing and residential care homes; non-institutional
- Pre-school attendance
- Preventive care practices
- Propensity score
- Renal diseases
- Sex
- Smoking
- Socio-economic status
- Type of medical coverage
- Underlying chronic conditions
- Vaccination of caregiver
- Washing hands and gargling

**FIGURE 1**

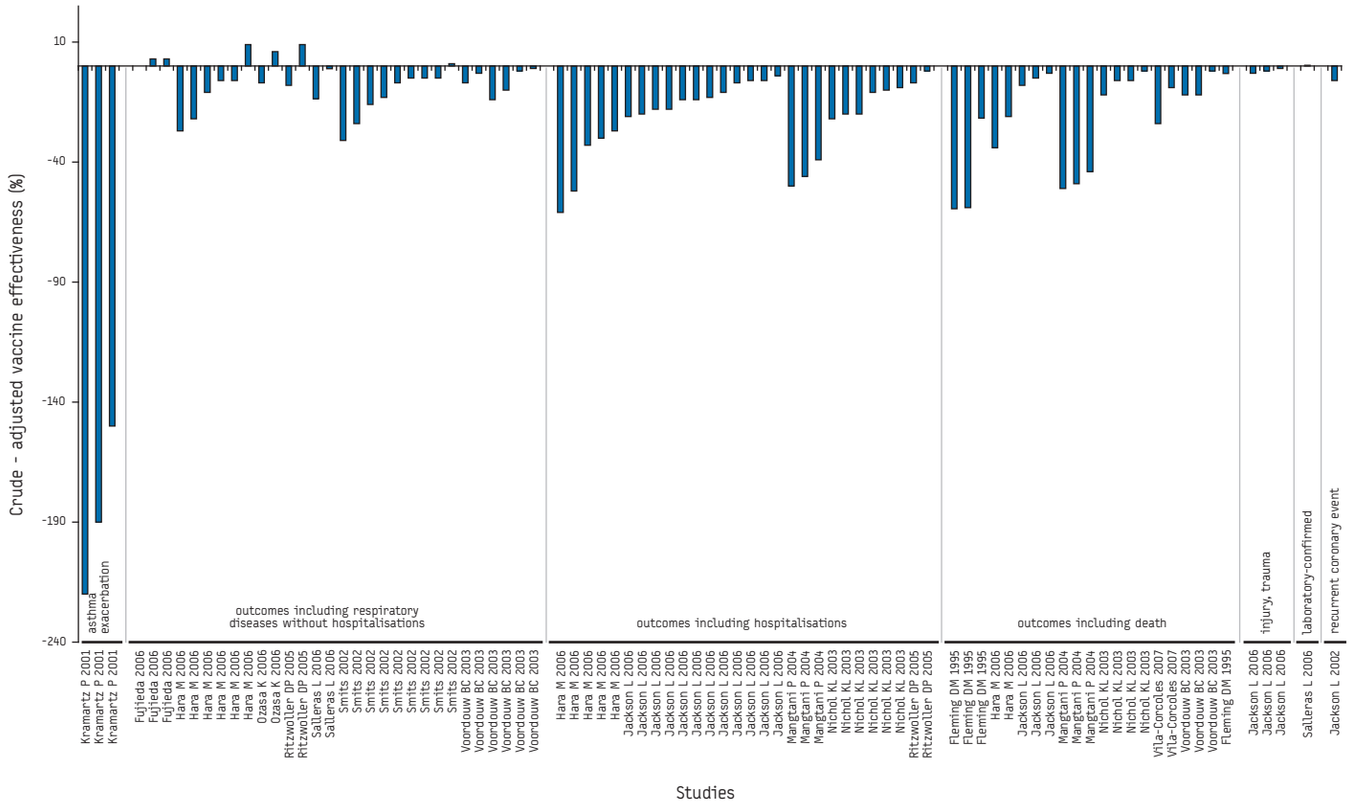
**Difference between crude and adjusted influenza vaccine effectiveness estimated in case control studies, by study outcome**




ARD, acute respiratory disease including acute bronchitis or exacerbations of chronic lung disease, influenza, pneumonia, and acute otitis media;
CVD, cerebrovascular disease including myocardial infarction, stroke, and heart failure;
GP, general practitioner;
ILI: influenza-like illness

**FIGURE 2**

**Difference between crude and adjusted influenza vaccine effectiveness estimated in cohort studies, by study outcome**



no influenza. The rationale behind this is that the vaccine should not have an effect in non-influenza seasons.

Several studies using this approach compared IVE during and after the influenza season. Most of the results showed a lower IVE after the season suggesting that there was no positive confounding [21,24,59-61]. Other authors, however, found a greater reduction in the risk of death and pneumonia hospitalisation in the period before the influenza season compared to the time during the influenza season, suggesting positive confounding [62]. They argue that studies that did not find an association between vaccine and disease outcome (low IVE) after the influenza season had assumed the difference in underlying characteristics to be constant over time. They suggest that the differences between vaccinated and unvaccinated individuals may diminish over time and the data should therefore be compared not only with the post-influenza season, but also with the pre-influenza season.

### Expert consultations

During the first phase of the project, we organised several workshops for experts participating in the consortium and additional invited influenza experts.

The first workshop was held in April 2008. The aim was to present and discuss the results of the literature review and survey as described above and to consider the feasibility of the various observational methods to estimate real-time IVE at EU/EEA level. The participants included 25 experts from institutions participating in the consortium, four external influenza experts (London School of Hygiene and Tropical Medicine, Instituto de Salud Pública de Castellón, Sanofi Pasteur MSD, United States-Centers for Disease Control and Prevention Influenza division), four staff members from the ECDC Scientific Advice Unit and two EpiConcept epidemiologists.

The participants worked in three groups to discuss cohort studies, case control studies, and screening method studies. For each study design, the groups made recommendations to be considered in the development of generic protocols for the pilot studies. The experts' recommendations were to determine IVE in various population subgroups, to control for positive and negative confounding and to use laboratory-confirmed influenza as outcome. The group recommended measuring IVE in a homogenous population for a period of several years, using the same design each year. The participating MS and ECDC expressed their interest in supporting this project in the long term.

Following the first workshop, we developed two generic protocols (see below) for case control and cohort studies to be adapted to the situation of each MS.

The second set of consultations was held in June 2008 with the MS that were interested in conducting pilot studies in the season 2008-9. The objective was to further discuss methodological issues related to the two generic protocols for measuring IVE. Specific sessions were held for each study design.

The group agreed that, during the first season of the pilot phase, 2008-9, the following study designs were to be considered:

- Case-control studies based on influenza sentinel surveillance systems with laboratory-confirmed influenza-positive ILI as cases and influenza-negative ILI as controls.
- Prospective cohort studies using computerised databases and providing IVE estimates for different periods (pre-/during/post-influenza season). At least a subset of the cases would be laboratory-confirmed.

### Conclusion

The survey showed that data sources to conduct IVE studies vary from MS to MS and in some MS from region to region. Computerised databases are available in few countries and, where available, are a good basis for cohort studies as they include large populations. Sentinel GP networks are present in all 24 EU/EEA MS that participated in the survey; they include laboratory confirmation of influenza cases and data on vaccination status for a subset of the population.

The literature review underlined the difficulty to interpret IVE estimates. IVE estimates vary with age, risk group and the specificity of the disease outcome. In addition, IVE estimates can be heavily biased by positive or negative confounding.

The expert consultations led to specific recommendations to be applied in the next phase of the project. Eight studies will be piloted in the 2008-9 season: two cohort studies, one case control nested in one of the cohorts, and five case control studies.

The two cohort studies will be conducted in England and Scotland, and in the Comunidad Autónoma de Navarra, Spain, using GP databases. These two studies will provide IVE for the pre- and post-influenza season and will allow to further analyse confounding factors included in the GP database. IVE will be estimated against ILI (both studies), all respiratory infections (England and Scotland), pneumonia and influenza hospitalisations (Navarra), all respiratory hospitalisations (Navarra), and all deaths (Navarra). In Navarra, a subset of patients will be laboratory-confirmed.

A case control study with laboratory-confirmed outcome will be nested in the England and Scotland cohort.

In addition, five case control studies among the elderly population will be conducted during the influenza season in Denmark, Hungary, Portugal, Romania and Spain. The vaccine status of ILI cases that are laboratory-confirmed for influenza will be compared to various sets of controls including influenza-negative ILI cases, controls from GP patients and controls from GP catchment areas.

The five studies will use the recommended European Commission case definition for ILI and a common definition for potential confounding factors such as functional status, underlying diseases, severity, smoking, previous influenza vaccination and pneumococcal vaccination. Therefore, the possibility of pooling the results from those five studies to have a multicentre IVE estimate will be explored.

Results of the 2008-9 pilot studies will be presented in an expert meeting in June 2009. Based on those results, amendments to the protocols will be proposed and implemented in the next round of pilot studies in the same eight countries in the season 2009-10. Subject to available resources, at least two additional pilot studies will start in 2009.

The results of the pilot studies will guide the establishment of a system capable to provide and share rapid and reliable information on IVE on an annual basis. The intention is for this information to be integrated as an essential part of the routine influenza surveillance outputs/data. In order to achieve the successful inclusion of IVE

data in regular influenza surveillance, sustained commitment from all partners as well as secured funding is fundamental.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# Review articles

## The scientific basis for offering seasonal influenza immunisation to risk groups in Europe

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This paper summarises the scientific evidence supporting selection of risk groups that would benefit from annual seasonal influenza immunisation in European Union (EU) countries. Risk groups are defined restrictively as *persons in Europe at higher than average risk of adverse outcomes should they be infected with seasonal influenza and for whom use of vaccine is demonstrated to be effective in reducing the risk of those outcomes*. Existing evidence indicate that older people and those with chronic disease are at higher risk of severe adverse outcome and that immunisation reduces this risk. There is thus good scientific evidence for routinely offering annual immunisation to all older people (at least those aged 65 years and older), and people with certain groups of chronic medical conditions. We estimated that these two groups account for between 19% and 28% of the population of EU countries. Thus in 2006, an estimated 84 million older people aged 65 years and over and 41 million people younger than 65 years of age with chronic conditions were living in these countries. There is also strong evidence for immunising staff caring for patients belonging to these two risk groups in residential (care home) settings in order to protect the patients. There are as yet no strong data on whether or not immunising other healthcare workers and carers protect patients though immunisation of healthcare workers can be justified on occupational health grounds. At present the scientific evidence for immunising other suggested risk groups, notably children and pregnant women is not strong for Europe though equally there is no evidence against immunising these groups.

### Introduction

Most people are susceptible to influenza infection and there are various estimates of the numbers that are infected each year, the resulting burden of ill-health and to what extent this burden can be reduced. All of these conclude that human seasonal influenza is a serious public health threat which occurs annually but can be significantly ameliorated [1,2]. Influenza vaccines are the most effective preventive tools available for reducing that burden and the risk to individuals [3-5]. The immunisation strategy for preventing human seasonal influenza aims at protecting vulnerable individuals, rather than trying to achieve herd immunity and reduce transmission in the community [6]. Some individuals and groups are more likely to develop severe disease and even die as a result of their infection [2,7-12]. Hence, since the first influenza vaccines were developed the strategy has been to immunise certain so-called 'risk groups' rather than whole populations [13].

Another reason for this selective strategy is the frequent change in circulating viruses and subsequently the need to regularly review the composition of influenza vaccines and to conduct immunisation annually. This introduces an unusually high level of expense and logistical considerations into vaccine production and delivery [14]. In addition to the traditional 'risk groups' (older people and people with chronic illnesses [6]) influenza vaccination is sometimes recommended to other groups and individuals who may or may not be at any higher than average risk of severe disease should they be infected. According to the VENICE study these groups in different EU countries include: pregnant women, children (under age of two or five years), persons living with those at higher risk, healthcare and other care workers, those working in essential, military and veterinary services, and poultry workers [15].

In 2003 the World Health Assembly (WHA) in a resolution concerning pandemic and seasonal influenza urged all its member states *"to establish and implement strategies to increase vaccination coverage of all people at high risk, including the elderly and persons with underlying diseases"* [16]. The resolution neither specified the age of the elderly nor any list of these underlying diseases and the scientific and public health background for the recommendation from the Assembly's secretariat in the World Health Organization (WHO) is unrecorded. Some subsequent specification can be found on the WHO web, where the high risk groups are described as: *the elderly, people with weakened immune systems and those with underlying chronic diseases where influenza often leads to severe pneumonia and other serious illness due to pre-existing chronic diseases* [17]. The WHA also recommended a coverage target for immunisation of the elderly of 50% by the year 2006 and 75% by the year 2010 [16]. No target for those with chronic illness was specified. All European Union (EU) countries are members of the WHA and none expressed a reservation to the resolution.

This paper is one of a series of outputs by the European Centre for Disease Prevention and Control (ECDC) providing scientifically-based public health information and advice concerning seasonal influenza vaccination in Europe, and its main aim is to summarise the scientific evidence supporting selection of risk groups. It also seeks to estimate the number of people in the two main identified risk groups and the proportion they constitute of the population in the EU countries and in EU as a whole.

## Methods

The term risk groups has been used in various ways in literature, e.g. persons at higher risk than average for acquiring influenza, persons at higher than average risk of transmitting influenza, persons at higher risk of having an adverse outcome (severe disease or death) should they acquire infection or persons who if they acquire influenza are more likely to transmit the infection to others who will then develop severe disease.

In this paper we employ a restrictive definition, namely *"persons in Europe at higher than average risk of adverse outcomes should they be infected with seasonal influenza and for whom use of seasonal influenza vaccination is demonstrated to be effective in reducing the risk of those outcomes"*.

We did a review of published scientific literature in the field. The literature search firstly focused on articles mentioning risk factors for experiencing severe outcomes following influenza infection. Secondly publications were sought that investigated whether influenza immunisation reduced risks of severe outcome or that it was at least protective against any influenza infection. It was also investigated whether the literature supported the view that immunisation of others, notably healthcare staff and other carers, protected people in the risk groups.

The strategy was to search the PubMed database without date restriction up to September 2008, for relevant articles in English, using medical subject headings (MESH) identifying the disease (Human Influenza, Flu), the clinical outcome (hospitalisation/ hospital*, mortality, death, pneumonia, morbidity) and a list of pre-identified possible broad risk factors (cardiovascular, chronic respiratory/COPD, diabetes, immunosuppression/immunodeficiency, HIV, transplant, pregnancy/pregn*, renal failure/dialysis/ haemodialysis, elderly/old, child*/infant). To select the subset of studies also reporting "vaccine effectiveness" estimates we included this term in each search considering only articles where vaccine effectiveness was mentioned in the title or abstract. We screened the retrieved articles by reading their abstracts and selected those that were most relevant in terms of article type (reviews, guidelines, large cohorts, meta-analyses) and appropriateness of the content. The literature was screened to select studies based on European populations, and where possible we gave more emphasis to European studies on increased risk of severe clinical outcome in the various risk groups studies as there may be European specific features in terms of prevalence of risk factors and burden of disease that make the results of non-European studies difficult to generalise. This is less the case for vaccine effectiveness studies.

Articles included in the references of reviews, guidelines and meta-analyses were added where they had not been retrieved by the PubMed search. In addition, we drew on a review undertaken for an ECDC-convened scientific panel on immunisation of children in 2006-7 [18] and a systematic review commissioned by ECDC on methods for measuring influenza vaccine effectiveness and undertaken by the organisation Epiconcept (http:www.epiconcept.fr) [19].

The planning estimates of the size of population in the risk groups were made for the elderly and for those with chronic conditions in younger years. For the population aged 65 years and older we used published European population statistics for the year 2004 and with projections made forward to 2050 [20]. Estimating the number of people with chronic conditions in the influenza risk groups was more difficult, as estimates of chronic ill-health are usually not available in the routine statistics and what exists does not conform to the risk groups for influenza which do not comprise all persons with chronic medical and physical conditions.

A specific issue to address was to avoid double counting of persons both aged 65 years and older and with chronic conditions. A large cohort study in Sweden showed that the prevalence of multiple morbidity among older individuals reaches 55% [21]. To overcome this, we excluded European studies where the distribution of chronic conditions was not stratified by age or where double counting due to co-morbidity was not eliminated [22,23], which in some studies resulted in implausible differences between neighbouring countries [24]. Data available from the Global Burden of Disease and Risk Factors (GBD) project which overcomes double counting could not be used either because it does not directly describe the distribution of risk factors relevant to influenza in the general population [25].

The only survey identified that avoided double counting and selected the risk factors for influenza was the one undertaken in the United Kingdom, which used primary care data specifically for planning the needs for influenza vaccine [26]. This study was therefore selected as most likely to provide the accurate age-specific estimates of the proportion of the population suffering from relevant chronic diseases in the EU countries. The survey was undertaken with government support, gave age-stratified results, avoided double counting and included medical validation through doctors' opinions on whether a patient's illness was significant enough to deserve immunisation. These age-specific proportions were then applied to the 2006 populations of all EU countries (derived from Eurostat; http://epp.eurostat.ec.europa.eu/) to provide country-specific estimates of those under age 65 with one or more conditions that would put them into the chronic disease risk group category. These totals were added to the Eurostat estimates of the number of the elderly aged 65 years and older to estimate the proportion of the population that was either suffering from one or more chronic diseases or was aged 65 years and older for each EU country and the EU as a whole.

## Results

Literature providing evidence on whether persons in certain categories are at higher than average risk of experiencing severe disease when infected with influenza are summarised in Table 1 along with relevant studies showing the effectiveness of vaccination in reducing this risk. The Table does not attempt to show all the studies but selects typical studies or describes the conclusions of reviews.

### Older people

The data strongly support the WHO position that older people are at higher risk of severe illness, hospitalisation and death if they are infected with influenza, compared to younger adults. The data also show that immunisation significantly reduced this risk of adverse outcomes, though the protection afforded is lower than for younger people. The protection was somewhat less for the more severe outcomes (hospitalisation, pneumonia and death) than it is for all influenza but it was still significant both statistically and from a public health perspective.

**Table 1**

**Selected articles providing evidence on the risk groups for influenza vaccination**

| Target population Risk group | Study type | Outcome measure provided | Comments |
|---|---|---|---|
| **Individuals aged 65 years and older (Group 1)** | | | |
| | Guidelines [27] | Not applicable | US-CDC updated recommendations for seasonal vaccination. Includes a comprehensive review of articles supporting vaccination of various risk groups. It is mainly based on evidence coming from the United States (US). |
| | Cohort [5] | VE against hospitalisation 21% (95% CI: 17%-26%). VE against death 12% (95% CI: 8%-16%). | Large cohort study conducted in the United Kingdom (UK) covering a 10-year period. Provides robust data on the effectiveness of vaccination in the elderly (≥65 years old) against hospitalisation and death. |
| | Cohort [3] | Incidence of hospitalisation for pneumonia/ influenza or death: 8.2/1,000 for healthy and 38.4/1,000 for high-risk individuals. VE against hospitalisation 48% (95% CI: 42%-52%) | Large cohort study conducted in the US. Provides rates of death/hospitalisation for healthy and high-risk elderly as well as VE data. |
| | Time series analysis [28] | Excess hospitalisations higher in persons ≥65 years old (10 per 100,000) | Large study based on hospital discharge records from all public hospitals in Spain covering four influenza seasons. Excess hospitalisations attributable to influenza significantly higher in those ≥65 years old. |
| **Chronic illness (Group 2)** | | | |
| Chronic respiratory diseases | Review [29] | Influenza vaccination reduced the development of severe respiratory complications and hospitalisation by 50-80%, and death from both respiratory disease and all causes by 40-55%. | |
| | RCT [8] | VE against influenza-confirmed ARI 76% among individuals with COPD. | VE was not influenced by the severity of COPD. None of the vaccinated patients required mechanical ventilation because of influenza-related ARI. By contrast, all the unvaccinated patients with moderate-to-severe COPD who were hospitalised because of influenza-related ARI needed assisted ventilation. |
| Chronic cardiovascular disease | Cohort [8] | Vaccination reduced the risk of cardiovascular death - RR 0.34 (95% CI: 0.17%-0.7%1) in individuals with stable coronary hearth disease. | |
| | Restrospective cohort [30-32] | Higher risk of acute myocardial infarction shortly after an acute respiratory infection (not necessarily influenza) RR 4.95 (95% CI: 4.43%-5.53%) | The study was based on the United Kingdom General Practice Research Database, which contains computerised medical records of more than five million patients. |
| Metabolic disorders (Including diabetes mellitus) | Case control [10-11] | Influenza vaccine effectiveness in diabetics was 79% (95% CI: 19%-95%) | |
| | Cohort [9] | Higher risk of hospitalisations, OR: 2.19 (95% CI: 1.08%-4.47%), and of any complication, OR: 1.74 (95% CI: 1.16%-2.61%), among non-elderly adults with diabetes. | |
| Chronic renal and hepatic diseases | Case series analysis [33,34] | Excess influenza-attributable mortality in patients on dialysis. | |
| | Literature review [34] | Increased incidence of respiratory infections in patients with chronic kidney disease. | |
| Immunosuppressed | Review [35] | Higher incidence of complication among organ and haematopoietic stem cell recipients. | |
| HIV | Meta-analysis [36-38] | Pooled relative risk reduction of 66% (95% CI: 36%-82%). | The study of the highest quality, an RCT, yielded the most conservative estimate (RRR 41%; 95% CI: 2%-64%) |
| | Cohort [37] | Influenza accounted for 42% of ARI among HIV-infected individuals followed up in a single clinic. | Probably high incidence of disease, but no evidence of more severe disease than in healthy population. |
| Young people taking salicylates long-term | Review [39] | Theoretical risk of developing severe disease (Reye syndrome) among people under the age of 20 taking salicylates. | A causal association was never established. |
| **Other groups** | | | |
| Pregnant women (Group 3) | Review [12] | Not applicable | Evidence is contradictory on pregnancy as risk factor for more severe influenza disease in women who are otherwise healthy. |
| Pregnant women with risk factors (Group 3) | Review [12] | Occurrence of acute respiratory illness was more likely than among healthy pregnant women OR: 3.2 (95% CI: 3%-3.5%). Influenza-attributable rate of hospital admission was increasing with pregnancy trimester: 3.9 (-6.4 to 14.2), 6.7 (-4.1 to 17.5), and 35.6 (21.1 to 50·1) respectively/per 10,000 woman-months. | |
| Children (Group 4) | ECDC technical report [18] | Data for young children, particularly under two years of age, are scant from European countries. Routine immunisation of school-age children has an indirect beneficial effect for adults and the elderly in terms of reduced disease burden. | This report was developed by a panel of experts who reviewed the available literature up to January 2007. |

Abbreviations: ARI, acute respiratory tract illness; CI, confidence interval; COPD, chronic obstructive pulmonary disease; ECDC, European Centre for Disease Prevention and Control; HIV, human immunodeficieancy virus; OR; odds ratio; RCT, randomised controlled trial; RR, relative risk; RRR, relative risk reduction; US-CDC, United States Centers for Disease Control and Prevention; VE, vaccine effectiveness;

There is uncertainty concerning the age 'cut-off', the lower age threshold above which all people should be recommended the vaccine and the data are not consistent with any precise age although as people get older the risk rises [28,40]. The age group most commonly stated as being routinely offered immunisation is of persons aged 65 years and older [15]. There are some exceptions to this and a few European countries have adopted policies for immunising younger persons and have lower age thresholds, others still are at present reviewing their policies with a view to lowering their age-limits [15]. One analysis sponsored by industry suggested reducing the age cut-off to 50 years [24].

**Children**

In 2006-7, an independent scientific panel convened by ECDC found there was then insufficient data to support starting widespread immunisation of children though the vaccines did induce immunity [18]. That review found considerable data from outside Europe but little that was from Europe itself, notably on the burden of disease in children. Our present review finds that this has not changed, although there is equally no evidence against immunising children.

**Table 2**

**Country-specific estimates of the population in the two major risk groups for European Union countries*, 2006**

| | Number aged 65 years or over[1] | | Number under 65 years-old with one or more risk morbidities[2] | | Total "at risk" | |
|---|---|---|---|---|---|---|
| Country | No. of people | % of country's population | No. of people | % of country's population | No. of people | % of country's population |
| Austria | 1,403,000 | 16.9 | 689,000 | 8.3 | 2,091,000 | 25.2 |
| Belgium | 1,810,000 | 17.1 | 879,000 | 8.3 | 2,689,000 | 25.4 |
| Bulgaria | 1,325,000 | 17.3 | 637,000 | 8.3 | 1,962,000 | 25.6 |
| Cyprus | 96,000 | 12.3 | 65,000 | 8.3 | 160,000 | 20.6 |
| Czech Republic | 1,482,000 | 14.4 | 853,000 | 8.3 | 2,336,000 | 22.7 |
| Denmark | 835,000 | 15.3 | 452,000 | 8.3 | 1,287,000 | 23.6 |
| Estonia | 229,000 | 17.1 | 111,000 | 8.3 | 340,000 | 25.4 |
| Finland | 869,000 | 16.5 | 437,000 | 8.3 | 1,306,000 | 24.8 |
| France | 10,277,000 | 16.2 | 5,262,000 | 8.3 | 15,539,000 | 24.5 |
| Germany | 16,299,000 | 19.8 | 6,832,000 | 8.3 | 23,131,000 | 28.1 |
| Greece | 2,074,000 | 18.6 | 927,000 | 8.3 | 3,001,000 | 26.9 |
| Hungary | 1,605,000 | 15.9 | 835,000 | 8.3 | 2,441,000 | 24.2 |
| Ireland | 478,000 | 11.1 | 358,000 | 8.3 | 836,000 | 19.4 |
| Italy | 11,772,000 | 19.9 | 4,907,000 | 8.3 | 16,681,000 | 28.2 |
| Latvia | 389,000 | 17.1 | 189,000 | 8.3 | 579,000 | 25.4 |
| Lithuania | 527,000 | 15.6 | 280,000 | 8.3 | 808,000 | 23.9 |
| Luxemburg | 67,000 | 14.0 | 40,000 | 8.3 | 106,000 | 22.3 |
| Malta | 56,000 | 13.8 | 34,000 | 8.3 | 91,000 | 22.1 |
| Netherlands | 2,368,000 | 14.5 | 1,358,000 | 8.3 | 3,726,000 | 22.8 |
| Poland | 5,116,000 | 13.4 | 3,164,000 | 8.3 | 8,280,000 | 21.7 |
| Portugal | 1,828,000 | 17.3 | 879,000 | 8.3 | 2,708,000 | 25.6 |
| Romania | 3,204,000 | 14.9 | 1,789,000 | 8.3 | 4,993,000 | 23.2 |
| Slovakia | 640,000 | 11.9 | 447,000 | 8.3 | 1,087,000 | 20.2 |
| Slovenia | 320,000 | 15.9 | 166,000 | 8.3 | 486,000 | 24.2 |
| Spain | 7,407,000 | 16.7 | 3,691,000 | 8.3 | 11,098,000 | 25.0 |
| Sweden | 1,581,000 | 17.4 | 756,000 | 8.3 | 2,338,000 | 25.7 |
| United Kingdom | 9,752,000 | 16.0 | 5.051,000 | 8.3 | 14,802,000 | 24.3 |
| **Total EU 27** | **83,813,000** | **16.9%** | **41,095,000** | **8.3%** | **124,909,000** | **25.2%** |

* Note numbers have been rounded to the nearest thousand so column totals will not necessarily add up.
[1] Eurostat data, average population by sex and five-year age groups, 2006 (date of extraction: 11 Feb 2008)
[2] Based on methodology of Fleming and Elliot (2006) [26]

Two other sources of information show similar estimates for specific countries:
**Belgium:** Based on the Health Interview Survey (HIS) last conducted in 2004 in Belgium [46], where people at risk were elderly or those with a chronic disease, 30.2% of the total population were at risk and considered for immunisation in 2004 which is consistent with the estimate applying Fleming and Elliot's findings of 25.4%. In absolute numbers, the population aged 65 years or older amounted to 1,789,812 individuals in 2004, and the population between 15- and 64-years-old with chronic health problems was estimated at 1,353,366 individuals. People with more than one chronic disease are not counted twice. Chronic conditions that were taken into consideration were similar to the ones counted in Fleming and Elliot (2006) [26].
**France:** The estimated number of people aged 65 years or older was around 9,100,000 (14.4% of the population of France) in 2007. The number of people who have used the social security system (because of chronic illness) was estimated at 7,700,000 (13.6%) in 2006 (L'assurance maladie, Caise national 2007 [47]). This means that the proportion of people in risk groups was about 28.0% of the total population which is close to the ECDC estimate of 24.5% applying Fleming and Elliot's data.

**Persons with chronic medical conditions**

Our review also supports the position that people of all ages with certain broad categories (as listed in Table 1) of chronic medical conditions are at higher risk for severe disease. However, there are much fewer published data that demonstrate that vaccination can reduce the risk of adverse outcomes in this group than there are for the older age-groups. When it comes to specific conditions (rather than broad groups), there is usually insufficient epidemiological scientific information to support immunisation, unless the condition is relatively common such as diabetes.

Our review of the literature also found that patients with more common milder conditions such as mild hypertension, mild asthma, asymptomatic HIV infection or controlled HIV disease with normal immune function have not been investigated for either an increased risk from influenza infection or the impact of vaccination.

**Healthy pregnant women**

Healthy pregnant women are another group where the case has been made for offering immunisation. It is policy in eight EU countries to offer the vaccine to healthy pregnant women [12,15], based on more complex arguments than in the case of children, reflecting both whether there is evidence of increased risk of severe disease in the women and whether or not this is a mechanism for providing direct and indirect protection of newborn babies by protecting their pregnant and nursing mothers [27]. There is only limited evidence from Europe of increased risk for severe disease in healthy pregnant women and hardly any evidence as yet of impact of immunisation, though the vaccines do induce immunity [12]. What evidence exists is conflicting and much of it is from outside Europe [12]. There are no data against immunising healthy pregnant women, but equally few data from Europe on the burden of influenza in pregnant women and none on the effectiveness of vaccination in reducing that burden. One recent blinded randomised trial of immunisation of pregnant women showed benefit for both mother and child in terms of reduced acute respiratory infection. But that study was conducted in a tropical country [41].

**Other groups to whom vaccination is recommended**

Many countries recommend immunising healthcare workers and there are occupational health reasons for doing so in order to protect the health of staff themselves [15], but that issue is outside the scope of this paper [42,43]. However immunisation of staff to protect people in risk groups is important to recognise. Randomised community trials (one conclusive community trial and another giving supportive evidence) of immunising care home staff have convincingly demonstrated that this reduces mortality in the elderly and chronically ill patients and therefore can be recommended [44,45]. In terms of protecting risk groups, we could identify no conclusive data that would support or refute policies for immunising other groups of staff or family carers.

**Proportion of the population targeted by immunisation**

Broad estimates of the number of people and the proportion of the population falling under the two main risk groups for influenza in EU countries and in the EU as a whole are shown in Table 2. The national range is from 19% to 28% depending on the proportion of the elderly in the population in each country. The EU total is estimated to be around 125 million people, with around 84 million persons aged 65 years or older and around 41 million younger persons living with chronic illness.

## Discussion

Although there are a number of published studies on burden of disease and vaccination effectiveness in risk groups, relatively few of these are based on data from European countries. Therefore, evidence was considered also from other countries, especially on the effectiveness of vaccination in protecting risk groups. A particular gap is the lack of data on burden of severe disease due to influenza in Europe and surveillance for so called severe acute respiratory infection (SARI) in particular in children and pregnant women. It is notable that while there is good laboratory surveillance and surveillance of those presenting to primary care services with influenza in Europe (so far undertaken through the European Influenza Surveillance System (EISS; http://www.eiss.org/) and WHO National Influenza Centres (http://www.who.int/csr/disease/influenza/centres/en/index.html) working with WHO Global Influenza Surveillance Network (GISN; http://www.who.int/csr/disease/influenza/influenzanetwork/en/index.html) there are no routine European systems of surveillance for persons with severe adverse outcomes due to influenza. Similarly, there is no routine evaluation of influenza vaccine effectiveness in Europe. Therefore, the task of objectively determining the burden of influenza disease, which groups are at risk of severe disease from influenza in Europe and of these which would gain most from immunisation is not as straightforward an exercise as it could be. This is especially pertinent as the characteristics of influenza can change annually leading to significant short term and perhaps longer term variations in the severity of disease and the vaccine effectiveness [6].

Estimates of the impact of influenza vaccines on morbidity and mortality are variable [4,5,48,49]. This is inevitable when citing studies with non-specific outcomes (e.g. all cause or respiratory-related deaths) which always dilute the effects generally found in studies with laboratory-confirmed outcomes. Even in the latter studies it is important to allow for the role of confounding factors. Both positive confounding due for example to the "healthy vaccinee effect", as well as negative confounding associated with serious pre-existing medical conditions being more frequent among vaccinees (counfounding by indication) can bias vaccine effectiveness up- and downwards respectively. The diluting effect and the predominance of negative confounding in a particular study population explains why some reviews of effect from the influenza vaccine may conclude by showing no protection [48].

That said, the evidence supporting the WHA policy for selectively immunising the two risk groups: older people and those with chronic ill-health in Europe is sufficiently strong. Though immunising older people is not a panacea in protecting them against influenza, on balance, it certainly reduces their risk of infection and the more severe outcomes. There is no consensus on what exactly is the age cut-off for 'older people' in Europe and there has been no EU level debate on this subject. Defining a cut-off is beyond the scope of this paper. It also needs to be borne in mind that the age-structure varies across EU countries as do the costs of healthcare and income levels and with these the relative costs and benefits of influenza disease and immunisation respectively. Hence it could be quite reasonable for national age cut-offs to differ. However what data and analyses there are suggest the age of 65 years and over as the current threshhold and this is at least a reasonable minimum recommendation for policy decisions. Concerning the youngest age groups the lack of data from Europe makes decisions over childhood vaccination difficult. It should be noted that three counties, Finland and neighbouring Estonia and Latvia have

recently started immunising children routinely and it is expected that this will provide information on both the burden and impact of immunisation [15].

There are difficulties in defining the chronic conditions. Some national authorities take the approach of coming up with lists of medical and physical conditions for which immunisation is recommended. Others have taken the more pragmatic approach of defining broad categories, e.g. "all chronic metabolic conditions" [50,51]. In our view, the latter broad brush approach is preferable for two reasons. When it comes to individual rare conditions the numbers are always too low to research and so there can only be presumed evidence of increased risk, and even less of the effectiveness of vaccination in reducing that risk. Also there are always uncommon conditions that may have been omitted from the lists. Finally comparison between various EU countries show differences between the detailed national lists while the broad-brush lists all look the same along the lines of Table 1. A problem with both approaches is whether to include mild conditions that are technically chronic diseases but for which there is in fact no demonstrated evidence of increased risk of benefit from immunisation.

When it comes to estimating the number of persons at risk, more credibility should be afforded to the data in our review for the elderly population than that for the people under age of 65 years with chronic illnesses, since the latter data rely on application of results obtained from one country's survey to all other countries. However, the results for chronic illness are similar to what is found in an independent study undertaken by Ryan *et al.* though the overall estimates are greater in Ryan *et al.* because they include people down to the age of 50 years [24] and prevalence surveys in Belgium [46] and France [47] came up with results that were within a few percentage points of what we derived for those countries applying Flemings estimates (Table 2). Both the two independent country estimates were somewhat higher than our estimate but that may reflect that their surveys were without medical verification.

Our calculations suggest that EU countries would currently need to immunise about one quarter of their population annually covering the two major risk groups. Projections of expected demographic trends to 2050 indicate that the absolute numbers and proportions of the older age groups will rise inexorably over time in Europe because of aging populations; from the range of 11-19% in 2004 to 22-35% in 2050 [20,52] (Figure 1). It is less clear what will happen with the size of younger populations with chronic illness. Common sense suggests that the success of modern medicine in permitting people with chronic illness like HIV infection to live productive lives will also result in the increase of the proportion of the population with chronic illnesses. Also some secular changes like increasing obesity and declining levels of exercise may independently increase the prevalence of conditions like maturity onset diabetes and cardiovascular disease. Some limited confirmation of this hypothesis comes from the surveys undertaken by the University of Zurich which show a slow increase in prevalence of people with self-reported ill-health in telephone surveys [53].

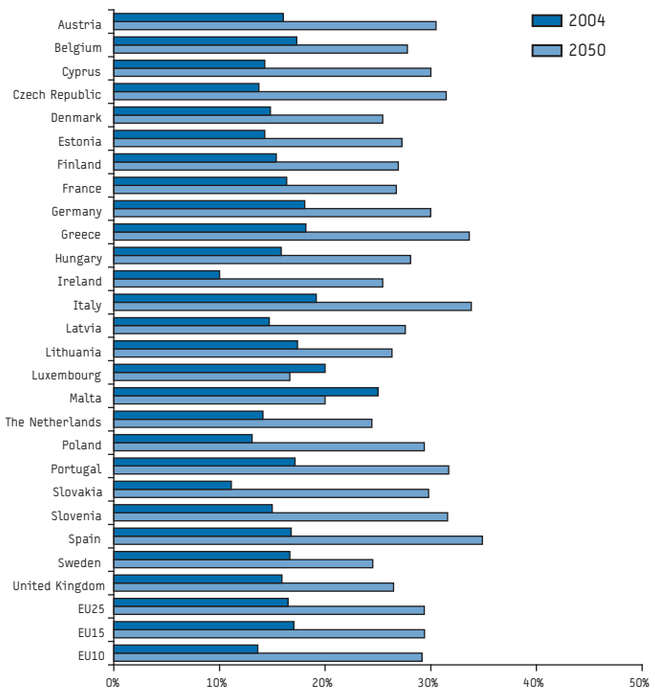
Despite the limited scientific basis for recommending influenza vaccination to healthcare workers in general there is no evidence against it either. Therefore the decisions taken by some countries to recommend immunisation to such groups are reasonable, even if they cannot yet be scientifically supported and conclusively shown to protect patients [54].

In conclusion, existing evidence indicate that the elderly and people with chronic diseases are at higher risk of severe adverse outcome of influenza and that immunisation reduces this risk. Our work has also highlighted a number of gaps in the evidence thus suggesting a number of obvious priorities for studies that could be performed in individual countries or at EU level. Specifically these are:

- Surveillance development – routine surveillance for severe manifestations of influenza and other respiratory infections in Europe (hospitalisations and death). This can be referred to as severe acute respiratory infection (SARI).
- Routine monitoring of the effectiveness of influenza vaccination against different outcomes. Such monitoring is currently piloted by ECDC, Epiconcept and EU Member States [55].
- Estimation of the burden of disease from influenza in pregnant women and children and evaluation of the impact of immunising these groups.
- Development of projects for stronger promotion of influenza immunisation among healthcare workers both for their own benefit and for that of their patients coupled with studies to investigate whether or not immunisation of healthcare staff and household members reduces risk in vulnerable people in the two main risk groups.

**Figure 1**

**Percentage of population aged 65 years and older: 2004 census data compared with 2050 projected data**




Data not stated for: Bulgaria, Romania (joined EU in 2007), Iceland and Norway
Data as published for Luxembourg and Malta
Source: The Economic Policy Committee (EPC) and European Commission (EC), December 2005 [20]

- Specific investigation as to whether or not there are higher levels of risk of severe disease from influenza infection in HIV-infected persons in Europe and similar studies for other more common conditions such as mild asthma.
- Development of cross-European health impact and health economic frameworks for policy-informing studies on influenza immunisation, for example regarding the cut-off ages of immunisation in the elderly recognising that there may be reasons for variation between countries.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# Editorials

## Turning the tide of antimicrobial resistance: Europe shows the way

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Ten years ago, European officials, experts and other stakeholders met in Copenhagen, Denmark, at the invitation of the Danish Ministry of Health and the Danish Ministry of Food, Agriculture and Fisheries. This European conference on "The Microbial Threat" due to antimicrobial resistance resulted in the publication of "Copenhagen Recommendations" calling for action to limit the emerging problem of antimicrobial-resistant microorganisms [1]. Following the conference, the European Commission prepared a comprehensive Community strategy against antimicrobial resistance, which was published in 2001 [2] and presented in Eurosurveillance [3]. Later the same year, European Union (EU) Health Ministers adopted a Council Recommendation on the prudent use of antimicrobial agents in human medicine with a series of specific measures aimed at containing the spread of antimicrobial resistance by prudent use of antimicrobial agents [4].

A review article published in this journal in 2001 showed that only six European countries had a national action plan to contain antimicrobial resistance [5]. An evaluation of implementation of the Council Recommendation performed by the European Commission showed that, by 2003, 16 countries had developed a national strategy to contain antimicrobial resistance and nine countries had an action plan [6,7]. The European Commission is currently performing another evaluation of the implementation of the Council Recommendation and its results will be available in 2009.

**The challenge is now to get all European countries take similar action.**

Historically, Denmark was the first European country to report on the control of methicillin-resistant *Staphylococcus aureus* (MRSA), which took place at the end of the 1960s and in the 1970s. Although the interventions were never fully documented, this decrease in the percentage of MRSA in *S. aureus* blood isolates from more than 30% to less than 1% - a figure that still holds today - has been attributed to a more prudent use of antbiotics combined with increased awareness of hospital hygiene [8]. In Iceland, a public media campaign on the prudent use of antibiotics in children in the mid-1990s led to a change in parents' attitudes, to a reduction in antimicrobial use and, subsequently, to a decrease in the incidence of penicillin-non-susceptible *Streptococcus pneumoniae* which had increased rapidly at the beginning of the decade [9]. This issue of Eurosurveillance is the first of two special issues on antimicrobial resistance and focuses on the recent successes of several EU Member States in reverting trends in antimicrobial resistance or, for the Netherlands, in maintaining already low antimicrobial resistance rates.

Among the six countries reporting in this issue of Eurosurveillance, the French success is remarkable because this country, which had the highest outpatient antibiotic consumption per capita in the EU, has been able to reduce this consumption by 16% between 2000 and 2006 following repeated annual public awareness campaigns on the prudent use of antibiotics combined with interventions targeted at general practitioners, including academic detailing and promotion of rapid testing for *Streptococcus pyogenes* tonsillitis [10]. This decrease in antibiotic use combined with the introduction of the 7-valent protein conjugated pneumococcal vaccine for young children in 2002 resulted in reverting trends in penicillin resistance in *S. pneumoniae* [10]. Additionally, several data sources confirm a decrease in the incidence and the prevalence of MRSA. For example, data from the European Antimicrobial Resistance Surveillance System (EARSS) show a decrease in the proportion of MRSA in *S. aureus* from blood cultures from France, from 33% in 2001 to 26% in 2007 [11]. This decrease has been attributed to the gradual expansion of infection control structures as well as implementation of specific MRSA control measures in French hospitals [10]. In Belgium, national activities to contain antimicrobial resistance have been coordinated by the Belgian Antibiotic Policy Coordination Committee (BAPCOC) since 1999. Yearly public awareness campaigns on antibiotics since 2000 have resulted in a 32% decrease in antibiotic consumption when expressed in packages and a concomitant decrease in, e.g. macrolide resistance in *S. pneumoniae* and *S. pyogenes* [12]. However, France and Belgium remain among the European countries with the highest consumption of antibiotics per capita and have therefore decided to continue organising national public awareness campaigns each year to consolidate their progress towards prudent use of antibiotics.

Other European countries with much lower levels of antimicrobial consumption and resistance have shown success with their national actions on prudent use of antibiotics and infection control. Through repeated reports in the media and the introduction of rapid diagnostic tests, Slovenia was able to show a 20% decrease

in antibiotic consumption in outpatients, although this decrease has so far not been followed by a concomitant decrease in resistance. In Slovenian healthcare facilities, the introduction of a comprehensive national strategy for MRSA control resulted in a decrease in the proportion of MRSA in *S. aureus* from blood cultures from 21% in 2000 to 8% in 2007 [13]. In the Czech Republic, an education programme targeted at primary care paediatricians, including repeated audits of prescribing practices and feedback, was implemented in 2001 as a control measure following increasing antibiotic consumption and resistance in the community in the 1990s [14]. In Sweden, national activities are coordinated by the Swedish Strategic Programme Against Antibiotic Resistance (STRAMA) and relayed at county level by a network of local STRAMA groups. Regular collaboration with national and regional media combined with local activities resulted in a 22% decrease in outpatient antibiotic consumption between 1994 and 2004 [15]. Finally, the Netherlands still have the lowest outpatient antibiotic consumption per capita in the EU as reported by European Surveillance of Antimicrobial Consumption (ESAC) [16], with antimicrobial resistance proportions that are among the lowest registered by the EARSS [11]. A Dutch Working Party on Antibiotic Policy (SWAB) was created in 1996 to ensure that the low level of antimicrobial resistance is preserved while improving the quality of antimicrobial prescriptions through the development of guidelines education and surveillance [17].

These experiences from European countries are encouraging. They show that it is possible to turn the tide of antimicrobial resistance through prudent use of antibiotics, better infection control practices and use of vaccines. The challenge is now to get all European countries take similar action. On 10 June 2008, EU Health Ministers adopted the Council Conclusions on antimicrobial resistance that reiterated their call for action to contain antimicrobial resistance and called upon Member States "to ensure that structures and resources for the implementation of the Council recommendation on the prudent use of antimicrobial agents in human medicine are in place and to continue with the implementation of specific strategies targeted towards the containment of the antimicrobial resistance" [18]. The Council also called upon the Commission and Member States "to coordinate an annual European initiative to increase awareness of the general public and veterinary and healthcare professionals about antimicrobial resistance, the prudent use of antibiotics in humans and animals and infection control practices". On 18 November 2008, the first European Antibiotic Awareness Day will be launched at the European Parliament in Strasbourg and marked in 29 European countries. This European health initiative coordinated by the European Centre for Disease Prevention and Control will in 2008 focus on increasing awareness of the general public about prudent use of antibiotics, based on the experience of a number of pioneer Member States reporting in this issue of Eurosurveillance. More information about European Antibiotic Awareness Day can be found at: http://antibiotic.ecdc.europa.eu.

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# Perspectives

## Recent trends in antimicrobial resistance among *Streptococcus pneumoniae* and *Staphylococcus aureus* isolates: the French experience

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In France, the overall proportion of penicillin-non-susceptible *Streptococcus pneumoniae* has decreased from 53% in 2002 to 38% in 2006, and the proportion of methicillin-resistant *Staphylococcus aureus* from 33% in 2001 to 26% in 2007. Although the rates remain very high compared to northern European countries, these trends suggest that the prevention efforts implemented since 2000 through two national programmes (the national plan for preserving the efficacy of antibiotics and the national infection control programme) and updated recommendations for pneumococcal vaccination are successful.

### Introduction

Antimicrobial resistance is a multifaceted threat of global concern in the European Union. In this article, we illustrate results and efforts to counteract its spread in France through two microorganisms, *Streptococcus pneumoniae* and *Staphylococcus aureus*, that are frequently isolated from community-acquired or hospital-acquired infections, respectively*. The proportion of resistance in these species is a good indicator of the evolution of antimicrobial resistance in France and these bacteria are key targets of two national programmes: the national plan for preserving the efficacy of antibiotics [1] and the national programme for infection control [2]. Quantitative targets were included in these programmes in 2004 [3], aiming to reduce, by 2008, the proportion of penicillin-non-susceptible strains among *S. pneumoniae* isolates to under 30% and the proportion of methicillin-resistant (MRSA) strains among *S. aureus* isolates to under 25%.

### *Streptococcus pneumoniae* resistance trends

#### Data sources

Antimicrobial susceptibility in *S. pneumoniae* is studied by a group of 22 regional laboratory networks (*Observatoires Régionaux du Pneumocoque*), covering the 22 French metropolitan regions (excluding overseas regions) and coordinated by the French national reference centre for *S. pneumoniae* (CNRP). The CNRP collects all blood or cerebrospinal fluid (CSF) isolates from children under the age of 15 years, all CSF isolates from adults, and a selection of strains isolated from adults with respiratory tract infections (respiratory or blood isolates) or from children with acute otitis media [4].

Since 2001, susceptibility testing results for invasive isolates (blood or CSF) have been submitted to the European Antimicrobial Resistance Surveillance System (EARSS; http://www.rivm.nl/earss/). All laboratories use agar dilution and recommendations from the Antibiogram Committee of the French Society for Microbiology (CA-SFM, http://www.sfm.asso.fr/) for antimicrobial susceptibility testing and breakpoints. However, yearly data submitted by France to EARSS only included the first six months of a given year due to time constraints in the European data collection process; the data presented in the following include all strains received annually by the CNRP.

#### Results

Participation of laboratories has been stable since 2001. In 2006, for instance, the CNRP collected 1,411 strains from 406 private or public microbiological laboratories that provide support for 444 healthcare facilities covering 61.4% of admissions to French medical wards. Among those strains, 857 (61%) were isolated from invasive infections (blood or CSF) and 554 (39%) were isolated from respiratory tract infections.

Overall, the proportion of penicillin-non-susceptible *S. pneumoniae* (PNSP) was negligible before 1987 and then increased regularly every year, up to 53% in 2002 (48% and 46% of blood and CSF isolates, respectively). Between 2003 and 2005, the proportion of PNSP decreased, and remained stable (38%) in 2006 (34% for blood and CSF isolates) (Figure 1) [4].

Among invasive *S. pneumoniae* isolates, the overall proportion of PNSP decreased from 47% in 2001 to 34% in 2006. This corresponded to a decrease from 51% to less than 32% in children under the age of 15 years, and from 45% to 35% in adults (Table 1). A sharp reduction was noted in the proportion of PNSP (from 67% to 27%) among CSF isolates from children under the age of two years. The change in blood isolates in the same age group was less pronounced, with the proportion of PSNP remaining at or above 40% throughout this period and even increasing in 2006.

#### Discussion: prevention and control activities

The observed decrease in PNSP started after the implementation in November 2001 by French public health authorities of the first national plan for preserving the efficacy of antibiotics (Figure 1). Two studies helped to define actions of this plan targeting the community: In 2000, a controlled, population-based trial was conducted in three French regions and demonstrated that intensive

educational strategies aimed at optimising antibiotic use could significantly reduce the rate of PNSP colonisation [5]. In 2002, a study conducted by the French National Insurance Fund for Salaried Workers (CNAMTS) showed that both physicians and patients had little knowledge on antibiotics, resulting in poor antibiotic practices.

A multifaceted programme was then initiated by CNAMTS to avoid inappropriate antibiotic use in outpatients. The "Antibiotics aren't automatic!" campaign (http://www.antibiotiquespasautomatiques.com/) aimed at increasing awareness of physicians as well as the public on good antibiotic practices. Using humoristic television commercials, it targeted specific populations likely to ask for antibiotics (young mothers, young workers, the elderly) and promoted prudent use of antibiotics. The campaign has been repeated every winter since 2002 and become widely known and popular, parents becoming more and more aware of the benefits and limits of antibiotics [6].

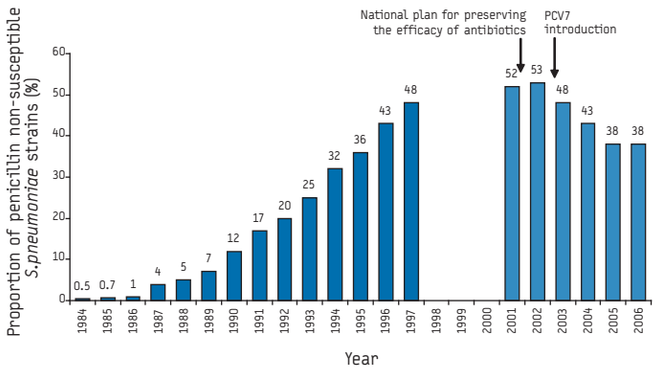
Other interventions since 2002 have been aimed at general practitioners, including academic detailing, peer-to-peer visits by health insurance delegates and the promotion of the streptococcal group A rapid diagnostic test for sore throat, that CNAMTS distributed to physicians free of charge. Data sent to the European surveillance of antimicrobial consumption (ESAC) network by the French Health Product Safety Agency (Afssaps) show that the overall antimicrobial consumption in ambulatory care in France has decreased from 33.0 defined daily doses per 1,000 inhabitants per day in 2001 to 27.9 in 2006, a reduction of 15%; the consumption of broad-spectrum penicillins (ATC4 code J01CA) has decreased by 20% and the consumption of macrolides (ATC4 code J01FA) by 39% (http://www.esac.ua.ac.be/). CNAMTS later demonstrated that its campaign was cost-effective [7].

In addition to reduced consumption of antibiotics, the introduction in March 2002 of the 7-valent protein conjugate vaccine (PCV7) for children under the age of two years [8] is likely to have contributed to the larger and faster decrease of PNSP rates among this age group than among adults. In 2002, serotypes covered by PCV7 (4, 6B, 9V, 14, 18C, 19F and 23F) accounted for 71% of invasive pneumococcal disease in France; most of them (68%) were PNSP, as compared to 44% for non-vaccine serotypes [4]. From 2004 to 2007, PCV7 vaccine coverage increased from 27% in six-month-old children to 56% in six- to 12-month-old children [9,10]. In children under the age of two years, the incidence between 2001/02 and 2006 of pneumococcal meningitis and bacteraemia decreased from 8.0 to 6.0 and from 21.8 to 17.5 cases per 100,000, respectively [11].

A partial replacement of vaccine serotypes by non-vaccine serotypes such as 19A, a serotype with a proportion of 85% PNSP in 2006, may explain why the decrease in the proportion of PNSP was not sustained in 2006 [12].

**FIGURE 1**

**Proportion of penicillin non-susceptible *S. pneumoniae* among all strains studied by CNRP, France, 1984 to 2006 (n=50,300)**




Note. no national figures from 1998 to 2000, as CNRP activities were interrupted.
CNRP: national reference centre for *S. pneumoniae*; PCV7: 7-valent pneumococcal protein conjugate vaccine.

**TABLE 1**

**Proportion of penicillin non-susceptible *S. pneumoniae* among invasive isolates, by age and type of isolate, France, 2001 to 2006**

| | 2001 | | 2002 | | 2003 | | 2004 | | 2005 | | 2006 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | % | N | % | N | % | N | % | N | % | N | % |
| **Children** | | | | | | | | | | | | |
| <2 years | | | | | | | | | | | | |
| Blood isolates | 143 | 62.2 | 104 | 59.6 | 170 | 58.8 | 83 | 39.8 | 145 | 41.4 | 99 | 46.5 |
| CSF isolates | 87 | 66.7 | 69 | 62.3 | 99 | 44.4 | 72 | 50.0 | 76 | 39.5 | 67 | 26.9 |
| 2-15 years | | | | | | | | | | | | |
| Blood isolates | 150 | 30.7 | 87 | 37.9 | 183 | 33.9 | 123 | 31.7 | 206 | 23.8 | 133 | 23.3 |
| CSF isolates | 39 | 51.3 | 37 | 37.8 | 37 | 35.1 | 41 | 29.3 | 55 | 30.9 | 33 | 30.3 |
| All isolates from children | 419 | 50.8 | 297 | 51.2 | 489 | 44.8 | 319 | 37.6 | 482 | 32.4 | 332 | 31.6 |
| **Adults (>15 years)** | | | | | | | | | | | | |
| Blood isolates | 828 | 46.0 | 678 | 46.0 | 635 | 41.6 | 232 | 44.8 | 461 | 36.2 | 308 | 34.1 |
| CSF isolates | 213 | 42.3 | 214 | 42.3 | 255 | 42.4 | 209 | 38.3 | 294 | 36.1 | 215 | 36.3 |
| All isolates from adults | 1,041 | 45.2 | 892 | 45.2 | 890 | 41.8 | 441 | 41.7 | 755 | 36.2 | 523 | 35.0 |
| Total | 1,460 | 46.8 | 1,189 | 47.5 | 1,379 | 42.9 | 760 | 40.0 | 1,237 | 34.7 | 855[1] | 33.7 |

[1] age missing for two of the 857 strains reported in 2006.
N: strains tested for susceptibility; %: proportion of PNSP among tested strains.

### *Staphylococcus aureus* resistance trends

**Data sources**

Data on methicillin resistance among *S. aureus* strains are issued from four different sources; all involved laboratories follow the recommendations from the Antibiogram Committee of the French Society for Microbiology (CA-SFM, http://www.sfm.asso.fr/) for antimicrobial susceptibility testing and breakpoints.

The first source is the data submitted each year since 2001 by France to EARSS (http://www.rivm.nl/earss/), collected by three microbiological networks that contribute to the *"Observatoire national de l'épidémiologie de la résistance bactérienne aux antibiotiques"* (Onerba). They include 19 teaching hospitals of the Azay-Resistance network, nine general hospitals of the Ile-de-France network, and, since 2004, 26 hospitals, mostly general hospitals, of the Reussir network (http://www.onerba.org/). These data allow calculating the proportion of methicillin-resistant *S. aureus* (MRSA) isolates among all *S. aureus* invasive isolates.

The second source is the national multidrug-resistant bacteria surveillance network (BMR-Raisin, http://www.invs.sante.fr/raisin/), which includes the five interregional infection control coordinating centres (CClin) and has been collecting data on MRSA isolates from all diagnostic specimens (excluding screening isolates) since 2002. More than 450 microbiological laboratories participate on a voluntary basis each year (between 478 in 2002 and 675 in 2006, when it accounted for 47% of all French hospital beds), making it possible to calculate the incidence density of MRSA infections in healthcare facilities per 1,000 patient days (pd) [13].

The third source is national prevalence surveys on nosocomial infections, which have been conducted every five years in French healthcare facilities since 1996. Antibiotic susceptibility profiles are recorded for selected pathogens (including *S. aureus*) that are recovered from any nosocomial infection, thus providing a measure of the prevalence of patients infected with MRSA [14].

The fourth and last source is a network of 39 teaching hospitals in the Paris area belonging to a single organisation, the *"Assistance publique - Hôpitaux de Paris"* (AP-HP); MRSA surveillance started there in 1993 and provides the longest continuous time series available on this topic in France.

**Results**

According to the latest EARSS report [15], France remained in 2006 one of the European countries with the highest proportion of MRSA among *S. aureus* isolates. However, while MRSA rates in most countries were increasing in 2006 (including those with the lowest rates), the report highlighted decreasing rates in two countries: France and Slovenia. In France, the MRSA proportion has decreased from 33% in 2001 to 26% in 2007. The additional 26 French laboratories enrolled in the EARSS data collection since 2004 actually slowed this downward trend, as they accounted for 38% of all *S. aureus* strains in 2006 and their MRSA proportions were higher than in other participating laboratories (Table 2).

The decreasing proportion of MRSA among *S. aureus*, as reported by EARSS, is confirmed by national incidence data collected through the BMR-Raisin network. Data from 227 laboratories that have participated in this network since 2003 (totalling more than 4,000,000 pd each year) point to a decreasing incidence density of MRSA infections in acute care wards, which fell from 0.89 MRSA infections per 1,000 pd in 2003 to 0.64 MRSA infections per 1,000 pd in 2007. This trend was even more pronounced in intensive care units, where the incidence density fell from 2.37 MRSA infections per 1,000 pd in 2003 to 1.59 MRSA infections per 1,000 pd in 2007 (Figure 2) [Raisin, unpublished data].

A decrease in MRSA rates was also noted in national prevalence surveys, through comparison of data from the 1,351 healthcare facilities having contributed to the surveys in 2001 and 2006 which included 550,637 patients (279,490 patients in 2001 and 271,147 in 2006). In these 1,351 healthcare facilities, the proportion of nosocomial infections with a microbiological diagnosis increased from 72% in 2001 to 78% in 2006, as did the proportion of *S. aureus* strains tested for antimicrobial susceptibility (93% in 2001 and 96% in 2006). The proportion of MRSA among *S. aureus* isolates decreased from 62% in 2001 to 50% in 2006. The prevalence of MRSA-infected patients decreased from 0.49% in 2001 to 0.29% in 2006, a reduction of 41%. This trend was

**TABLE 2**

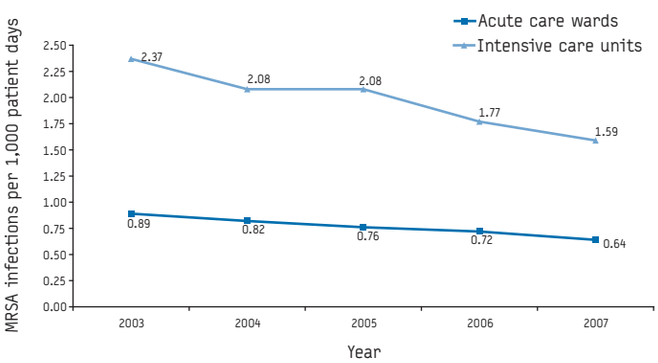
**Proportion of methicillin-resistant *S. aureus* among strains isolated from invasive isolates, by network contributing to EARSS, France, 2001 to 2007**

| Year | Azay-Resistance | | Ile-de-France | | Reussir | | Total | |
|---|---|---|---|---|---|---|---|---|
| | N | % | N | % | N | % | N | % |
| 2001 | 1,459 | 32.8 | 248 | 35.5 | - | - | 1,707 | 33.2 |
| 2002 | 1,425 | 32.9 | 238 | 33.2 | - | - | 1,663 | 32.9 |
| 2003 | 1,419 | 28.3 | 285 | 31.9 | - | - | 1,704 | 28.9 |
| 2004 | 1,596 | 26.4 | 319 | 28.2 | 1,409 | 31.6 | 3,324 | 28.8 |
| 2005 | 1,905 | 24.9 | 204 | 30.9 | 1,343 | 29.9 | 3,452 | 27.2 |
| 2006 | 2,078 | 25.7 | 276 | 25.0 | 1,444 | 28.4 | 3,798 | 26.7 |
| 2007* | 2,429 | 25.3 | 287 | 20.2 | 1,535 | 27.7 | 4,251 | 25.7 |

*preliminary data as of July 2008;
N: strains tested for susceptibility; %: proportion of MRSA among tested strains; EARSS: European Antimicrobial Resistance Surveillance System; MRSA: methicillin-resistant S. aureus;

**FIGURE 2**

**Methicillin-resistant *S. aureus* incidence density in healthcare facilities that have participated since 2003 in the BMR-Raisin Network, by type of unit, France, 2003 to 2007 (n=227)**



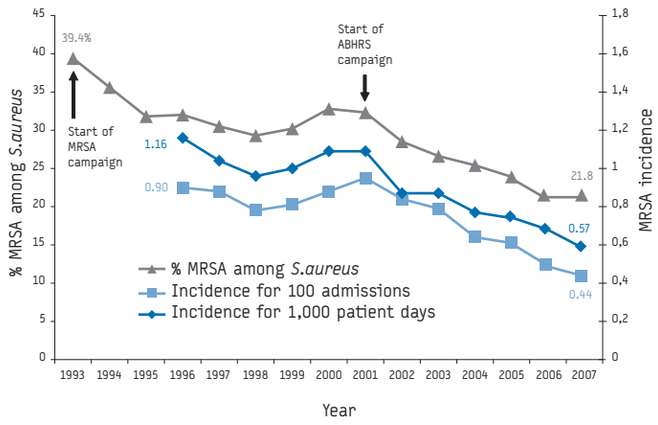

MRSA: methicillin-resistant *S. aureus*.

observed across all types of healthcare facility, from university hospitals to long-term care facilities, and across all subspecialties but obstetrics (Table 3); it remained significant after adjusting for the patients' case-mix in a multivariate analysis [14].

Finally, in the AP-HP group, the proportion of MRSA among *S. aureus* isolated from clinical specimens in acute care decreased from 39% in 1993 to 22% in 2007. At the same time, the incidence density of MRSA decreased from 1.16 MRSA infections per 1,000 pd in 1996 to 0.57 MRSA infections per 1,000 pd in 2007 (Figure 3) [AP-HP, unpublished data].

**FIGURE 3**

**MRSA proportion among *S. aureus*, and MRSA incidence, 39 teaching hospitals of the Paris area, 1993 to 2007**




Source: Assistance publique - Hôpitaux de Paris
MRSA: methicillin-resistant *S. aureus*. ABHRS: alcohol-based hand rub solutions

**Discussion: prevention and control activities**

Interventions that may account for the decrease in MRSA rates in France started in 1992, when the first European study on MRSA reported that the proportion of MRSA among *S. aureus* was 33.8% in France, the second highest proportion after Italy [16]. In 1995, a first multicenter survey in 43 hospitals showed that the median MRSA incidence in French intensive care units was 2.82 MRSA infections per 1,000 pd [17]. At that time, infection control teams were progressively implemented in French healthcare facilities, CClin had just been created, and antimicrobial resistance surveillance networks were being developed. A group of French intensive care specialists and microbiologists decided to start acting first in their own hospitals within the AP-HP group, and produced in 1993 (Figure 3) the first recommendations for prevention and control of multidrug-resistant bacteria [18].

The AP-HP recommendations provided the basis for the first national guidelines issued in 1999 by the French Ministry of Health and its Hospital Infection Control Advisory Committee [19]. They were disseminated to healthcare facilities and services through the CClin who coordinate regional networks of infection control teams and targeted diagnosis of multidrug-resistant bacteria, contact precautions, reinforcement of hand hygiene, isolation and cohorting, screening of patients, prudent antimicrobial use and evaluation through audits of practices and surveillance.

Interestingly, the fact that it is still necessary nowadays to include these key targets into national plans, shows that the fight against antimicrobial resistance is a long road. In addition, it takes time to provide the resources for adequate infection control nationwide – in 2006, 92% of French healthcare facilities had an infection control team, according to a yearly survey performed by the Ministry of Health [20] – and to integrate recommendations in the daily clinical practice – in 2001, a study assessing the implementation of recommendations in 395 French intensive care units found that 70% performed active surveillance cultures for MRSA and that 88% flagged and isolated carriers [21]. Even if there is still room for improvement, the situation appeared to be considerably better than the one in the United States, a country

**TABLE 3**

**Prevalence of methicillin-resistant *S. aureus* infected patients, by type of ward and year of survey; French national prevalence surveys, 2001 and 2006**

| Specialty | 2001 | | | 2006 | | | Δ (%) |
|---|---|---|---|---|---|---|---|
| | Patients | Infected | | Patients | Infected | | |
| | N | N | % | N | N | % | |
| Acute care | 146,445 | 708 | 0.48 | 147,908 | 437 | 0.30 | -39 |
| - medicine | 72,933 | 325 | 0.45 | 76,418 | 212 | 0.28 | -38 |
| - surgery | 49,086 | 253 | 0.52 | 47,776 | 148 | 0.31 | -40 |
| - obstetrics | 18,313 | 6 | 0.03 | 18,356 | 10 | 0.05 | |
| - intensive care | 6,113 | 124 | 2.03 | 5,358 | 67 | 1.25 | -38 |
| Rehabilitation | 42,737 | 331 | 0.77 | 43,203 | 173 | 0.40 | -48 |
| Long term care | 55,370 | 295 | 0.53 | 44,720 | 161 | 0.36 | -32 |
| Psychiatry | 34,867 | 24 | 0.07 | 33,791 | 8 | 0.02 | -66 |
| Other | 71 | 2 | 2.82 | 1,525 | 2 | 0.13 | |
| Total | 279,490 | 1 360 | 0.49 | 271,147 | 781 | 0.29 | -41 |

Note: This analysis was restricted to nosocomial infections acquired in the 1,351 healthcare facilities that participated in both surveys.
Δ (%) = relative difference in prevalence between 2006 and 2001

with very high MRSA rates, where only 18% of hospitals performed MRSA surveillance cultures in high risk units in 2003 [22].

More recently, MRSA control in France has been reinforced through the extensive promotion and use of alcohol-based hand rub solutions for hand hygiene. An intensive campaign to promote their use was launched within the AP-HP group (Figure 3), and the overall usage increased from 1 to 21 litres per 1,000 pd from 2000 to 2007 [AP-HP, unpublished data]. Similar campaigns were conducted in other hospitals and regions, e.g. in Western France where a survey recently reported that the usage of alcohol-based hand rub solutions has doubled in the period from 2002 to 2005 [23].

Other factors that possibly contributed to the decrease of MRSA in France may have been the strong and coordinated national infection control programme that allocates infection control resources and sets quantitative objectives through indicators, as well as patients' associations asking for more results and transparency. The benefits and pitfalls of public reporting of infection control indicators remain a matter of debate. Such indicators have been progressively implemented in France since 2006 by the Ministry of Health (http://www.icalin.sante.gouv.fr/). They include scores that rate nosocomial infection control organisation and activities in each hospital (ICALIN) and the overall consumption of alcohol-based hand rub products (ICSHA) [24]. Our experience suggests that they provide a strong incentive for healthcare facilities to develop infection control activities and may be a key element for a sustainable decrease in MRSA rates.

## Conclusion

PNSP and MRSA rates remain very high in France compared to Northern Europe countries [15]. Although the recent trends are encouraging, it is difficult to relate them to specific actions, as the interventions were multifaceted and implemented simultaneously. However, they suggest that the prevention efforts implemented since 2000 were successful and the national targets set in 2004 for 2008 will hopefully be reached.

According to a modelling study published in 2006, it may take more than 10 years to lower MRSA rates in countries with high prevalence [25]. The trends observed in France confirm that the fight against antimicrobial resistance is a long and demanding challenge and suggest that the dissemination of recommendations for a rational use of antibiotics, infection control and vaccination should be actively pursued.

* Data on other multidrug-resistant bacteria in France are available through the InVS website at http://www.invs.sante.fr/ratb/ (French and English versions).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# Perspectives

## Achievements of the Belgian Antibiotic Policy Coordination Committee (BAPCOC)

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A Belgian Antibiotic Policy Coordination Committee (BAPCOC) was officially established in 1999 by Royal Decree. The overall objective of BAPCOC is to promote judicious use of antibiotics in humans and animals and to promote infection control and hospital hygiene, with the overall aim to reduce antibiotic resistance. BAPCOC fostered strong and interdisciplinary public health, scientific and political leadership, which led to many evidence-based interventions such as multimedia campaigns to promote the prudent use of antibiotics in the community, national campaigns to promote hand hygiene in hospitals, publication of clinical practice guidelines, staffing and technical support for establishment of antibiotic management teams in all Belgian hospitals, surveillance programmes on antibiotic use and resistance in humans and animals and the promotion of research. These activities and interventions resulted in a measurable decrease in antibiotic use and resistance in the community and hospitals.

### Introduction

Belgium is a small federal country with 10.5 million inhabitants living in three regions. In 1999, the Belgian Ministry of Health established by Royal Decree an official committee, called the Belgian Antibiotic Policy Coordination Committee (BAPCOC) [1].

The specific objectives of BAPCOC are to promote judicious use of antibiotics in humans and animals and enhance infection control and hospital hygiene, with the overall aim of reducing antibiotic resistance.

The specific tasks of BAPCOC are to:

- collect information on antibiotic use and resistance to antibiotics in humans and animals;
- publish reports on the evolution of antibiotic use and resistance;
- create awareness of the evolution of antibiotic resistance and the risks for public health;
- publish recommendations on the detection and surveillance of antibiotic resistance, on the appropriate use of antibiotics, on indications for prophylactic and therapeutic use of antibiotics, on the evaluation and the surveillance of antibiotic use in humans and animals, and on the implementation of international recommendations on the prudent use of antibiotics in humans and animals;
- and to publish recommendations on future research into the emergence, spread and control of antibiotic resistance.

**Table**

**Key activities of the Belgian Antibiotic Policy Coordination Committee (BAPCOC)**

| Activity | Budget |
|---|---|
| Multimedia campaigns to promote the prudent use of antibiotics in the community | 400,000 € per campaign |
| National campaigns to promote hand hygiene in hospitals | 125,000 € per campaign |
| Staffing and technical support for establishment of antibiotic management teams in all Belgian hospitals | 3.6 million € each year |
| Publication of clinical practice guidelines | 25,000 € per guideline |
| Publication of guide to antibiotic prescribing in ambulatory care | 100,000 € per guide |
| Surveillance programmes on antibiotic use and resistance in humans and animals | 100,000 € each year |
| Promotion of research e.g.<br>- prevalence of MRSA among nursing home residents<br>- prevalence of MRSA ST398 in pigs and pig farmers | <br>100,000 €<br>150,000 € |
| Support infection control practices (better funding and clear organisation in hospitals) | 3.4 million € additional funding in 2007 |

To address these specific tasks BAPCOC founded the following five multidisciplinary working groups: ambulatory care, hospital care, awareness campaigns, infection control and veterinary medicine. The working groups are composed of microbiologists, infectious diseases' and infection control specialists, epidemiologists, general practitioners (GPs), pharmacists, nurses, veterinarians, basic researchers, public health experts and health economists. The (scientific) secretariat, responsible for their day-to-day management, is hosted by the Federal Public Service Health, Food Chain Safety and Environment, Brussels, Belgium. A Steering Committee, composed of the presidents of the working groups, the chair and vice-chair of BAPCOC, meets monthly. The Steering Committee is responsible for the continuity, interaction and follow-up of initiatives and projects. At plenary meetings of BAPCOC, which are held every four months, the working groups report on their activities so that all stakeholders, including policy makers, scientific organisations, public health institutes are informed about the BAPCOC activities and results.

The key BAPCOC activities and corresponding budgets are listed in the Table. BAPCOC's annual budget in 2007 was 7.8 million EUR. Furthermore, BAPCOC participates in European projects, such as European Surveillance of Antimicrobial Consumption (ESAC; www.esac.ua.ac.be), European Antimicrobial Resistance Surveillance System (EARSS; www.rivm.nl/earss), ABS International (www.abs-international.eu), and e-bug (www.e-bug.eu).

This paper discusses selected examples of the activities and achievements of BAPCOC.

## Public antibiotic awareness campaigns

The BAPCOC working group for public awareness campaigns set the following goals:

- to provide the general public with a better understanding of the natural course of minor and self-limiting infections, such as common cold, acute bronchitis, or influenza;
- to explain when the use of antibiotics is needed, i.e. in case of serious bacterial infections;
- to underline the consequences of emergence of resistance to antibiotics;
- and to facilitate a discussion between patients doctors and pharmacists on the need for appropriate antibiotic use.

No specific target for reductions in antibiotic sales was set.

In December 2000, BAPCOC launched a media campaign which ran over three consecutive winter seasons and concentrated on simple messages that were conveyed through booklets, handouts, posters, prime-time television and radio spots, and websites, like "Use antibiotics less frequently, but better", "Save antibiotics, they may save your life", and "Talk to your doctor, talk to your pharmacist" (www.red-antibiotica.org) [2,3]. The involvement of GPs, paediatricians, pneumologists, ear, nose and throat specialists as well as of retail pharmacists was sought through personalised letters accompanied by campaign materials for presentation to patients. In November 2004, a new media campaign was launched, using the slogan "Antibiotics are ineffective for the common cold, acute bronchitis and flu"; this ran until last winter season (www.antibiotics-info.be). On 18 November 2008, a new media campaign will be launched to mark the European Antibiotic Awareness Day.

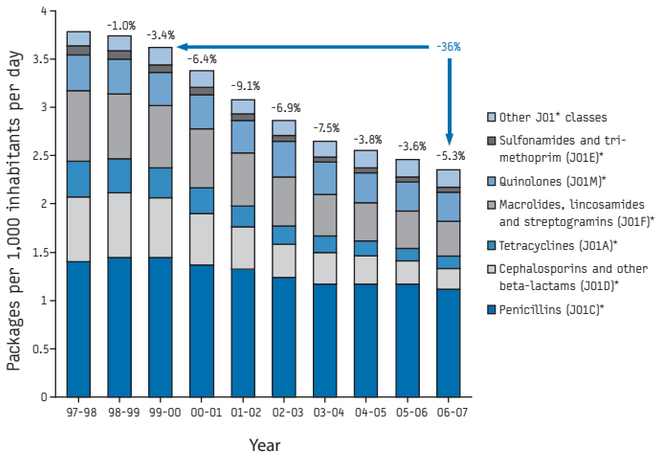
The impact of these activities has been evaluated through pre-and post-campaign face-to face interviews with the public, post-campaign surveys of the GPs, records of antibiotic sales and prescriptions in the retail pharmacies, and evolution of antibiotic resistance among pathogens frequently affecting the community. Outpatient antibiotic use, expressed by the number of reimbursed packages per 1,000 inhabitants per day, decreased by 36% between the winter season 1997-8 and 2006-7 in Belgium (Figure 1) [4]. Penicillin, tetracycline and macrolide resistance in *Streptococcus pneumoniae* increased up to the year 2000, after which it decreased substantially (Figure 2). Similarly, macrolide resistance in *Streptococcus pyogenes* decreased dramatically from 17% in 2001 to 2% in 2007 (Figure 3).

## National hand hygiene campaigns

BAPCOC has organised two countrywide campaigns – in 2005 and in 2007 – for the prevention of nosocomial infections by improving

**Figure 1**

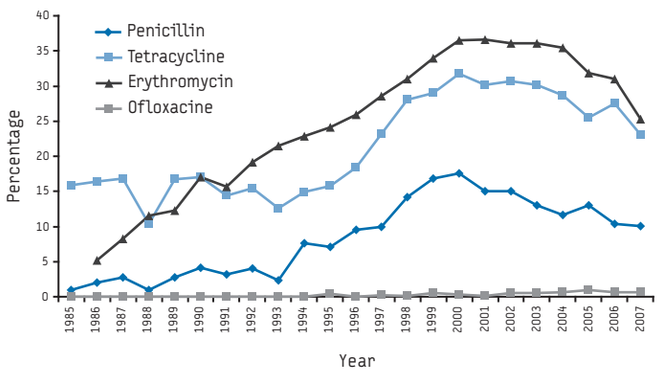
**Outpatient antibiotic use in packages per 1,000 inhabitants per day, Belgium, July 1997 to June 2007**




*Anatomical Therapeutic Chemical (ATC) classification code

**Figure 2**

**Penicillin, tetracycline, macrolide (erythromycin) and ofloxacine resistance in *Streptococcus pneumoniae*, Belgium, 1985-2007**




Number of strains tested varied between 1,218 in 2002 and 1,744 in 2005.
Source: National Reference Centre *S. pneumoniae* (University of Leuven)

hand hygiene compliance in Belgian hospitals. Key components of these campaigns were audit with performance feedback, reminders (posters), educational sessions for healthcare workers, promotion of alcohol-based hand rubs, and patient awareness (folders). Participation, on a voluntary basis, was excellent for both campaigns: 97% for acute care hospitals, 66% for long-term care hospitals and 63% for psychiatric hospitals. Overall compliance with hand hygiene (measured by direct observation) increased significantly from 49% to 69% for the first campaign and from 53% to 69% for the second campaign. The third campaign will be held in December of 2008.

### Antibiotic Management Teams in hospitals

Since the 1990s, there has been a move in Belgian hospitals to establish multidisciplinary antibiotic management teams (AMT) to contain antibiotic resistance and improve antibiotic prescribing. The BAPCOC working group on hospital care developed an implementation plan for this strategy by mobilising federal funding for AMTs and antibiotic managers. In 2002, a pilot project started with a yearly budget of 0.93 million euros for hiring trained antibiotic managers in 37 hospitals, selected from 69 candidate hospitals for their expertise and experience with a local antibiotic stewardship programme. Based on successful activity reports of these pilot hospitals [5], the project was extended to 61 hospitals in 2006 by doubling the financial support to 1.83 million euros and since July 2007 to all acute care hospitals with an annual budget of 3.61 million euros. The AMTs will remain in the BAPCOC programme and their tasks have been defined by Royal Decree. BAPCOC provides scientific support to the participating hospitals by means of a dedicated training course, national study days, standardised evaluation of local progress reports and national surveillance of antibiotic consumption in hospitals. In the years 2002-7, over 600 hospital pharmacists and physicians participated in BAPCOC-supported interuniversity teaching courses in antibiotic management. National workshops were held twice to share good practices between hospital AMTs. Analysis of the recent reports of the 61 pilot phase hospitals clearly demonstrates general adoption of well- established quality improvement interventions, such as an antibiotic formulary, guidelines for prophylactic and therapeutic antibiotic use, regular analysis of local antibiotic consumption and resistance profiles, and increasing conductance of clinical audits and drug use evaluations. In a recent international survey of structural indicators of antibiotic stewardship programmes, Belgian hospitals scored high on average (3.75/5). The survey showed that 90% of hospitals had key structural resources and tools in place for effective antibiotic management and infection control [6].

The impact of these activities in hospital infection control and antibiotic stewardship is monitored through a newly developed surveillance of hospital-anti-infective drug consumption as well as by several longstanding surveillance schemes of nosocomial infection and multidrug resistant pathogens coordinated by the Scientific Institute of Public Health. These include methicillin-resistant *Staphylococcus aureus* (MRSA), extended spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae* and pathogenic *Clostridium difficile*. An example of encouraging results is the 35 % relative reduction since 2004 in the incidence of nosocomial acquisition of MRSA among patients admitted to acute care hospitals [7].

### Publication of guidelines and a guide for appropriate use of antibiotics

BAPCOC, in collaboration with scientific experts and organisations, produced evidence-based guidelines for the appropriate use of antibiotics in hospitals and ambulatory care for important infectious disease syndromes such as acute sore throat, otitis media, sinusitis, community-acquired pneumonia, uncomplicated and complicated urinary tract infections. All BAPCOC guidelines were disseminated free of charge to all relevant physicians (GPs and/or specialists) in Belgium. The guideline recommendations for ambulatory care were supplemented by conclusions of systematic literature reviews. BAPCOC also produced a booklet in 2004 on antibiotic treatment of community-acquired infectious diseases. All Belgian GPs received a copy of this first antibiotic booklet in 2004. A copy of the second edition of this antibiotic guide will be distributed among all primary care physicians in November 2008. The impact of this antibiotic guide for outpatients is monitored based on antibiotic prescribing in the community by indication, age and class of antibiotics.

### Surveillance programmes on antibiotic use and resistance in humans and animals

BAPCOC provides support and additional funding for surveillance of MRSA, vancomycin-resistant enterococci (VRE), ESBL-producing *Enterobacteriaceae*, *S. pneumoniae* and *S. pyogenes*. BAPCOC also supports surveillance programmes on antibiotic use in hospitals, such as point prevalence surveys. The results of these surveillance programmes are published in an annual report, published by the Public Health Institute in Brussels.

### Research projects funded by BAPCOC

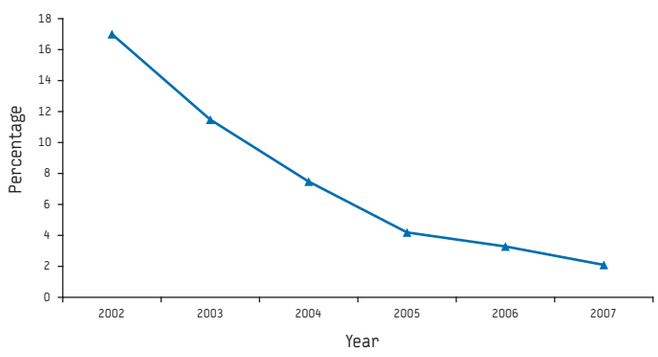
BAPCOC funded a number of research projects to provide scientific evidence for national policies and guidelines of which examples are given below.

#### National survey of prevalence of MRSA carriage among residents of nursing homes

BAPCOC funded the first national survey of prevalence of MRSA carriage among residents of nursing homes in Belgium. The objectives of this survey were to study the national prevalence of MRSA colonisation in Belgian nursing home residents, to identify risk factors for MRSA carriage among nursing home residents, in respect of both the individual resident and of the nursing home

**FIGURE 3**

**Macrolide (erythromycin) resistance in *Streptococcus pyogenes*, Belgium, 2002 to 2007**




Number of strains tested varied between 1298 in 2002 and 934 in 2007.
Source: National Reference Centre *Streptococcus pyogenes* (University of Antwerp)

facility, and to study the molecular epidemiology and susceptibility of MRSA strains isolated from residents living in these facilities. Based on a representative sample of 3,000 residents in 60 institutions, a prevalence of 19% carriage was noted (unpublished data). Importantly, 90% of MRSA carriers found in the survey were not identified as such by the nursing and medical personnel in spite of routine MRSA screening on transfer from hospitals and a discharge letter being sent by the hospital, in the majority of cases, to the participating nursing homes at the time of patient referral. A better understanding of the MRSA reservoir in nursing homes and analysis of risk factors permitted the adaptation of an MRSA control policy. National guidelines to prevent the spread of MRSA in nursing homes have been developed and the impact of these guidelines on the evolution of MRSA carriage in nursing homes will be evaluated by a new prevalence survey in the near future.

**Prevalence survey of MRSA in swine farmers in Belgium**

BAPCOC funded a survey on MRSA in swine farmers and their household contacts, to determine if MRSA strains in those farmers are related to those in swine, to characterise and compare phenotypes and genotypes of MRSA strains from humans and swine, and to study risk factors for MRSA colonisation and assess levels of personal hygiene in farm workers. Extensive colonisation with two subtypes of the livestock-associated ST398 MRSA strain was found in swine (44% carriers from 68% of farms) and farmers (38%) in contact with swine and other animals. Reported use of personal protection equipment and decontamination showed no difference in the rates of colonisation [8]. As a result of this survey, a National MRSA Med Vet Task Force was established to coordinate further investigation of the epidemiology of MRSA in animals and persons in contact with animals and to develop guidelines for risk management.

**National investigation on the infection control practices in surgery**

BAPCOC funded and supported a national investigation on infection control practices in operating rooms. More than half of all acute care hospitals participated in a national inventory evaluating the extent to which internationally suggested infection prevention precautions are defined, carried out and followed-up. A list of essential precautions was grouped into different categories: architecture and structure, environmental cleaning, peri-operative procedures, sterilisation, logistic activities and surveillance of postoperative wound infections.

For each essential item, participants reported the estimated degree of compliance in their institution, the existence of a standard operating procedure (SOP) for the performance for this precaution, and finally whether the respective items are written down in institutional guidelines or procedures. The investigation clearly demonstrated the extensive variability in infection control practices in Belgian operating rooms, with respect to standards described in institutional operating procedures as well as to actual compliance with local and/or (inter)national precaution measures. The results are detailed in an advisory document reported to the authorities, stating the necessity of quality control standards implementation and official follow-up of procedures regarding peri-operative infection control.

## Conclusions

Our experience demonstrates that strong joint and interdisciplinary public health, scientific and political engagement in Belgium led to many evidence-based interventions, aimed at both the general public and healthcare professionals and those interventions in return resulted in a decrease in antibiotic use and resistance in the community and hospitals. They also show that creating awareness for the factors driving antimicrobial resistance and providing a knowledge base for physicians, public health experts and scientists is crucial in containing antibiotic resistance. A number of scientific conferences and public health workshops organised by BAPCOC were helpful in this respect.

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# Perspectives

## Experiences in prevention and control of antibiotic resistance in Slovenia

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During 1991-1999 a significant increase of consumption of macrolides and fluoroquinolones was observed in Slovenia, and this was associated with significant increase of resistance of *Streptococcus pneumoniae* and *Streptococcus pyogenes* to macrolides and *Escherichia coli* to fluoroquinolones, respectively. Between 1999 and 2007 the prevalence of *S. pneumoniae* resistant to erythromycin increased from 3.7% to 16.8% even though the use of macrolides in the same period decreased from 3.81 to 2.43 defined daily doses (DDD) per 1,000 inhabitants and per day. The co-resistance and the spread of resistant clones were the reason for constant increase in macrolide resistance. Slovenia is one of the few European countries with decreasing prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in hospital care during the last years. As a result of control measures introduced in 1999, the MRSA prevalence rates decreased from 21.4% in 2000 to 8.3% in 2007.

### Background

Slovenia is a small central European country with over two million inhabitants [1]. The country has a centralised compulsory Bismarck-style* health insurance system, which is administered by the Health Insurance Institute of Slovenia and includes almost all inhabitants (>99%). However, approximately 1.4 million residents also have supplementary health insurance provided by three private insurance providers. Prescription is needed for every antibiotic purchase, and in human medicine antibiotics may only be prescribed by physicians. The consumption of antibiotics in ambulatory care has been monitored in Slovenia since 1974, and the consumption of antibiotics in hospital care has been monitored since 1985. Since 2000 Slovenia has participated in EARSS (European Antibiotic Resistance Surveillance System) and since 2001 in ESAC (European Surveillance of Antibiotic Consumption) projects.

Resistance to antibiotics is a global public health problem. Selective antibiotic pressure and transferable resistance (clonal spread or horizontal resistance genes transfer) are major determinants of resistance development. It is irrefutable that the use of antibiotics promotes resistance. In this paper we describe the experiences in prevention and control of antibiotic resistance in ambulatory care, focusing on methicillin-resistant *Staphylococcus aureus* (MRSA) in hospital care.

### Ambulatory care

In Slovenia the increased use of antibiotics in ambulatory care during the 1990s was associated with increased resistance of some respiratory infection pathogens. An increase in macrolide prescriptions by 3.5 between 1991 and 1996 was associated with significant increase in macrolide resistance in *Streptococcus pyogenes* and non-invasive *Streptococcus pneumoniae* between 1994 and 1997 [2]. Between 1994 and 1999, the macrolide consumption increased twofold, from 1.89 to 3.84 defined daily doses (DDD) per 1,000 inhabitants and per day, and at the same time the macrolide resistance in *S. pyogenes* increased from 0 to 7.4% and of non-invasive strains of *S. pneumoniae* from 0 to 9% [3]. The outpatient consumption of fluoroquinolones increased by 2.5, from 0.59 DDD per 1,000 inhabitants and per day in 1992 to 1.50 DDD per 1,000 inhabitants and per day in 1999 and this was paralleled by an increase of resistance of *Escherichia coli* to ciprofloxacin in adult patients from 3.6% in 1996 to 9.2% in 1999 [4].

Seeing that the resistance of some pathogens (*S. pneumoniae*, *S. pyogenes*, *E. coli*) had been increasing constantly, interventions were introduced to decrease their prevalence in the community. In June 2000, based on suggestions from infectious disease specialists, the Health Insurance Institute of Slovenia introduced administrative restrictive measures for the prescription of amoxicillin and clavulanic acid (co-amoxiclav) and fluoroquinolones [5]. Co-amoxiclav could no longer be prescribed for patients with *S. pyogenes* infections diagnosed clinically or documented microbiologically. Fluoroquinolones could only be prescribed as an alternative treatment for therapy of acute respiratory and urinary tract infections after clinical failure of first-choice antibiotics, or on the basis of tests showing susceptibility to fluoroquinolones and resistance to first-choice antibiotics [5]. In May 2004, the prescription of respiratory fluoroquinolones (moxifloxacin, levofloxacin) was modified. They could be prescribed in an unrestrictive manner only for severe community-acquired pneumonia and chronic obstructive pulmonary disease and when respiratory infection with resistant pathogens was expected. The staff of Health Insurance Institute of Slovenia checked the implementation of restrictive interventions by controlling individual prescriptions of physicians.

In Slovenia the overall consumption of antibiotics in outpatients decreased during the period between 2000 and 2007 by 20.32% [unpublished data]. However a greater decrease was observed for restricted than for non-restricted antibiotics (27.7% vs. 16.1%) [unpublished data]. This result shows that restrictive intervention can be efficient. To date a focused campaign directed at public and health care professionals has not been organised.

A detailed analysis of the causes and consequences of decreased antibiotic consumption over five years (1999-2003) showed a positive correlation between antibiotic consumption and repeated media reports and a negative correlation with the number of rapid diagnostic tests (C-reactive protein test (CRP), streptococcal antigen tests) [5]. Professional communication (scientific articles) and media reports for general public (lay articles) showed small negative correlation with antibiotic consumption. No increase in mastoiditis cases was observed in spite of reduced antibiotic consumption [5]. Reduced antibiotic consumption was paralleled by a decrease in penicillin resistance among invasive pneumococci and lower costs of antibacterials for systemic use. In contrast, reduced macrolide resistance rates of *S. pneumoniae* and *S. pyogenes* was not observed despite the 21.3% decline of total macrolide use during the period 1999 – 2004 [6].

A recent analysis showed that the prevalence of erythromycin resistance among invasive *S. pneumoniae* isolates increased from 3.7% in 1999 to 16.8% in 2007 in spite of a decrease of consumption of macrolides by 36.3% in the same period (from 3.81 to 2.43 DDD per 1,000 inhabitants and per day) [7]. The resistance increased almost eightfold among isolates from children (from 3.1% in 1999 to 24.6% in 2007). The most likely explanation for the continuous increase in macrolide resistance was co-resistance and the spread of resistant clones. The most frequent co-resistance pattern in the erythromycin-resistant strains of invasive *S. pneumoniae* isolates with erm(B) gene was resistance to penicillin, tetracycline and trimethoprim-sulfametoxazole.

To decrease the resistance of respiratory infection pathogens to macrolides, measures to reduce the use of macrolides especially new ones (by educational and/or restrictive interventions) and/or the introduction of conjugated pneumococcal vaccine are being discussed.

In addition, according to EARSS data the prevalence of resistance of *E. coli* to quinolones doubled (from 8.5% to 17.4%) between 2001 and 2007 in spite of reduced (15%) use of fluoroquinolones in the community (from 1.3 to 1.11 DDD per 1,000 inhabitants and per day) [8]. This data shows the complex correlation between antibiotic use and antibiotic resistance and indicates that the reduction of antibiotic use alone does not guarantee that lower prevalence of resistance can be achieved.

## Hospital care

In 2008 Slovenia has 29 hospitals including two teaching hospitals, 10 general hospitals and 14 specialised hospitals providing orthopedic (1), pulmonary (2), gynecological (2), psychiatric (5), nursing (1), rehabilitation (2) and oncology (1) care, and three hospitals providing diagnostic or surgical procedures. All but three hospitals providing diagnostic or surgical procedures are state owned. In this section we focus exclusively on MRSA as an example to control antibiotic resistant bacteria. Slovenia is one of the few European countries which succeeded to reduce the prevalence of MRSA. In the period from 2000 to 2007 the prevalence decreased from 21.4% to 8.3% (by 61.3%).

In 1997 and 2001 two point prevalence studies showed high (75% and 60%) prevalence of MRSA in the adult intensive care units (ICU) in Slovenia [9]. Also a national point prevalence study of hospital-acquired infections in acute care hospitals in 2001 showed high (61.8%) prevalence of MRSA [10]. After the high prevalence of MRSA in Slovenia had been recognised two studies showed that with the comprehensive infection control program the number of MRSA cases could be decreased. In the first study the proportion of MRSA cases acquired in the hospital decreased from 50% to 6.1% during 1999 – 2002, and in the second the incidence of ICU-acquired MRSA decreased from 7.8% to 1.9% [11,12]. The legislation and regulation of the infection control program in health care institutions published in 1999 and the audit of infection control implementations in health care institutions published in 2006 also had an impact on the decline of the prevalence of MRSA in Slovenia [13,14].

Currently, the components of Slovenian strategy for MRSA control are:

- active surveillance – selective screening for MRSA in patients at risk of carrying MRSA on admission;
- contact isolation of patients with MRSA (not always possible because of single bedroom shortage);
- promotion of hand hygiene – use of alcohol-based hand rub;
- selective decolonisation;
- improved communication (reporting) about patients with MRSA within and between health care facilities;
- continuous education of health care workers (HCW) on appropriate hygiene procedures in health care institutions (hospitals, nursing homes);
- use of hospital computer system to record MRSA carriers;
- education of professionals (postgraduate educational courses have been organised by the Medical Faculty in Ljubljana since 1984 (162 physicians and 290 nurses have participated till 2008), national scientific meetings;
- education of patients (newspapers, magazines, TV, leaflets);
- spread of information to media and politicians.

The greatest obstacle for a further decrease in the prevalence of MRSA is the shortage of single-bed rooms and staff.

Notwithstanding the success in decreasing the prevalence of MRSA, a new resistant pathogen has emerged recently, the vancomycin-resistant enterococcus (VRE). Before the year 2005 according to EARSS data all invasive strains of *Enterococcus faecium* isolates had been susceptible to vancomycin [8]. In 2006, 6% and in 2007, 4.6 % of *E. faecium* invasive isolates were found to be resistant to vancomycin. The main source of resistant isolates are patients hospitalised in one haematological department in a tertiary care centre. In this department not enough single- or double-bed rooms are available, so the contact precautions are not possible to be provided.

In recent years higher use of linezolid was followed by the emergence of linezolid resistant enterococci (LRE) [unpublished data].

## Conclusion

In Slovenia, antibiotic resistance is a problem in outpatient as well as hospital settings [8]. In order to combat antibiotic resistance, antibiotic policy and infection control are needed. Despite reduced use of all antibiotics including macrolides and fluoroquinolones in outpatient care, resistance of *S. pneumoniae* to macrolides and *E. coli* to fluoroquinolones is still increasing. Hospital consumption of antibiotics in Slovenia is moderate and stable and we have observed a decrease in MRSA prevalence probably due to better infection control [8,15]. However the emergence of VRE isolates has become an increasing problem in the last three years. Reducing prevalence of resistance is a difficult task; apart from prudent antibiotic use and better infection control it requires many other sustained interventions.

## Acknowledgements

Thanks are due to Mrs Simona Rojs for technical support and Živa Čižman for grammatical review of the manuscript.

*Note: Bismarck model healthcare systems are systems based on social insurance, where there is a multitude of insurance organisations (e.g. Krankenkassen) that are organisationally independent of healthcare providers.

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# Perspectives

## Improvements in antibiotic prescribing by community paediatricians in the Czech Republic

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Repeated surveys among primary care paediatricians were performed annually from 1998 to 2002 in the Czech Republic. The task was to assess the prescription of antibiotics in treatment of respiratory infections in children. The results were evaluated in the light of existing guidelines and conclusions were used in a number of interventions aimed at reducing the inadequate use of antibiotics and hence preventing the potential increase of the antibiotic-resistant bacteria. In addition, data on overall consumption of antibiotics in outpatient care and trends in the prevalence of resistant strains of *Streptococcus pneumoniae* and *Streptococcus pyogenes* are discussed.

### Introduction

The centrally regulated healthcare system with limited financial resources, existing in the former communist Czechoslovakia, resulted in a low level of antibiotic consumption and rare occurrence of antimicrobial resistance. Despite the obvious defects of the system, some remarkable activities established during this period have proved beneficial from the long-term perspective. One of these was the establishment of "antibiotic centres" in the 1970s which contributed to promoting the prudent use of antibiotics. These organisational units were incorporated into the clinical microbiology departments and made responsible for local surveillance of antimicrobial resistance and supervision of the use of restricted antimicrobials. The network of antibiotic centres has remained active until the present time and currently represents a local structure ready to use for the organisation of systematic interventions.

A significant shift in antibiotic consumption was observed in the early 1990s, after the political changes. The most significant change was documented from 1992 to 1994, when the privatisation of primary and outpatient care took place, and sophisticated marketing of pharmaceutical industry was introduced as a new phenomenon. In this period, the total ambulatory consumption of antibiotics increased from 14 to 20 defined daily doses (DDD) per 1,000 inhabitants and per day, and at the same time also the proportion of second-line antibiotics increased significantly although prescribing of these costly drugs was not appropriate in the existing epidemiological context [1]. The first warning signs of growing antimicrobial resistance among pathogens in the community were recorded a few years later. The earliest signal was associated with the rapidly increasing resistance of *Streptococcus pyogenes* to macrolides [2], which was probably due to changes in prescribing habits in primary paediatric care.

In these changed conditions, the application of new methods and innovative tools to prevent antibiotic resistance was needed. We describe a series of multicentre interventions, including annual surveys, aimed at identifying the diagnostic and prescribing habits and promoting the prudent use of antibiotics among participating paediatricians. In addition, we present data on overall ambulatory antibiotic consumption and antimicrobial resistance available from other sources.

### Methods

#### Survey of antibiotic prescriptions in paediatric care

Annual surveys on outpatient antibiotic prescriptions in the treatment of respiratory infections in children were conducted among primary care paediatricians between 1998 and 2002. The surveys were organised every year during four weeks in November, with one week of follow up. The surveys in 1998, 1999 and 2000 were held in two Prague districts. The surveys in 2001 and 2002 were multicentre and involved 13 different districts across the Czech Republic. Participation in the survey was voluntary. Paediatricians were invited through regional coordinators of the Czech Society of Primary Care Paediatricians. Training sessions for participants were held before each annual survey: first centrally organised "training of trainers" later followed by local training seminars for participants in all regions. Two coordinators of the survey were established in every region including one expert from the antibiotic centre and one experienced paediatrician.

The questionnaire used in the survey focused on diagnostic and therapeutic approaches to the management of acute respiratory infections in children. The case definitions, criteria of appropriateness of antibiotic use and definitions of second-line antibiotics were adopted according to the national guidelines for antibiotic therapy in primary and ambulatory care issued, updated and disseminated by the Subcommittee on Antibiotic Policies of the Czech Medical Association [4]. Participating paediatricians were asked to fill in one questionnaire for each patient presenting with acute respiratory illness, irrespective of the prescription of antibiotics. Requested information included diagnostic approaches,

such as indication of laboratory or other diagnostic tests (e.g. throat swab culture, C-reactive protein test, X-ray examination). Aetiology of particular case of respiratory illness was presumed on the basis of clinical diagnosis. Computer software tools were developed for processing of questionnaires and validation and analysis of the data. The survey results were processed and communicated as individual, local and aggregated.

**Interventions, educational activities**

Feedback based on the results of the repeated surveys was the most important intervention tool used to promote good prescribing habits among participating doctors. This was accomplished by dissemination of printed survey results to individual doctors. In addition, a final conference and local seminars in regions were organised for participants to explain the obtained prescribing parameters. A comparison of individual approaches with a defined good practice was made.

Information obtained from the surveys was also used in educational activities organised by the Society for Primary Care Paediatricians, postgraduate training courses for all medical professionals and press conferences aimed at the general public.

**Surveillance of ambulatory antibiotic consumption**

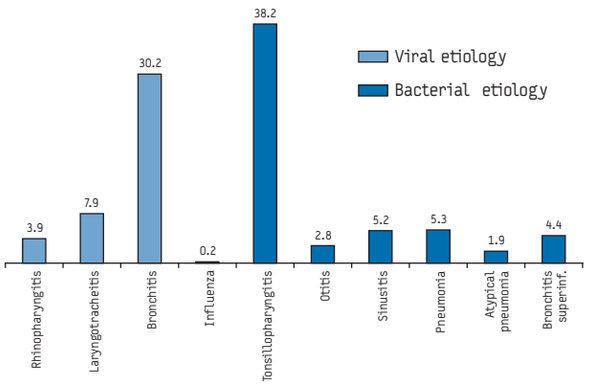
We used data on ambulatory antibiotic consumption that is reported yearly to the European Surveillance of Antimicrobial Consumption (ESAC). It is calculated on the basis of information obtained from health insurance system. Data obtained from this source are processed by the national ESAC co-ordinator according to the methods agreed by the WHO Collaborating Centre for Drug Statistics Methodology and ESAC [3]. Only aggregated data on the overall ambulatory consumption of antibiotics are available. Detailed sorting of the data according to a geographical area or medical specialisation is not possible.

**Surveillance of antimicrobial resistance in community**

Monitoring of the resistance of bacterial pathogens causing respiratory infections in the community has been organised by the National Reference Laboratory for Antibiotics since 1996. About 50 antibiotic centres are involved in this periodic surveillance every year. It takes place during the last quarter of the year and in 2007 covered a catchment population of about 80%. Every centre provides susceptibility data of 100 consecutive isolates of *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Streptococcus pyogenes* from clinically relevant samples.

FIGURE 1

**The proportion of antibiotic prescriptions in treatment of community-acquired respiratory tract infections in children, by clinical diagnosis. Output of multicentre survey of antibiotic use in primary paediatric care in the Czech Republic in 2001**




## Results

**Survey of antibiotic prescriptions in paediatric care**

The repeated surveys identified the respiratory infections most frequently diagnosed as the cause of patients' visits to primary care paediatricians and revealed the doctors' prescribing habits in the context of particular clinical diagnosis [5,6]. The number of participating doctors varied from year to year: 13 took part in 1998, 23 in 1999, 28 in 2000, 114 in 2001 and 57 in 2002. The numbers of registered visits/cases of acute respiratory illnesses were the following: 3,707/3,006 (1999), 4,230/3,273 (2000), 31,077/19,013 (2001), 14,801/9,373 (2002). The numbers of visits per practitioner were comparable.

In the first survey in 1998 only information on cases in which antibiotics were prescribed was collected. In the following surveys (1999-2002) all cases of acute respiratory infections were documented including those in which no antibiotics were administered.

Here we present the results of the largest and most representative multicentre survey performed in 2001, but similar output was obtained in other survey years as well. In the 2001 survey, 19,013 acute respiratory illness cases were registered by the participating paediatricians with the following clinical diagnoses: rhinopharyngitis (56.3% of the cases), laryngotracheitis (13.8%), bronchitis (16.8%), influenza (1.9%), tonsillopharyngitis (18.3%), otitis (2.2%), sinusitis (2.4%), pneumonia (1.9%), atypical pneumonia (0.7%), bronchitis-bacterial superinfection (1.5%). Several visits with different diagnoses could be linked to one case which is why the sum of these percentages exceeds 100%. We estimated that in 42.2% of the cases in which antibiotics were prescribed, the prescriptions were issued inappropriately for an inadequate treatment of predominantly viral illnesses. Prescribing preferences according to clinical diagnosis are described in Figure 1. Acute bronchitis and laryngotracheitis represented 38.1% of all indications for antibiotic treatment. This percentage indicates a large proportion of overuse and the opportunity for improvement. The treatment was initiated using second-line and more expensive drug in 47.2% of all cases in which antibiotics were prescribed while the first choice and mostly cheaper antibiotic recommended in an official guideline was not used.

**Interventions, educational activities**

Taking part in the survey and receiving feedback seems to have positively influenced the prescribing habits of participating physicians. The 57 doctors who participated in the survey in both 2001 and 2002 and reported a significant number of cases of acute bronchitis and laryngotracheitis, had changed their prescribing habits and considerably reduced the use of antibiotics in inadequate indications (Figure 2). A rapid improvement of prescribing preferences in the treatment of acute tonsillopharyngitis of 10 doctors participating in surveys in 1998, 1999 and 2000 is shown in Figure 3 as another example of the interventional effect.

Based on the results of the surveys, half-day seminars focused on prudent use of antibiotics in paediatrics were organised in all

regions of the Czech Republic during 2002, in the framework of official educational activities of the Society for Primary Care Paediatricians. The information obtained from the surveys was disseminated via this multicentre educational event to all interested paediatricians. Easy to understand messages explaining the priorities for improvement and ways to reach better prescribing practices were communicated.

In addition, since 2002, interdisciplinary training courses on the use of antibiotics have been regularly organised by the Institute for Postgraduate Medical Education.

No specific financial resources were available for the preparation of systematic public campaigns. Nevertheless two press conferences specifically focused on the overuse of antibiotics and on the threat of antimicrobial resistance were held in 2002 and 2003.

**Trends in ambulatory antibiotic consumption**

The quantitative trends in outpatient consumption of antibiotics in the Czech Republic are shown in Figure 4. In the early 1990s, the total antibiotic consumption was approximately 14 DDD per 1,000 inhabitants and per day, with penicillins predominating. An increase of consumption to 20 DDD per 1,000 inhabitants and per day occurred during 1994, and remained at that level till 2002.

A qualitative change was observed from 1990 to 1994: the proportion of penicillins decreased giving way to macrolides (increase from 0.33 to 1.75 DDD per 1,000 inhabitants and per day), aminopenicillins with beta-lactamase inhibitors (0.01 to 1.4 DDD per 1,000 inhabitants and per day) and cephalosporines (0.16 to 1.04 DDD per 1,000 inhabitants and per day). Subsequently, in

**Figure 2**

**The proportion of antibiotic prescriptions in treatment of predominantly viral respiratory tract infections in children. Comparison of results of repeated surveys including 57 primary care paediatricians in the Czech Republic in 2001 and 2002**

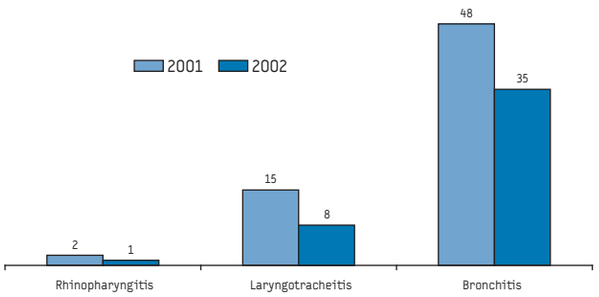



**Figure 4**

**Trends in ambulatory antibiotic consumption, Czech Republic, 1989 - 2007**


Source of data: 1989-2002 [1], 2003-2006 [3], 2007 - preliminary data
DID - Defined daily doses per 1,000 inhabitants and per day

**Figure 3**

**The proportion of various classes of antibiotics prescribed in treatment of acute tonsillopharyngitis in children. Comparison of results of repeated surveys including 10 primary care paediatricians, in the Czech Republic in 1998, 1999 and 2000**

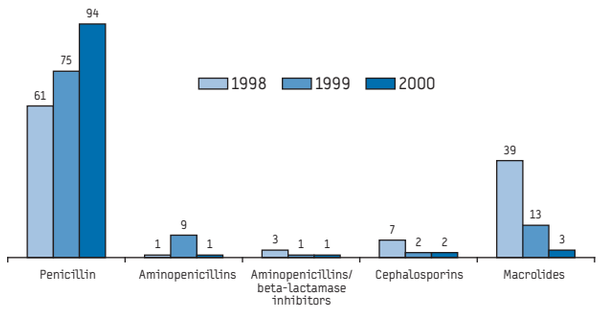
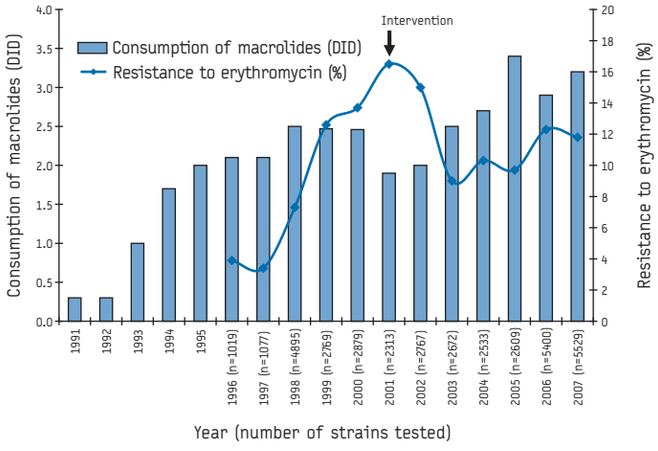



**Figure 5**

**Total consumption of macrolides in the community and resistance of *Streptococcus pyogenes* to erythromycin, Czech Republic, 1991-2007**




Note: The arrow indicates the beginning of official educational activities in prudent use of antibiotics in paediatric care organised by the Czech Medical Association
Source of data on consumption: 1989-2002 [1], 2003-2006 [3], 2007 - preliminary data.
DID - Defined daily doses per 1,000 inhabitants and per day

2003, a significant decrease of the overall antibiotic consumption was observed, however its qualitative structure remained inappropriate (high consumption of aminopenicillins with beta-lactamase inhibitors, macrolides and fluoroquinolones) [1,3].

**Trends in antimicrobial resistance in community**

The resistance of some bacterial pathogens causing community acquired respiratory infections has remained low in the past years. This is namely true for *Streptococcus pneumoniae*. The strains causing invasive infections [7], as well as respiratory isolates are mostly fully susceptible to penicillin, intermediate susceptibility is present only in 4-5% of the isolates, and a high-level resistance is extremely rare. The resistance of invasive isolates to erythromycin is one of the lowest in Europe [7]. The situation is different for *Streptococcus pyogenes*. A rapid increase of resistance to erythromycin from 3% to more than 16% was observed between 1996 and 2000. This dangerous trend was interrupted and the rates fell back to 9% in 2002-2003 [2]. A decrease in macrolide consumption during 2001-2002 was probably in the background of this phenomenon. However, from 2003, the consumption has been rapidly growing again, which is likely to cause increase in resistance.

## Discussion

The fact that more than half of the patients' visits to paediatricians are due to common cold (56% reported in 2001 survey) was surprising. At the same time, antibiotic prescription for common cold was rare. Nevertheless it is important to note that frequent visits due to this self-limited illness induce unnecessary overloading of paediatricians, when time and resources could be used to address more serious problems, including careful explanation of non-antibiotic management of viral illnesses to the patients and the parents.

In the surveys, the overuse of antibiotics for viral respiratory illnesses was especially marked in case of febrile, coughing children with diagnosis of acute bronchitis or laryngotracheitis. This clinical picture has a strong psychological impact influencing the decisions of doctors as well as treatment expectations of parents. Meta-analyses arguing in favour of non-antibiotic management of these diseases were available already during the 1990s but the traditional approach previously recommended antibiotic treatment.

The differentiation between bacterial and viral aetiology of acute tonsillopharyngitis and otitis media was not done in the surveys described here. However, an important fraction of these illnesses is caused by viruses and this fact represents another chance for improvement of diagnostics and antibiotic prescribing.

Our results clearly identified priorities for systematic interventions with a potential to eliminate about 40% of total antibiotic prescriptions in primary paediatric care. The influence of the repeated surveying showed good example of real improvement.

The limited availability of structured ambulatory consumption data makes it impossible to carry out a detailed analysis of antibiotic usage for particular medical specialities (primary care paediatricians, general practitioners for adults, outpatient specialists), for smaller geographical areas (regions, districts), and for the routine evaluation of prescribing patterns of individual doctors. Consequently, the impact of interventions is difficult to analyse, and the basis for routine performing of systematic activities are incomplete. This barrier seems to be an important obstacle in persuading health insurance companies to organise nationwide interventions.

No change in the healthcare reimbursement system was made which could have contributed to the decrease of antibiotic consumption observed from 2003. The change from "fee for service" to "per capita" payment was carried out for primary care in 1997, surprisingly without any influence on high ambulatory consumption of antibiotics during the late nineties. No significant differences in the occurrence of acute respiratory illnesses were observed in association with decreasing antibiotic consumption before or after 2003.

The decrease in the resistance of *Streptococcus pyogenes* to erythromycin seems to be in correlation with a time-limited decrease in macrolide consumption which may be at least partially attributed to the performed surveys and interventions (Figure 5). Nevertheless, more detailed analyses are needed to identify the reasons behind these resistance trends. Only a short interruption of the increasing macrolide consumption (2001-2002) indicates a limited sustainability of the outcome of performed interventions, which should be long lasting and nationwide. Otherwise, drug marketing can easily overcome its effectiveness.

The echo of press conferences has remained quite long lasting. There are more opportunities to address the general public in the media, including radio and television, in comparison with the previous period. The journalists seem to be still interested in this topic which facilitates the organisation of the first European Antibiotic Awareness Day this year.

**Conclusions and further developments**

The results of limited interventions performed in connection with the annual voluntary surveys among paediatricians in the Czech Republic provided good background for the planning of systematic nationwide activities focused on the prudent use of antibiotics and control of antimicrobial resistance in the community. The expectations regarding potential savings of the budget of the public health insurance system are fully justified. A specialised training of regional coordinators supporting good prescribing practice has been running since 2005 and a pool of competent professionals has been constantly growing. However, we are now at a starting point for further developments when routine operations of the existing know-how require regular support from the government and health insurance companies.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# Perspectives

## Strama - a Swedish working model for containment of antibiotic resistance

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The overall aim of Strama (The Swedish Strategic Programme Against Antibiotic Resistance) is to preserve the effectiveness of antibiotics in humans and animals. Strama is organised at two levels: a network of independent local multidisciplinary groups in each county that provide prescribers with feedback on antibiotic use and resistance and implement guidelines; and a national executive working group funded by the government. To gain an insight into antibiotic use, Strama has conducted several large diagnosis-prescribing surveys in primary care, in the hospital settings and in nursing homes. National antibiotic susceptibility data for Sweden and mandatory notification show that in recent years the proportion of *Streptococcus pneumoniae* with decreased sensitivity to penicillin V has stabilised (around 6 %), but the number of notified cases of meticillin-resistant *Staphylococcus aureus* (MRSA) has increased and ESBL-producing *Enterobacteraceae* have turned into an endemic situation. Still, Sweden is among the countries with the lowest rates of MRSA (<1 %), *S. pneumoniae* can still be treated with penicillin V and the rate of *Escherichia coli*-producing ESBLs is below 5 %. Strama´s activities have contributed to a steady decrease in antibiotic use from the mid 1990s until 2004 (when total use slowly started to increase again) without measurable negative consequences. Regular collaboration with national and regional news media has been one of the key strategies.

### Background

Increasing use of antibiotics and spread of penicillin-resistant pneumococcal clones in the beginning of the 1990s alarmed the medical profession and authorities in Sweden. Strama (The Swedish Strategic Programme against Antibiotic Resistance) started as an informal network between experts and authorities in 1994. In 2000, Strama, in close cooperation with the National Board of Health and Welfare, prepared a proposal for a national action plan to contain antibiotic resistance [1]. This proposal was later developed into a governmental bill "Strategy to prevent antibiotic resistance and health-care associated infections" [2], which was passed in 2006. Since then Strama has been institutionalised as an independent governmental body with an annual budget of 10 million Swedish crowns from the Ministry of Health and Social Affairs. Recently, a corresponding Strama VL (Veterinary and Food) coordinated by the National Veterinary Institute has been inaugurated.

The overall aim of Strama's activities is to preserve the effectiveness of antibiotics in humans and animals. Strama is organised at two levels: local groups in each county and a national executive working group funded by the government. Detailed overviews of the efforts to contain antibiotic resistance in Sweden and of the systems for the surveillance of antibiotic consumption and antibiotic resistance have been published [3-5].

### Local Strama groups

Strama developed as a network with nodes of independent local groups coordinated by each county department for communicable disease control. The local groups are the drivers of Strama activities. These local groups usually comprise specialists in communicable diseases, infectious diseases, clinical microbiology, infection control, general practice and pharmacy. Paediatricians as well as ear, nose and throat specialists are common additional members. In most counties there is a close link to the local drug and therapeutics committee.

The guiding principle underlying local Strama activities is to promote the rational use of antibiotics by providing prescribers with feedback on local or individual data on prescription for comparison with other prescribers and prevailing therapy recommendations. Local data on antibiotic resistance is provided by the clinical microbiology laboratory. Other important activities are to develop local therapeutic guidelines and to organise courses and lectures for local physicians and other health-care workers at different levels of training. While initially focussing on general practice, parallel groups targeting hospital care were recently developed in an increasing number of counties and regions.

There is no formal reporting on either activities or on budgets from the local groups to the national level. Data from local projects are shared and discussed at national meetings at least once annually. A problem is that many of the local activities rely on personal commitment from devoted physicians and that a formal mandate and financial support from the county council is still missing in many counties. However, the awareness of a need for such targeted and mandated activities to contain antibiotic resistance is slowly increasing and a growing number of Strama groups (or equivalent bodies) are now supported.

### Strama at national level

While the local groups coordinate activities targeting local prescribers, the national executive working group is responsible for national coordination of information and meetings, initiating studies in areas where knowledge gaps have been identified, disseminating Strama´s results and acting as a node for international collaboration. The executive working group is supported by a secretariat.

Strama has a formal regulatory instruction from the Swedish government. The chairman is appointed by the government and reports directly to the Ministry of Health and Social affairs. The Strama governance board has members from the Swedish Institute for Infectious Disease Control, the National Board of Health and Welfare, the National Veterinary Institute, the Medical Products Agency, the Swedish Corporation of Pharmacies (Apoteket AB), the Swedish Association of Local Authorities and Regions, the Swedish Reference Group for Antibiotics and the professional societies for infectious diseases, infection control and communicable diseases. The executive working group has a broad multisectorial composition including several clinicians and meets at least bimonthly to outline working-directions and priorities and to define areas needing further studies.

Although penicillin resistance in *S. pneumoniae* in the community was the first target of Strama, national activities have continuously expanded and today include many additional fields e.g. hospital care, intensive care ("ICU-Strama") [6-7], nursing homes, day-care centers and clinical trials. ICU-Strama has developed a close collaboration with the Swedish Intensive Care Registry (SIR) and is now integrated as a part of its national quality registry. Experiences from ICU-Strama have been incorporated into the European network care-ICU [8].

Strama has co-organised several workshops yielding national recommendations for the treatment of various diagnoses common in general practice such as acute otitis media, acute pharyngotonsillitis, impetigo, acute sinusitis, urinary tract infections and lower respiratory tract infections.

The national office supports the local groups, coordinates different activities, supplies national data and manages a national website (www.strama.se). A national meeting with annual updates on scientific and medical aspects of antibiotic resistance, statistics on antibiotic use and resistance as well as results and analysis of performed studies, interventions and educational programmes is held for the members of the local Strama groups and other interested parties. Abstracts and/or presentations are distributed via the website for further dissemination as a rule. News, regional and national data on antibiotic use and resistance are regularly updated as well as treatment guidelines and results from Strama-funded projects. A physician is contracted who regularly distributes relevant news in the field from the medical press and other sources and "Strama News" containing summaries of relevant recent scientific publications is distributed by email to listed subscribers about eight times a year.

Occasionally, a more acute situation unfolds and calls for more extensive actions. This was the case when it became evident that *Enterobacteriaceae* producing extended-spectrum beta-lactamases (ESBLs) rapidly became increasingly prevalent and caused outbreaks. This prompted Strama to organise a workshop whose findings were then translated into a proposal for a national action plan [9].

### Antibiotic utilisation

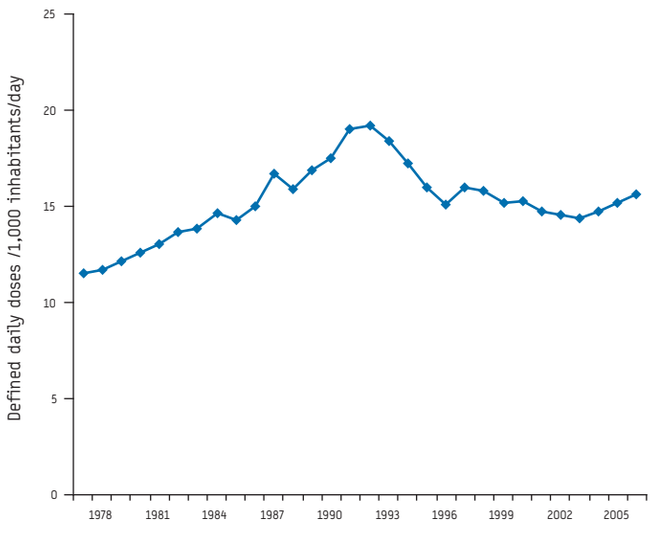
Strama has taken the responsibility for the regular analysis of antibiotic consumption at national level. A detailed description and analysis of antibiotic consumption and resistance: "SWEDRES - A report on Swedish antimicrobial utilisation and resistance in human medicine" is published yearly in collaboration with Swedish Institute for Infectious Disease Control (SMI) and is co-produced and packaged with the corresponding veterinary report SVARM [5]. Following the increased awareness and the inception of the Strama programme, the total antibiotic sales in general practice in Sweden continuously decreased in the 1990s until 2004. In contrast, the other Nordic countries have either remained at a comparatively higher level of antibiotic use (Iceland and Finland) or experienced an uninterrupted increasing trend. According to official figures from the respective medicine agencies in the Nordic countries, Sweden has had the lowest antibiotic use since 2003. However, since 2004 a slow rise, mainly attributable to increased prescription of penicillin to children, has been seen again (Figure 1).

### Drug-prescribing surveys and other studies

To learn more about compliance with guidelines in general practice and about antibiotic use in the hospital setting, Strama has initiated and coordinated several large diagnosis- prescribing surveys. The use of antibiotics in primary care and compliance with the recommendations from the workshops and the quality indicators defined by the General Practitioners Association (SFAM) have been studied in diagnosis-prescribing surveys conducted in 2000, 2002 and 2005 [10-15]. These studies comprised altogether 15,371 patients with infectious symptoms treated by around 600 general practitioners (GPs). The studies showed high antibiotic prescribing in acute otitis media, acute pharyngotonsillitis and acute bronchitis, indicating that the current treatment guidelines for these conditions had not been not fully implemented. For the treatment of uncomplicated urinary tract infections a shift from the use of trimethoprim and fluoroquinolones to pivmecillinam and nitrofurantoin is recommended. A restricted use of fluoroquinolones was advocated already in 1996, [16] leading to a decreasing trend as clearly documented in the surveys.

**FIGURE 1**

**Antibiotic use in outpatients, methenamine excluded, in defined daily doses (DDD) per 1,000 inhabitants and per day, Sweden, 1978-2007**




Source: Apoteket AB

To address antibiotic use in the hospital setting Strama initiated and coordinated nationwide point prevalence studies in 2003, 2004 and 2006. The number of participating hospitals was 54, 49 and 64 and the number of covered hospitalised patients (proportion of all hospitalised patients in somatic clinics in the respective years) was 13,536 (60 %), 11,348 (50%) and 17,113 (80 %), respectively [17]. Data in these studies were reported by a web-based system and results were likewise available for the participating Strama groups. The studies showed that approximately every third hospitalised patient on a given day received antibiotics. While almost 10 % were given antibiotics to treat a health-care associated infection, 6-7 % were given surgical or medical prophylaxis and the remaining 17-19 % treatment for a community acquired infection. The method and protocols used formed the basis for a pan-European study coordinated by ESAC [18].

Increasing antibiotic use in the elderly population prompted a separate study in 2004 on indications for antibiotic prescribing in 58 nursing homes [19].

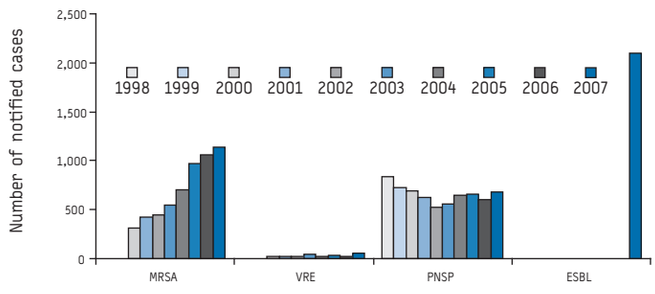
It is important that as a result of the efforts to improve antibiotic use, the reduction in prescriptions does not cause unwanted negative effects. A survey of hospital admissions recorded in the national registry of diagnosis in hospital care, showed no increase in the number of patients with acute sinusitis, quinsy and acute mastoiditis despite the reduction in antibiotic prescriptions for children between 1987 and 2003 [20]. Continuous systems for such monitoring need to be implemented. Another important task for Strama is to encourage studies aimed at preserving the efficacy of existing drugs e.g. through modified dosing regimens or drug combinations.

## Antibiotic resistance

A comparatively widespread practice of culturing clinical specimens in combination with well-functioning diagnostic laboratories using harmonised methods (www.srga.org) have formed the basis for the surveillance of antibiotic resistance in Sweden. Surveillance mainly relies on two major sources: notification of any resistance according to the Swedish Communicable Diseases Act and since 2002 a combined surveillance and quality control programme (RSQC surveys) that was further developed into the web-based ResNet (http://130.237.97.245/ResNet/index.jsp). National antibiotic susceptibility data are presented regularly on the internet. Figure 2 illustrates reporting according to the Swedish Communicable Diseases Act. While the proportion of *S. pneumoniae* with decreased sensitivity to penicillin V has stabilised, the number of notified cases of methicillin-resistant *Staphylococcus aureus* (MRSA) has increased, ESBL-producing Enterobacteraceae have turned into an endemic situation and, most recently, the hitherto largest outbreaks of vancomycin-resistant enterococci (VRE) is ongoing in the Stockholm region. Still, Sweden is among the countries with the lowest rates of MRSA (still below 1 %), *S. pneumoniae* can still be treated with penicillin V and the rate of *Escherichia coli*-producing ESBLs is below 5 %.

**FIGURE 2**

**Notifications of infections or colonisation with antibiotic-resistant pathogens notifiable by the Swedish Communicable Disease Act, 1998-2007**




MRSA: methicillin-resistant *S. aureus* since year 2000
VRE: vancomycin-resistant *E. feacalis* and *E. feacium* since year 2000
PNSP: penicillin-nonsusceptible *Streptococcus pneumoniae* (minimum inhibitory concentration (MIC) for penicillin G ≥ 0.5 mg/L, since 1996)
ESBL: extended spectrum beta-lactamase-producing Enterobacteriaceae, since 2007.

## Conclusions

Strama's multidisciplinary and multisectorial programme has developed into a coordinated national effort that has contributed to a decrease in antibiotic use without measurable negative consequences. Furthermore, resistance levels are still comparatively low in Sweden. Some factors that have paved the way for this success have been the utilisation and early involvement of pre-existing structures and resources such as the communicable disease officers, the multi-disciplinary approach, the collaboration with the local drug and therapeutics committees and microbiology laboratories and the political support at national level. The most suitable structure for such local nodes will no doubt differ from one country to the next and may take some extra resources to identify and put in place. Particular difficulties can be expected when trying to collect local data sent to different (remote) microbiology laboratories and to develop mechanisms to aggregate prescriptions from individual prescribers or health-care facilities. Not least, regular collaboration with national and regional news media has been one of the key strategies.

Recently, however, antibiotic sales seem to have started to rise again and resistance is increasing in several species. This must be met by intensified information and education campaigns, aimed at doctors as well as the general public, on the rational use of antibiotics and the promotion of compliance with basic hygiene in the health-care sector. Examples of areas which call for further attention are antibiotic use in long-term care facilities, among private health-care providers, to treat sexually transmitted diseases (STIs) and for some chronic conditions such as acne, chronic obstructive pulmonary disease and diabetic foot infections. To achieve this goal, all local groups should be formally supported with a defined mission incorporated in the patient safety and quality work by 2010.

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# Perspectives

## Experiences with the Dutch Working Party on Antibiotic Policy (SWAB)

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The Dutch Working Party on Antibiotic Policy (Stichting Werkgroep AntibioticaBeleid, SWAB) was founded in 1996 as an initiative of the Society for Infectious Diseases, the Dutch Society for Medical Microbiology, and the Dutch Association of Hospital Pharmacists. Its primary goal is to contribute to the containment of antimicrobial resistance and the expanding costs incurred for the use of antibiotics. SWAB is the Intersectoral Coordinating Mechanism (ICM) for the Netherlands, and it is at present the National Antimicrobial Resistance (AMR) Focal Point. It coordinates the national surveillance of antibiotic resistance, in collaboration with the National Institute for Public Health and the Environment (RIVM), coordinates the surveillance of the use of antibiotics, and runs a guideline development programme. Information about consumption of antimicrobial agents and antimicrobial resistance among medically important bacteria is presented annually in NethMap. Over the past decade, outpatient consumption of antibiotics has risen only slightly, but in the hospital setting there was an overall significant increase in antibiotic use, due mainly to the steady reduction in the average length of patient hospital stays. In 2006 we introduced our electronic national antibiotic guide 'SWAB-ID' for the antibiotic treatment and prophylaxis of common infectious diseases in hospitals.

### Background

The Dutch Working Party on Antibiotic Policy (Stichting Werkgroep Antibiotica Beleid, SWAB) was founded in 1996 as an initiative of the Society for Infectious Diseases (VIZ), the Dutch Society for Medical Microbiology (NVMM), and the Dutch Association of Hospital Pharmacists (NVZA). Its primary goal is to contribute to the containment of antimicrobial resistance and the expanding costs incurred for the use of antibiotics. This was to be achieved by optimising the use of antibiotics through guideline development, education, and surveillance of antibiotic use and resistance. Following advice by the Dutch Advisory Council on Health Research in 2000 on the containment of Antibiotic Resistance, in 2001 SWAB was appointed by the Dutch Ministry of Health, Welfare and Sports to coordinate the national surveillance of antibiotic resistance, in collaboration with the National Institute for Public Health and the Environment (RIVM) (currently: the Centre for Infectious Disease Control Netherlands, CiB), and to coordinate the surveillance of the use of antibiotics. Structural funds were provided, also for the guideline development programme. Finally, a platform with the Veterinary Antibiotic Usage and Resistance Surveillance Working Group (VANTURES) was created. When the "Council Recommendation on the prudent use of antimicrobial agents in human medicine" (2002/77/EC) [1] was issued, SWAB became the Intersectoral Coordinating Mechanism (ICM) for the Netherlands, and it is at present the National AMR Focal Point.

### NethMap – the annual report on antimicrobial use and resistance

NethMap 2003 was the first epidemiological report with information about consumption of antimicrobial agents and antimicrobial resistance among medically important bacteria in the Netherlands. It was presented in April 2003 during the SWAB symposium 2003 [2]. NethMap was named after a similar yearly report from Denmark (DANMAP). In contrast to the DANMAP report, veterinary data in the Netherlands are published in separate reports, called MARAN (http://www.cvi.wur.nl/NL/publicaties/rapporten/maranrapportage/).

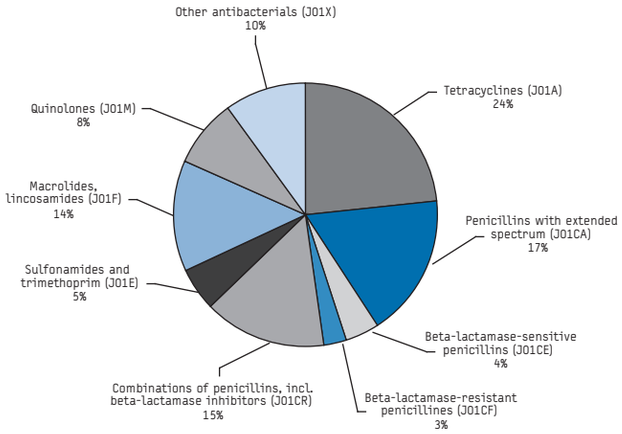
From 2003 through 2008 NethMap has been updated annually, and can be downloaded from http://www.swab.nl >professional>NethMap. NethMap is published by SWAB in collaboration with the RIVM. Data on delivery of antimicrobials from hospital pharmacies are collected by SWAB (Figure 1), and data from 90% of all community pharmacies are provided by the Foundation for Pharmaceutical Statistics (Figure 2). Data on antimicrobial resistance in hospitals are collected from local laboratories by RIVM. The susceptibility of strains collected from outpatients and hospital departments (Urology, Pulmonology and Intensive Care) is determined quantitatively by the Laboratory of Medical Microbiology of Maastricht University. The population coverage for the resistance surveillance programmes is approximately 30%.

Over the past decade, outpatient consumption of antibiotics has risen only slightly, from 9.9 to 11.1 defined daily doses

(DDD)/1,000 inhabitants/day. In hospitals overall antibiotic use increased significantly from 47.8 to 62.2 DDD per 100 patient-days, but this increase was due to the steady reduction in the average length of patient hospital stays. Overall antibiotic use per 100 admitted patients remained constant. Outside hospitals, co-amoxiclav gradually replaced amoxicillin (Figure 3) and, according to revised guidelines for general practitioners (GPs) for the treatment of cystitis, more nitrofurantoin was used at the expense of trimethoprim. The percentage of *Streptococcus pneumoniae* which was of intermediate susceptibility or resistant to penicillin and the percentage of *Staphylococcus aureus* resistant to oxacillin (MRSA) remained lower than 3%, but resistance of *S. pneumoniae* and *S. aureus* to macrolides has increased to nearly 10% of the investigated isolates. *Escherichia coli* resistance to ciprofloxacin among hospitalised patients increased to 9% in 2007 (Figure 4). Occasionally, hospital epidemics of *Klebsiella pneumoniae* resistant to third-generation cephalosporins occurred, but the overall percentage of such strains remained low at 3-6%. In contrast, the ciprofloxacin resistance in *Neisseria gonorrhoeae* from sexually transmitted diseases' (STD) clinics has increased to such a high level (44%) that fluoroquinolones cannot be advised anymore for first-line treatment. The Dutch guidelines for treating gonorrhoea have been adapted accordingly.

**Figure 1**

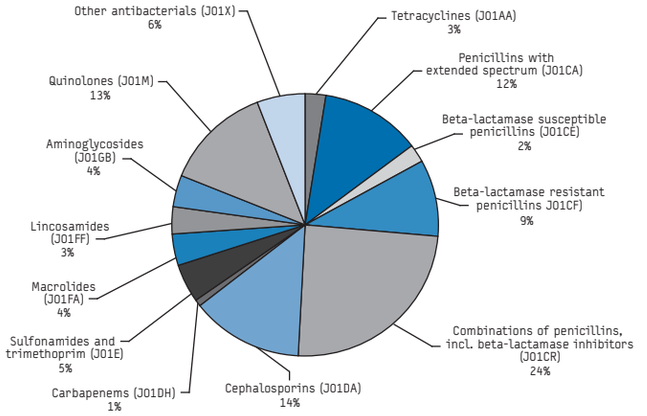
**Distribution of antibiotics administered for systemic use* (defined daily doses (DDD)/1,000 inhabitants/day) in primary health care, the Netherlands 2007**




Source: Stichting Farmaceutische Kengetallen - SFK.
* Anatomical Therapeutic Chemical (ATC) Classification System J01

**Figure 2**

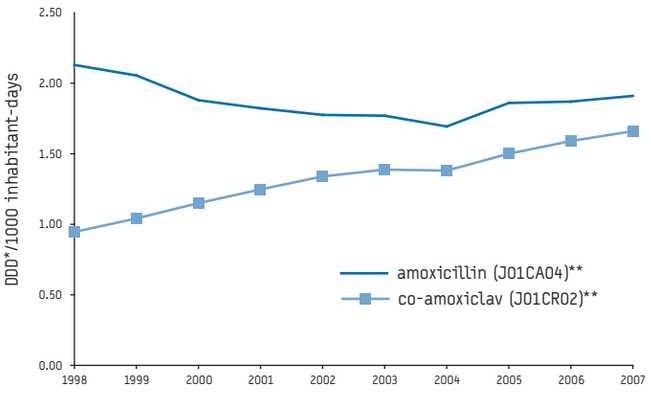
**Distribution of antibiotics administered for systemic use* (Defined Daily Doses (DDD)/100 patient-days) in hospitals, the Netherlands, 2006**




Source: Dutch Working Party on Antibiotic Policy - SWAB.
* Anatomical Therapeutic Chemical (ATC) Classification System J01

**Figure 3**

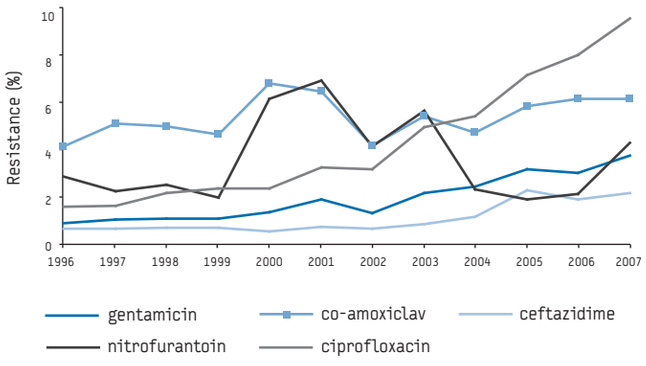
**Use of amoxicillin and co-amoxiclav in primary health care, the Netherlands 1998 – 2007**




Source: Stichting Farmaceutische Kengetallen – SFK.
* Defined Daily Doses
** Anatomical Therapeutic Chemical (ATC) classification code

**Figure 4**

**Trends in resistance to antibiotics among *Escherichia coli* from unselected hospital departments, the Netherlands, 1996-2007**



### Guideline development in the Netherlands

Since the late 1980s, the Dutch College for General Practitioners has been running a guideline programme. Guidelines have been developed for e.g. otitis media in children, sinusitis, sore throat and pneumonia. These guidelines are regularly updated. In addition, the GPs have in place a "peer review group" system: a nationwide structure of general practitioners' peer review groups, with collaborating pharmacists, which aims to promote rational prescribing through audit and feedback [3].

Since its conception, SWAB has developed national guidelines for the use of antibiotics, which are aimed at the hospitalised adult patient. Initially, the draft guidelines were prepared by a writing committee, selected by SWAB, consisting of five to ten experts: medical microbiologists, infectious diseases' specialists, hospital pharmacists and medical specialists relevant to the specific topic. After review by another 25 experts, the guidelines were finalised and published. Guidelines were published in the major national Dutch medical journal (Nederlands Tijdschrift voor Geneeskunde) [4].

In 2001, a survey among hospital antibiotic policy committees revealed that the majority of respondents were aware of SWAB guidelines, but it was suggested that the draft guidelines should be made more broadly available, e.g. on the internet, and with a clearer method for grading the strength of the evidence on which the guideline was based. A particular feature of infectious diseases' guidelines is that local epidemiology and resistance data must be taken into account, and NethMap has provided this information since 2003. As a result of our survey and following the principles of evidence-based guideline development [5] we revised the procedures for the development of SWAB guidelines in 2005 [6,7]. The new procedure includes the consultation of the concerned professional societies for delegating experts to the writing committee, and all their members are given an opportunity to comment on draft guidelines. There are now also GPs on the writing committee to ensure that there is consistency between the guidelines for ambulatory care and hospitals. After final approval by the board, SWAB guidelines are posted on the SWAB website (www.swab.nl). For most of the guidelines English versions are freely available from the internet. Implementation of the guidelines in hospitals is studied by government-funded research projects [8].

### The national electronic antibiotic guide 'SWAB-ID' for use in hospitals

The survey (unpublished data) among Dutch hospital antibiotic policy committees also revealed their wish to compile a comprehensive, national antibiotic treatment guide. SWAB took up this challenge, and in 2006 we introduced our electronic national antibiotic guide 'SWAB-ID' for the antibiotic treatment and prophylaxis of common infectious diseases in hospitals [9]. This guide also contains a formulary for all antimicrobial drugs available in the Netherlands. Treatment choices and dose regimens are based on existing national evidence-based guidelines, where available. Where no guideline is available, the advice is based on an inventory of the antibiotic policies of the 12 Dutch centres with an infectious diseases' or medical microbiology training programme. The national antibiotic guide can be accessed through the SWAB website (http://customid.duhs.duke.edu/NL/Main/Start.asp) and can also be downloaded on PDA/PocketPC, free of charge. The guide is updated regularly, for instance when new guidelines are issued or new antimicrobial agents become available.

Every hospital antibiotic policy committee in the Netherlands is offered the opportunity to edit the national version for local use. For a relatively small fee, SWAB provides a copy of the national version, in which adaptations can be made if local circumstances so demand, and this local version is again accessible through the internet, and downloadable on PDA. So far, six out of eight university hospitals, and ten non-academic hospitals / hospital groups are now using a local version of the national SWAB guide.

### SWAB, Europe, and the First European Antibiotic Awareness Day

SWAB assisted Croatia in implementing EU directives and recommendations in the field of antimicrobial resistance and the sound use of antibiotics within a framework project launched by the Dutch Ministry of Foreign Affairs in 2006 [10]. Although antibiotic use and resistance rates in the Netherlands are relatively low compared to almost any other country [11], the NethMap surveillance 2008 report shows that figures are rising slowly. The reasons for this are not fully understood. Up to now, SWAB initiatives to maintain prudent antibiotic use have addressed healthcare professionals only. The Council document [1] included recommendations to make consumers aware of the risks posed by antimicrobial resistance. In the spirit of the first European Antibiotic Awareness Day that will take place across Europe on 18 November 2008 [12], SWAB will soon develop activities to increase awareness among the general public of the importance of consolidating the prudent use of antibiotics.

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# Editorials

## Increasing multidrug resistance and limited treatment options: situation and initiatives in Europe

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Antimicrobial resistance due to the continuous selective pressure from widespread use of antimicrobials in humans, animals and agriculture has been a growing problem for decades. In 2001, European Union Ministers of Health adopted Council Recommendations on the prudent use of antimicrobial agents in human medicine with a number of specific measures aimed at containing the spread of antimicrobial resistance by prudent use of antimicrobial agents [1]. The first recommendation was that Member States should establish and strengthen surveillance systems on antimicrobial resistance and the use of antimicrobial agents. Since 1999, the European Antimicrobial Surveillance System (EARSS, http://www.rivm.nl/earss/) provides validated data on the prevalence and spread of major disease-causing bacteria with resistance to one or more antibiotics. It has since become one of the most successful dedicated infectious disease surveillance systems in Europe. In order to be able to compare resistance rates of individual countries, the study sample and methods must be comparable. In this respect the variety of susceptibility testing methods in Europe represents a challenge; however, the quality of antimicrobial susceptibility testing of EARSS participating laboratories is regularly checked through external quality assessment exercises. EARSS has so far only gathered information on antimicrobial resistance in seven bacteria of clinical relevance and isolated from invasive infections (blood and cerebrospinal fluid samples). In its recently published Annual Report 2007, the EARSS reiterated its previous conclusion that "the data that EARSS has gathered over the years bring an unpleasant, but important message: antimicrobial resistance is becoming a larger public health problem year after year and only a concerted effort might turn the tide" [2].

This issue of Eurosurveillance is the second one this month dedicated to antimicrobial resistance, in connection with the first-ever European Antibiotic Awareness Day - a European Union (EU) health initiative involving all key players to increase awareness of Europeans about antimicrobial resistance and prudent use of antibiotics. While the first issue reported on encouraging examples of countries that took corrective actions and show decreasing trends in resistance [3-8], this issue focuses on bacteria that are not among the classical human pathogens, yet are, due to resistance to multiple antibiotics, increasingly complicating patient management in hospitals and other healthcare institutions. These pathogens also contribute considerably to the morbidity and mortality of healthcare-associated infections in Europe.

Enterococci are frequently responsible for healthcare-associated infections. They show an increasing prevalence of acquired resistance to ampicillin, aminoglycosides and glycopeptides, leaving the therapeutic alternatives to few antibiotics that were recently introduced into clinical practice and have limited indications, i.e. quinupristin-dalfopristin, linezolid, tigecycline and daptomycin. In this issue, G. Werner et al. review the situation in Europe [9] where vancomycin-resistant enterococci appear to be a serious and growing problem in most countries with the highest rates being reported by Greece, Ireland, Portugal, Cyprus and the United Kingdom [2]. The highest resistance rates are seen in the species *Enterococcus faecium* of which defined clonal groups have shown an enhanced capacity to disseminate in the nosocomial setting. Despite this clonality, the population of hospital-acquired, vancomycin-resistant *E. faecium* isolates tends to be polyclonal with highly mobile resistance determinants. The control of vancomycin-resistant enterococci remains a formidable task for hospital infection control practitioners. Both prudent use of antibiotics and compliance with hand hygiene and other infection control measures are essential to reduce selection and spread of multidrug-resistant enterococci.

**Multidrug resistance has serious consequences on the outcome of serious infections because it usually delays administration of appropriate antibiotic therapy.**

Multidrug resistance is also increasing in Gram-negative bacilli [2]. In this issue, T.M. Coque et al. highlight the growing threat posed by increasing prevalence of extended-spectrum beta-lactamase (ESBL) producing Enterobacteriaceae all over Europe, even in countries traditionally showing low prevalence rates of resistance [10]. The highest prevalence rates are being reported by eastern and south-eastern European countries. Although originally, ESBLs were mainly found in bacteria responsible for healthcare-associated infections, their prevalence is now increasing in the community. In particular, emergence and spread of the CTX-M-15 ESBL enzyme is reported in most European countries, both in hospitals and the community. The patient risk factors for colonisation and/or infection are not only prior use of third-generation cephalosporins, but also of other antibiotics, and the ESBL reservoir is not limited to humans as ESBLs have been isolated from animals, food and environmental samples.

The relentless increase in resistance to third-generation cephalosporins and fluoroquinolones in Enterobacteriaceae such as *Escherichia coli* and *Klebsiella pneumoniae* in Europe [2] has led to increasing use of carbapenems in hospitals, one of the most potent class of antibiotics against Gram-negative bacilli infections. Outbreaks due to metallo-beta-lactamase (MBL) producing, thus carbapenem-resistant, *K. pneumoniae* is therefore of great concern [11]. Isolates of Gram-negative bacilli simultaneously containing plasmids encoding various ESBLs, MBLs or AmpC beta-lactamases are now increasingly being reported in Europe. The acronym XDR, which was originally coined for extensively drug-resistant *Mycobacterium tuberculosis*, is now used, though with various definitions, to describe such multidrug-resistant Gram-negative bacilli isolates for which only one or two antibiotic alternatives are available for therapy [12,13]. This increasing number of reports of XDR Gram-negative bacilli is particularly worrisome, especially because it has not been paralleled by development and availability of alternative therapeutic options. There are very few new antibiotics with a novel mechanism of action in the pharmaceutical industry research and development pipeline.

In this issue, M. Souli et al. review the emergence of such XDR, or even pandrug-resistant, i.e. resistant to all available antibiotics, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and Enterobacteriaceae in Europe [14]. Unfortunately, common official definitions and recommendations on how to detect and report such isolates are still being developed and surveillance systems such as the EARSS do not specifically report such data. As a consequence, we presently do not fully know the prevalence of such isolates in European countries, but it looks like the highest, hospital-specific prevalence rates of XDR and pandrug-resistant isolates have been reported from centres in southern and eastern European countries. However, patients are regularly transferred between hospitals from different European countries and the issue is relevant for all Member States. M. Souli et al. [14] quote two recent studies where mortality attributable to XDR and pandrug-resistant Enterobacteriaceae was 19% and 33%, respectively. The antibiotics that usually remain active against XDR isolates are colistin and tigecycline, yet resistance to these last-line drugs is increasingly being reported [15,16].

Prompt treatment with appropriate antibiotics is essential in serious bacterial infections to prevent complications and death. Multidrug resistance has serious consequences on the outcome of serious infections because it usually delays administration of appropriate antibiotic therapy. Several studies have demonstrated an increased mortality for infections due to multidrug-resistant and XDR bacteria in high-income countries [17-20]. This is also true in many low-income countries where the surge in antimicrobial resistance is seen as disastrous because of the lack of resources for purchasing expensive second-line drugs. This was recently documented in a paediatric ward of a tertiary care hospital in Tanzania where the fatality rate in patients with septicaemia due to ESBL-producing Gram-negative bacteria was significantly higher than in those with non-ESBL isolates [21].

European physicians are increasingly being faced with infections caused by bacteria for which limited or no adequate therapeutic options exists. Although Europe appears to have relatively good information about prevalence of resistance compared to other parts of the world, coverage could be improved and should include surveillance of XDR and pandrug-resistant bacteria. European laboratories and hospitals should be able to rapidly detect such strains to adjust patient therapy and put in place adequate local control measures. Additionally, similar to other communicable diseases, multidrug-resistant bacteria do not respect borders. Physicians and laboratories should be aware of the risk posed by transfer of patients from hospitals in other countries [22]. In this context, rapid and effective international communication is important to prevent further spread of emerging, multidrug resistant microorganisms.

Interventions are urgently needed to control and prevent further spread of multidrug-resistant bacteria through improvement of antimicrobial prescribing and infection control practices in Europe. But so far these interventions, though quite successful, have been few and far between, and limited to community prescribing or to the control of specific hospital bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA) [3-8]. Member States have taken various actions following Council Recommendation of November 2001 [1] and the Council Conclusions on antimicrobial resistance recently adopted by EU health ministers during the Slovenian Presidency of the EU [23]. The European Commission has put considerable attention on this issue at the EU level. Based on EARSS data and articles in this issue of Eurosurveillance, control programmes could consider including other multidrug-resistant microorganism targets in addition to MRSA. The European Commission is finalising its proposal for a Council Recommendation on patient safety and quality of health services, including the prevention and control of healthcare associated infections [24]. Once adopted, this recommendation will contribute to strengthening national infection control programmes, including actions aimed at preventing spread of multidrug-resistant bacteria. Finally, the successive EU presidencies of Slovenia, France, the Czech Republic and Sweden have decided to make antimicrobial resistance a health priority. On 15 and 16 April 2009, a conference on "The Microbial Threat to Patient Safety in Europe" will be organised by the Czech Presidency of the EU. This European conference will cover standards and indicators for antibiotic stewardship in European hospitals, the influence of healthcare systems characteristics on antimicrobial resistance and healthcare-associated infections, as well as the importance of leadership and accountability to reduce patient risks linked to these infections, and will contribute to containing antimicrobial resistance in European hospitals. During the second part of 2009, the Swedish Presidency of the EU will organise a follow-up conference focusing more specifically on the gap between increasing multidrug resistance, the need for new antibiotics with a novel mechanism of action and incentives for research and development of such antibiotics. These partnership approaches between all the relevant stakeholders are expected to bring further positive progress in the containment of antimicrobial resistance at the EU level.

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# Review articles

## Emergence and spread of vancomycin resistance among enterococci in Europe

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Vancomycin-resistant enterococci (VRE) first appeared in the late 1980s in a few European countries. Nowadays, six types of acquired vancomycin resistance in enterococci are known; however, only *VanA* and to a lesser extent *VanB* are widely prevalent. Various genes encode acquired vancomycin resistance and these are typically associated with mobile genetic elements which allow resistance to spread clonally and laterally. The major reservoir of acquired vancomycin resistance is *Enterococcus faecium*; vancomycin-resistant *Enterococcus faecalis* are still rare. Population analysis of *E. faecium* has revealed a distinct subpopulation of hospital-acquired strain types, which can be differentiated by molecular typing methods (MLVA, MLST) from human commensal and animal strains. Hospital-acquired *E. faecium* have additional genomic content (accessory genome) including several factors known or supposed to be virulence-associated. Acquired ampicillin resistance is a major phenotypic marker of hospital-acquired *E. faecium* in Europe and experience has shown that it often precedes increasing rates of VRE with a delay of several years. Several factors are known to promote VRE colonisation and transmission; however, despite having populations with similar predispositions and preconditions, rates of VRE vary all over Europe.

### Introduction

Enterococci are important hospital-acquired pathogens. Isolates of *Enterococcus faecalis* and *Enterococcus faecium* are the third- to fourth-most prevalent nosocomial pathogen worldwide. Acquired resistance, most prominently to penicilin/ampicillin, aminoglycosides (high-level resistance) and glycopeptides are reported in an increasing number of isolates and the therapeutic spectrum in these cases is limited. Therapeutic alternatives to treat infections with multi- and vancomycin-resistant enterococci (VRE) are restricted to antibiotics introduced recently into clinical practice such as quinupristin/dalfopristin, linezolid, tigecycline, daptomycin. However, these drugs are only approved for certain indications and resistance has already been reported [1-5].

Acquired resistance to glycopeptides is mediated by various mechanisms (types VanA/B/D/E/G/L; Table 1); the *vanA* and *vanB* resistance genotypes are by far the most prevalent in Europe. The reservoir for *vanA*- and *vanB*-type resistance in humans is *E. faecium* [6;7]. Consequently, increasing rates of VRE in several European countries are due to an increasing prevalence of vancomycin-resistant *E. faecium* (VREfm). Ampicillin- and/or vancomycin-resistant *E. faecalis* (VREfs) are still rare [8]. Defined clonal groups of *E. faecium* show an enhanced capacity to disseminate in the nosocomial setting and are thus called epidemic or hospital-acquired [7]. These strains can be assigned to distinct clonal groups or complexes based on DNA sequence-based typing (multi-locus sequence typing - MLST) and phylogenetic analyses (eBURST) [6;7]. Hospital-acquired *E. faecium* are mostly ampicillin-resistant, partly high-level ciprofloxacin-resistant and possess additional genomic content, which includes putative virulence traits such as a gene for an enterococcal surface protein, *esp*, genes encoding different cell wall-anchored surface proteins, a putative hyaluronidase gene, $hyl_{Efm}$ and a gene encoding a collagen-binding protein, *acm* [6;7;9-12].

The current model predicts that spread of ampicillin-resistant, hospital-acquired *E. faecium* strains is a pre-requisite for successful establishment of VRE and further dissemination of vancomycin resistance among the hospital *E. faecium* population in general (see also following chapters). To a larger or lesser extent, non-

microbiological factors such as antibiotic consumption (particular classes and in general); "colonisation pressure", "understaffing", compliance with hand hygiene and other infection control measures also influence this development [13-16]. Therefore, it might not come as a big surprise that despite having similar starting points and preconditions different countries experienced diverse trends in VRE prevalence. Already during the early and mid-1990s, epidemic clonal types of *E. faecium* were prevalent in hospitals in many countries, and this coincided in some European countries with a high prevalence of vancomycin resistance among *E. faecium* from animals and healthy volunteers linked to a widespread use of avoparcin as a growth promoter in commercial animal husbandry [14;17;18]. However, VRE rates in clinical isolates increased in many countries and peaked only almost ten years later when glycopeptide resistance had already declined in the non-hospital reservoir. Retrospective epidemiological analyses in hospitals experiencing larger VRE outbreaks revealed that changes in specific procedures such as antibiotic policy, staffing, infection prevention and control regimes were, in some instances, significantly associated with increasing VRE rates, whereas in other settings this could not be shown unambiguously. In addition, increased VRE prevalence is only partly associated with spread of single, distinct epidemic clones or types as known for pneumococci or methicillin-resistant *Staphylococcus aureus* (MRSA) [18-20]. VRE outbreaks in single centres tend to be polyclonal suggesting a highly diverse population of hospital-acquired *E. faecium* strains and a highly mobile resistance determinant capable of spreading widely among suitable recipient strains [21-23]. Many facets of VRE and vancomycin resistance epidemiology are currently not fully understood and the question why vancomycin resistance is still mainly limited to *E. faecium* remains unanswered.

Several national and European surveillance systems collect data on vancomycin resistance in enterococci. In some countries mandatory VRE surveillance is already established, in others coverage for the general population or selected settings is rather limited and the available data do not allow sound statistical analyses and in some countries data are completely lacking (see chapter 2). The most successful European antibiotic resistance surveillance scheme is the European Antimicrobial Resistance Surveillance System (EARSS) (http://www.rivm.nl/earss/) [8], which was established in 1998 and is partly funded by the European Commission. EARSS collects data for selected antibiotic resistances in indicator bacteria exclusively from invasive (bloodstream) infections currently covering *S. aureus*, *Escherichia coli*, *E. faecalis* and *E. faecium*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. In 2006 over 800 microbiological laboratories serving more than 1,300 hospitals from 31 countries provided susceptibility data from more than 500,000 invasive isolates. Quality assessment is confirmed by annual external quality exercises. Despite the many advantages of an active European antimicrobial surveillance scheme, the huge amount of collected data cannot mask some of its drawbacks and limitations. Data collection and interpretation rely on different standards (Clinical and Laboratory Standards Institute - CLSI; European Committee on Antimicrobial Susceptibility Testing - EUCAST, British Society for Antimicrobial Chemotherapy - BSAC; etc.) and different methods (minimal inhibitory concentration - MIC determination; disk diffusion tests) used in the participating laboratories. What this means in practice has been documented by Leegaard *et al.* [24]. They tested a representative collection of strains and demonstrated, for instance, rates of MRSA among *S. aureus* isolates varying between 0 and 15 % depending on the standard applied. In an attempt to harmonise and standardise procedures for testing each bacterium/resistance combination, an EARSS manual was written in 2005; however, different methods and various standards are still being used which complicates the overall comparison of results. As the number of participating laboratories changes over time, distinct "resistance trends" may in some cases simply reflect organisational changes. Statistical coverage of the general population according to the number and country-wide distribution of contributing laboratories varies greatly between countries. Due to these limitations simple comparisons of surveillance data over time between countries or even within single countries should be done carefully (see also chapter 4 in

**Table 1**

**Vancomycin resistance in enterococci. See cited reviews for details [96;97]**

| | Acquired resistance | | | | | | Intrinsic resistance |
|---|---|---|---|---|---|---|---|
| **phenotype** | **VanA** | **VanB** | **VanD** | **VanE** | **VanG** | **VanL** | **VanC** |
| **ligase gene** | *vanA* | *vanB*[2] | *vanD*[2] | *vanE* | *vanG*[2] | *vanL* | *vanC* |
| **MIC $_{vancomycin}$ in mg/L** | 16 - 1000 | 4 - 32 (-1000) | 64 - 128 | 8 - 32 | 16 | 8 | 2 - 32 |
| **MIC $_{teicoplanin}$ in mg/L** | (4-) 16 - 512 | 0,5 - 1 | 4 - 64 | 0,5 | 0,5 | S | 0,5 - 1 |
| **expression** | inducible | inducible | constitutive | inducible | inducible | inducible | constitutive/ inducible |
| **localisation** | plasmid/ chromosome | plasmid/ chromosome | chromosome | chromosome | chromosome | chromosome? | chromosome |
| **transferable by conjugation** | +/- | +/- | - | - | + | - | - |
| **distribution among enterococcal species** | *E. faecium*<br>*E. faecalis*<br>*E. durans*<br>*E. hirae*<br>*E. gallinarum*[1]<br>*E. casseliflavus*[1]<br>*E. raffinosus*<br>*E. avium*<br>*E. mundtii* | *E. faecium*<br>*E. faecalis*<br>*E. durans*<br>*E. gallinarum*[1] | *E. faecium*<br>*E. faecalis*<br>*E. raffinosus* | *E. faecalis* | *E. faecalis* | *E. faecalis* | *E. gallinarum: vanC1*<br>*E. casseliflavus: vanC2/3* |

[1] acquisition of *vanA* or *vanB* cluster in addition to *vanC1* or *vanC2/3* genes – rare event
[2] subtypes exist (*vanB*1-3, *vanD*1-5, *vanG*1-2); S, susceptible to teicoplanin (no value given in the corresponding paper)

the EARSS Annual Report 2006) [25]. A thorough study of the annual EARSS reports including all the available country-specific parameters provided in the annexes is essential for a critical and sound evaluation and interpretation of resistance data and trends.

The following chapters give a detailed description of the current and past epidemiological VRE situation for different regions and countries in Europe. Several national experts were invited to describe local and regional differences and measures undertaken when facing first and limited VRE outbreaks or country-wide trends of VRE rates over the years.

## Description of the epidemiological situation in Europe

### Northern Europe

VRE surveillance in the Nordic countries, Norway, Denmark, Sweden, Finland and Iceland, is based on national public health programmes for containment of antimicrobial resistance, participation in EARSS and in some countries case notification from laboratories and clinicians. The Nordic countries have traditionally had a low prevalence of antimicrobial resistance, and this is also true for VRE.

Since the mid 1990s, Norway, Denmark and Iceland have only registered sporadic cases and minor outbreaks of VRE infection or colonisation, often among patients transferred from hospitals in high-prevalence countries in Europe or the United States of America [26;27]. The annual number of cases has been 10–20 in Denmark, 5–10 in Norway and single individual cases have been detected in Iceland. Hospital outbreaks of VREfm have in some cases been associated with concomitant dissemination of vancomycin-susceptible, ampicillin-resistant strains of the same clone [28-30]. As a consequence of previous exposure to the growth promotor avoparcin in animal husbandry, significant animal reservoirs of VREfm have been reported from both Denmark and Norway. Individual examples of a possible clonal relationship between human clinical strains and isolates of animal origin have been detected [31], but the clinical impact in terms of human VRE infections has been limited. The VRE reservoirs in animal husbandry have been substantially reduced since avoparcin was banned in 1996.

The epidemiology of VRE colonisation and infections is somewhat different in Sweden and Finland. The Helsinki area experienced an epidemic of VRE affecting patients in haematological and other internal medicine wards in several hospitals in 1996-1997 [32;33]. The outbreak involved two different *E. faecium* clones which harboured either *vanA*, *vanB* or both determinants. A number of vancomycin-susceptible *E. faecium* (VSEfm) isolates shared the same macrorestriction pattern in pulsed-field gel electrophoresis (PFGE) as the outbreak strains. Investigation of the outbreak suggested that *vanA* and *vanB* clusters were incorporated into an endemic ampicillin-resistant VSEfm strain. Over the last ten years, the situation in Finland has been stable with 30–60 cases of VRE infection or colonisation each year being reported from different counties.

In Sweden, the situation has been stable with 18–53 cases of VRE infections and colonisations being reported annually between 2000 and 2007, and with a prevalence of VRE among Swedish enterococcal bloodstream isolates below 0.5% until 2006 [34;35]. However, the situation is rapidly changing with the predominant spread of a *vanB E. faecium* clone, but also of other strains, among more than 200 patients in Stockholm and several other counties since autumn 2007 (http://www.smittskyddsinstitutet.se/in-english/statistics/vancomycin-resistant-enterococc-infection-vre/). Given this situation one may fear that VRE will become established as an endemic hospital pathogen in parts of Sweden.

The Nordic countries have been relatively successful in containing MRSA. This has been achieved through strict enforcement of infection control measures such as contact isolation of known cases, screening for MRSA among patients and healthcare workers exposed to MRSA or arriving from high-prevalence areas, and eradication of MRSA colonisation. These strategies have been written into local guidelines and national regulations. Finland issued specific national guidelines for VRE in conjunction with the outbreak in 1996-1997, and patients in Sweden are presently screened for VRE applying the MRSA guidelines. In Denmark, Norway and Iceland VRE is not subject to the same level of regulation as MRSA. Many institutions will screen patients who may have been exposed to VRE, but the extent of screening as well as the isolation regimen used is based on local assessment. One can expect more explicit national guidelines in these countries if the prevalence of hospital VRE increases further.

### United Kingdom and Ireland

There is no single comprehensive surveillance scheme for monitoring VRE infections in the United Kingdom (UK). However, bacteraemia caused by VRE is monitored by four complementary surveillance programmes, with varying degrees of coverage and participation:

- Department of Health mandatory glycopeptide-resistant enterococcal bacteraemia reporting scheme [36;37], collecting the total number of VRE bacteraemias in England each year;
- Health Protection Agency (HPA) LabBase2 reporting, voluntary surveillance scheme, collecting VRE data from England, Wales and Northern Ireland [38]; ascertainment of cases not as complete as in mandatory reporting;
- British Society for Antimicrobial Chemotherapy (BSAC) Bacteraemia Surveillance Programme [39], sentinel surveillance programme, collecting isolates from 25 centres in the UK and Ireland each year, providing high-quality centralised investigation of the isolates; and
- EARSS [8], collecting VRE data from England and Wales.

Based on data from all four surveillance programmes estimates for the proportion of enterococcal bacteraemia attributable to VRE for the UK as a whole in 2007 are 8.5-12.5% for all enterococci, 20 - 25% for *E. faecium* and 1.6-2.5% for *E. faecalis* [8;37;39]. There are other surveillance programmes monitoring VRE prevalence in Wales and Scotland but, although some recent data from these are available, more data are required to assess trends over time. However, the VRE rate reported for Wales in 2006 was similar to that determined in the BSAC surveillance for Wales, 15.5% versus 11.9% respectively [40]. The HPA's Laboratory of Healthcare-Associated Infections offers to 'type' VRE to assist local outbreak investigations, but currently there is no initiative to undertake detailed molecular epidemiological investigations of VRE on a national level in the UK.

Between October 2006 and September 2007, 910 VRE bacteraemia cases were reported by English hospitals via the mandatory VRE surveillance [36]. Among the acute National Health Service (NHS) Trusts that reported data, 24 (14%) reported >10 cases, 94 (55%) reported 1-10 cases, and 53 (31%) had no cases. The majority of Trusts reporting >10 cases were acute teaching

Trusts. VRE is not a high profile cause of invasive infection in the UK; VRE is eclipsed by more profuse pathogens with, for example, 4,438 MRSA bacteraemias [41] and 50,392 Clostridium difficile cases reported by the Department of Health's mandatory reporting schemes over the same time period [42]. In consequence, VRE does not "enjoy" the same degree of political and press attention as MRSA and C. difficile.

Table 2 shows the prevalence of VRE found by three of the surveillance programmes operating in the UK, which provide sufficient data to show trends over time and the proportion of overall enterococcal bacteraemias they comprise. As the data in Table 2 is derived from surveillance programmes with differing coverage of UK regions and levels of participation, it is not possible to compare the figures directly. However, the data allow VRE trends to be approximated and similar trends present in various datasets add to its validity. The LabBase and BSAC surveillance data show that the prevalence of VRE among enterococcal bacteraemias has increased from 2001–2006. EARSS only started to determine VRE prevalence from 2005 and VRE numbers from this survey appear to have dropped by approximately 50% from 2005 to 2007. However, it is too early to conclude whether this represents a reliable downward trend since, unlike the mandatory and LabBase programmes, EARSS collects data from a relatively small number (n= 23) of study centres, and is therefore more susceptible to year-to-year variation within a single centre. The same applies for the BSAC study. Moreover, mandatory data show that the numbers of cases vary between hospitals from 0 to >10. Variation between the surveillance schemes might thus reflect regional variation and the types of hospitals participating in the different schemes. As the mandatory reporting scheme does not collect total numbers of enterococcal bacteraemias, it is not possible to determine VRE prevalence from this dataset. However, mandatory reporting has shown an increase in the number of VRE bacteraemias since the inception of the scheme in 2004 [36].

Unlike the mandatory reporting scheme, the LabBase, BSAC and EARSS surveillance programmes record the identification of VRE to species level and collect susceptibility data on antibacterial agents in addition to vancomycin. Figure 1 compares the resistance to vancomycin in *E. faecium* and *E. faecalis* as seen in the LabBase surveillance 1994-2007. As with the LabBAse data the other surveillance programmes show that the majority of VRE in the UK are *E. faecium*, and that the bulk of VRE have the *VanA* phenotype, with non-susceptibility to both vancomycin and teicoplanin [37;38]. A recent review of data from 2001-2006 from the BSAC bacteraemia survey [37;39] showed that VRE bacteraemia isolates were most likely to be from patients who had been in hospital for more than 48h, and were associated with haematology/oncology patients. Inter-centre variation of VRE prevalence was also highlighted, with 54.1% of vancomycin non-susceptible isolates coming from just six out of all 29 centres participating in the study [37]. None of the current VRE surveillance programmes collect data on antibiotic prescribing so it is not possible to tell whether high rates of VRE are related to prescribing policy at these centres.

Ireland has been contributing resistance data for enterococci to EARSS since 2002 with an excellent coverage of almost 100% in the last years. Rates of VREfm increased the first years of reporting from 2002–2005 due to new laboratories joining and lower coverage and levelled off at 30 – 35% from 2005 on. Rates of VREfs increased slightly but remained below 5%.

**France**

Before 2005, only sporadic cases or outbreaks with a limited number of cases due to VRE were reported in France. The incidence of glycopeptide resistance in *E. faecium* from bacteraemia remained below 5% [8]. Despite this reassuring picture, large outbreaks affecting several hundreds of patients occurred in 2005 in a few hospitals and these prompted the French authorities to recommend in 2005 and 2006 notification of all cases of infections/colonisations due to VRE. Furthermore the implementation of strict infection control measures was also recommended (http://cclin-sudest.chu-lyon.fr/Alertes/ficheERV_CAT_112006.pdf) [43]. In addition, isolates should be sent for analysis to the Laboratory for Enterococci, which is part of the French Reference Centre for Antimicrobial Resistance. In 2006, 93% (26/28) of hospitals that notified VRE cases also sent the isolates to the Reference Centre; this percentage decreased to 50% in 2007 but reached 100% in the first six months of 2008. Overall, it is assumed that the

**Table 2**

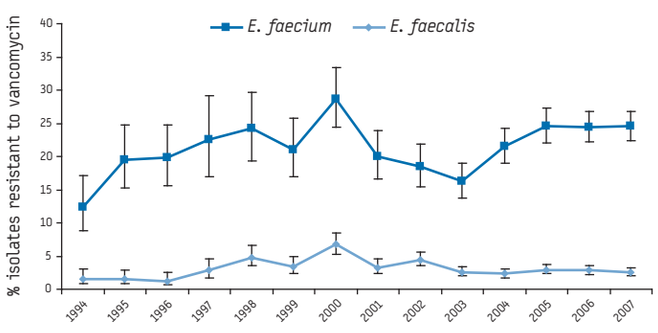
**Prevalence levels of vancomycin-resistant entrococci (VRE) among enterococcal bacteraemia cases as reported by three different surveillance programmes with varying amounts of region coverage and participation, United Kingdom, 2001-2007**

| Year | LabBase[a] | BSAC[b] | EARSS[c] |
|---|---|---|---|
| 2001 | 9.1% | 8.1% | N/A |
| 2002 | 9.1% | 8.5% | N/A |
| 2003 | 9.3% | 10.2% | N/A |
| 2004 | 10% | 11% | N/A |
| 2005 | 10.7% | 16% | 14.9% |
| 2006 | 11.5% | 12.6% | 6.9% |
| 2007 | 12.2% | Data not yet available | 8.5% |

[a] LabBase data obtained from English, Welsh and Northern Irish (from 2002) hospitals
[b] British Society for Antimicrobial Chemotherapy (BSAC) sentinel surveillance covering the United Kingdom and Ireland
[c] European Antimicrobial Resistance Surveillance System (EARSS) sentinel surveillance covering England and Wales
N/A, data not provided

**Figure 1**

**Resistance to vancomycin in *Enterococcus faecium* and *E. faecalis* from bacteraemia, England, Wales and Northern Ireland, 1994-2007**




Source: LabBase voluntary laboratory reporting [36]

Reference Centre has analysed isolates from the major outbreaks occurring in France since 2005. Among 507 isolates analysed, 27 were obtained from blood cultures, 30 from various suppurations (mostly intra-abdominal), 10 from intravenous catheters, 68 from urine and 372 from rectal swabs. The latter isolates were obtained during faecal screening, which is part of infection control measures. This distribution confirms the low ratio of infection versus colonisation for VRE. The vast majority of isolates were *E. faecium* and *E. faecalis* and contained the *vanA* or *vanB* genes; *vanA E. faecium*, *vanB E. faecium*, *vanA E. faecalis* and *vanB E. faecalis* represented 78.2%, 18%, 3% and 0.8% of isolates, respectively.

Variation in the number of isolates received by the Reference Centre in different years corresponds to changes in the type and numbers of hospitals affected by outbreaks. In 2005–2006, most isolates were from large outbreaks of *vanA E. faecium* occurring in hospitals in Paris and central France (Clermont-Ferrand) and smaller outbreaks in other hospitals. In 2007, the number of isolates sent by these hospitals markedly decreased suggesting that these outbreaks were controlled. However, other hospitals, in the east of France in 2007, and then in the north and east of France in 2008, faced *vanA E. faecium* outbreaks. In the beginning of 2008, spread of *vanB E. faecium* isolates was observed in several hospitals from the north of France.

PFGE analysis revealed clonal diversity among VRE. Generally, a few (one to four) predominant clones and several other clones (up to twelve) spread in an affected hospital. In general, each hospital has specific clones, distinct from those of other hospitals. However, as expected, we observed that, in several cases, strains can spread between neighbouring hospitals that frequently exchange patients. Typing by MLST and subsequent eBURST analysis showed that all typed isolates from the predominant clones in France of the French major clones belonged to hospital-acquired clonal types (clonal complex CC17). Sequence types ST78 and ST18 are the most frequently isolated. The presence of the *esp* and $hyl_{Efm}$ genes is variable.

As already reported for these hospital-acquired *E. faecium* strains, the studied *E. faecium* isolates were highly resistant to ampicillin and fluoroquinolones, no matter whether they contained the *vanA* or the *vanB* gene. Vancomycin resistance was usually expressed at high levels for isolates containing the *vanA* gene. However, a particular clone isolated in Paris had a heterogeneous and low-level expression of vancomycin resistance [44]. High-level resistance to gentamicin was expressed by 59.6% of the tested strains and was associated with specific clones. All isolates were susceptible to linezolid, tigecycline and daptomycin.

In conclusion, *vanA*-carrying *E. faecium* are highly predominant in France although outbreaks due to *vanB E. faecium* recently emerged. Isolates share the characteristics of representatives of the clonal complex of hospital-acquired types (CC17) but sometimes lack the *esp* and $hyl_{Efm}$ genes.

**Central Europe**

Austria has reported resistance data for enterococci to EARSS since 2001. Austrian EARSS data are also included with a more detailed description in the National Antibiotic Resistance and Consumption Report AURES (http://www.ages.at/uploads/media/AURES_2004_04.pdf; accessed 20 October 2008). The number of laboratories participating in EARSS increased annually. In 2006 a total of 33 laboratories participated, serving a balanced mixture of hospitals of primary, secondary and tertiary care and provide a high coverage of the total population (87%; [25]). Resistance to vancomycin is rare; rates of VREfs or VREfm were ≤1% from 2003-2006 with a slight increase for VREfm in 2007 (1.9%). VREfm rates of 4% in 2001 and 5% in 2002 may be related to and thus biased by the low number of participating laboratories in the beginning. There is one report of an outbreak caused by a single VRE clone in a large teaching hospital attributed to inadequate infection control measures [45]. The increasing rate of ampicillin-resistant *E. faecium*, from 67% in 2001 to 89% in 2006, suggests a wide dissemination of hospital-acquired clonal types similar to many other European countries. AURES also reports resistance in indicator bacteria showing that the reservoir of vancomycin resistance among colonising *E. faecalis* and *E. faecium* in animal husbandry (poultry, pigs and cows) is low (<1%).

EARSS data from Germany are based on a varying number of participating laboratories since 1999 and are associated mainly with tertiary care hospitals. The number of participants dropped after 2004 to 15 reporting laboratories in 2006. This corresponds to a catchment population of only 2%. Hence it is questionable how representative those figures are on a national scale and it is important to compare them with data from other surveillance schemes. German EARSS data state an increase in VREfm from 1% in 2001 via 11% in 2004 to 8 % in 2006 rising again to 15% in 2007. It can be expected that rates vary due to annual differences in the number and composition of participating laboratories and do not reflect true epidemiological trends. The percentage of ampicillin-resistant *E. faecium* (AREfm); however, constantly increased to reach a level of >90% after 2004 suggesting wide distribution of hospital-acquired *E. faecium* strains. The prevalence of VREfs remains at <1%.

There are several German resistance surveillance systems reporting vancomycin resistance rates and resistance development in enterococci supporting or adding to the results of EARSS. The longest established surveillance project is that founded by the Paul Ehrlich Society for Chemotherapy Task Force Susceptibility Testing and Resistance (http://www.p-e-g.org/ag_resistenz/main.htm; accessed 20.10.2008). Around 30 laboratories in Germany, Austria (n= 3) and Switzerland (n= 3) participate. Every three years consecutive isolates exclusively from infections (no repeat isolates) are collected for several weeks and antimicrobial resistance is determined using standardised broth microdilution methods. Results for enterococci have been reported since 1990 (for *E. faecium* since 1995). The two main findings showing that rates of VREfs are still below 1% and rates for VREfm increased during the last three studies from 2.7% in 2001 to 13.5% in 2004 and 11.2% in 2007 confirm results of other surveillance schemes (http://www.p-e-g.org/ag_resistenz/main.htm).

Founded in 1999 the German Network for Antimicrobial Resistance Surveillance (GENARS; http://www.genars.de/index.htm) collected data on clinical and surveillance isolates from five to seven major German tertiary care hospitals. All participants use the same methodology (MIC testing by broth microdilution), data/isolates are collected permanently and evaluated biannually. Results for 2002 to 2006 show an increase in the rates of VREfm from 0.9% in the first half of 2002 to 15.3% in the second half of 2006. Vancomycin resistance is rare in *E. faecalis* from GENARS hospitals (<1%).

Increased VREfm prevalence in Germany was first noted in south-western German hospitals in 2003 and marked by several outbreaks in hospitals in Baden-Württemberg. In this context, data from a major laboratory service provider (laboratory Dr. Limbach and colleagues, Heidelberg, Germany) supporting a large number of hospitals in different neighbouring federal states in this area are of special interest. They showed increasing VREfm rates several months before this manifested as a national trend (compared to GENARS and EARSS data). Between the first and second half of 2003 VREfm rates increased threefold (4% versus 13%) whereas the number of sampled *E. faecium* isolates remained constant. About 10% of all sampled enterococci were *E. faecium* (1998: 2.6%; 2002: 3.5%) and VREfm rates vary between 18% and 28% indicating still the highest VRE prevalence in this part of Germany.

In February 2000 an interdisciplinary project called Surveillance of Antibiotic Use and Resistance in Intensive Care Units (SARI) was initiated (www.antibiotika-sari.de). SARI collects data on antibiotic resistance in nosocomial pathogens exclusively from intensive care units (ICU) (n= 47 ICUs from 25 hospitals in 2006) and links them with numbers for antibiotic consumption. Rates for VREfm vary between 0.6% in 2002 and 5.6% in 2005, with a rate of 2.6% in 2007. So far, a definite trend could not be demonstrated in the data and the peak in 2004-2005 was due to VREfm outbreaks in single, participating ICUs in south-west German hospitals. Intriguingly, VRE outbreaks could not be linked statistically to changing antibiotic policies, increasing antibiotic consumption in general or for special substances, change in staffing, changes in infection control measures, etc. Interestingly, the VRE trend did also not follow the MRSA trend in the corresponding SARI ICUs.

Molecular epidemiological investigations of several outbreaks and clusters of infections in German hospitals indicated that clonal spread of different epidemic VREfm strains and lateral gene (plasmid) transfer between unrelated enterococcal recipient strains contributed to increasing VREfm rates (not described in details) [20;46].

Initiatives are currently underway to consolidate the different national surveillance schemes under a single coordinating centre - the Robert Koch Institute- and with funding by the Federal Ministry of Health, Germany. The eventual goal is to combine all efforts into a single national surveillance scheme for antimicrobial resistance and consumption providing up-to-date, reliable and comparable data with high coverage.

For Belgium, 24 laboratories submitted data for enterococci to EARSS. Belgium has had high MRSA rates in recent years and several national initiatives and campaigns have been started to target this problem. According to EARSS data, rates of VREfm increased sharply from 2004 to 2005 from 0 to 14% but decreased again to <1% in 2007. Fluctuations may be related to the varying number of participating laboratories and a few outbreaks during the study period in single institutions [22] that biased the strain collection. The disproportionate numbers for MRSA and VRE rates indicate that high MRSA prevalence over a longer time does not necessarily lead to increasing VRE rates.

Switzerland, not being a member of the European Union (EU), established its own resistance surveillance project called SEARCH (Surveillance of Antibiotic Resistance in Switzerland; http://www.search.ifik.unibe.ch/de/index.shtml). This project was established as part of the National Research Programme NRP49 "Antibiotic Resistance". Corresponding resistance data from 2007 onwards will be integrated into the EARSS platform. SEARCH will be extended later on to data on antibiotic consumption. In general, antibiotic resistance is low in Switzerland. Results for 2007 show 1.5% and 1.1% vancomycin resistance among *E. faecium* and *E. faecalis*, respectively. About 80% of all *E. faecium* isolates are ampicillin-resistant showing wide distribution of hospital-acquired clonal types for Switzerland.

**Southern Europe**

The highest rates of VRE associated with nosocomial infections in Europe were reported in some countries of southern Europe with levels up to 45% detected in recent years in Greece and Portugal [8]. As observed in other geographical regions, *vanA E. faecium* isolates were mainly responsible for the high rates of infections caused by VRE in Greece, Portugal and Italy [8;47-50].

The System for the Surveillance of Antimicrobial Resistance in Greece has provided VRE data to EARSS through the participation of an increasing number of hospital laboratories (n=12 in 2000, n=39 in 2004), mostly associated with hospitals providing secondary care and now covering around 75% of the population [8]. VREfm rates significantly increased from <1% in 2000 to 42% in 2006, with a slight decrease registered in 2007 (37%). As in other European countries, lower glycopeptide resistance rates for *E. faecalis* (<10 %) have been maintained in most years [8]. The few available studies concerning molecular characterisation of Greek VRE described a polyclonal multidrug-resistant *E. faecium* population with hospital-acquired, epidemic strains [47;49]. There is one report of an outbreak caused by a single VREfs clone in a large hospital attributed to inadequate infection control measures [51].

The first large VRE surveillance study in Portugal which included data from ten participating hospitals was performed in 1994 and revealed rates of 1% of VREfs and 9% of VREfm among isolates causing urinary tract and invasive infections [52]. A remarkable increase in VREfm was documented in subsequent years with rates rising from 20% in 1996 (for the same 10 hospitals screened in 1994) to 47% in 2003 [8;53]. Decreasing VREfm rates reported by EARSS in 2007 (29%) may indicate the implementation of successful infection control measures. In Portugal, antibiotic resistance data have been collected by an increasing number of EARSS-participating laboratories: 12 in 2001, 20 in 2006, mostly from tertiary care hospitals providing nowadays a coverage of almost 90% of the total population. Although polyclonality was frequently observed among VREfm, intra- and interhospital dissemination of persisting *E. faecium* and *E. faecalis* clones and specific *vanA* transposon (Tn*1546*) types seemed to have contributed to the rapid and extensive spread of VRE in Portuguese hospitals [48;54;55]. A high proportion of VREfm isolates was also resistant to ampicillin (70 - 74% between 1994 and 2006) [8;52], which together with MLST data suggests wide dissemination of epidemic clones among Portuguese hospitals [48;56; unpublished results].

In Italy, a large multicenter study carried out between 1993 and 1995 reported 9% of *E. faecium* isolates were resistant to vancomycin [57]. Since 2001, the Italian Antibiotico-resistenza-Istituto Superiore di Sanitá has provided VRE data to EARSS through laboratories of secondary care hospitals (35 participating laboratories in 2006 and 49 in 2002), which currently cover around

10% of the population. VREfm rates increased from 15% in 2001 to 24% in 2003, but decreased to 11% in 2007. The frequency of VREfs has increased but has remained below 5% during the entire period (from <1% in 2002 to 4% in 2006) [8]. The first clonal outbreak caused by VRE in Italy was reported in an ICU in 1996 and since then clonal outbreaks have been reported in different hospitals [50;58;59]. Nationwide spread of an *E. faecium vanA* strain causing infections in different cities from 2001 to 2003 was also described [50]. Most VREfm strains associated with human infections which were characterised since 1993 have been multidrug-resistant and have clustered with hospital-acquired clonal types [19;50;60]. Horizontal transfer of Tn*1546* also seemed to contribute to the recent spread of VRE in Italy [61].

EARSS data from Spain have been available since 2001 and are provided by a constant number of approximately 35 laboratories of secondary care hospitals [8]. Rates of VRE in Spain remain among the lowest in EU Member States: <1% of VREfs and 1-3% of VREfm between 2001 and 2003. However, self-limited hospital clonal outbreaks caused by *vanA E. faecalis* have been reported between 1994 and 2006 [62;63]. *VanB E. faecium* clonal outbreaks were initially described in 2001 but remained rare until recently. The description of two large clonal outbreaks caused by *vanB E. faecium* in different cities in the north-west area in 2004 and 2006 and the recent interhospital dissemination of a particular clone deserve attention [64-67]. Representative isolates of most of these outbreak strains belong to *E. faecium* and *E. faecalis* epidemic clonal types (VREfm: CC17 and VREfc: CC2/CC9) [68].

Despite the very low prevalence of VREfm in Spain, a dramatic increase in *E. faecium* resistant to high levels of ampicillin has been detected, rising from 49% in 2001 to 73% in 2006 [8]. These epidemic AREfm strains might facilitate a future increase in VREfm in this country [65;66;69].

**Eastern and south-eastern Europe**

The first reports of VRE in Poland date back to the second half of the 1990s when the first vancomycin- and teicoplanin-resistant (*VanA* phenotype) isolates of *E. faecium* were obtained from three patients in the adult haematology ward of Gdansk Medical University in late 1996/early 1997 [70]. All these isolates showed the presence of the *vanA* gene, but were genetically unrelated in PFGE analysis. A subsequent study in the same ward showed that *vanA*-positive *E. faecium* accounted for almost 50% of this species (49 VREfm from 29 patients) [71]. The 1997–1999 VRE outbreaks in the adult and paediatric haematological wards of the Gdansk Medical University showed the involvement of two distinct polymorphs of the *vanA* gene cluster and two types of Tn*1546*-like transposons [72]. These determinants were most probably introduced into the hospital independently, resulting in a complex epidemiological situation involving both horizontal gene transfer among unrelated strains of *E. faecium* and a single isolate of *E. faecalis*, as well as the clonal spread of VRE in the two wards. The first *vanB E. faecium*, harbouring the *vanB2* gene variant, was found in a patient undergoing prolonged vancomycin therapy in an ICU ward of one of Warsaw's hospitals in 1999 [73]. The introduction of appropriate infection control procedures prevented the further spread of VRE within the hospital. During the period of 1999–2000, an outbreak of *vanB* enterococci occurred independently in another Warsaw hospital which specialised in haematological disorders [74]. PFGE and MLST analyses of VREfm and VSEfm recovered concomitantly in the same hospital suggested that the resistance determinant was introduced into a locally persisting strain (unpublished results). Similar to other countries, most of the recorded VRE outbreaks in Poland were caused by *E. faecium* and *E. faecalis*. In contrast, an unusual mixed outbreak of *E. faecium* and *E. raffinosus*, both of which carried the *vanA* gene occurred in 2005 in the haematology, nephrology and surgery wards in Krakow [23]. Despite these sporadic outbreaks and documented local VRE prevalence, EARSS data for Poland do not suggest a general VRE problem in the country. However, data have to be used with caution since coverage and the number of investigated isolates per year is low, especially those for *E. faecium* [8].

In the Czech Republic, systematic screening for VRE in patients hospitalised at the Department of Haemato-Oncology, Olomunc University Hospital (Moravia region), started in 1997 [75], and the first isolates of VRE were identified the same year [76]. Between 1998 and 2002, VRE remained at the level of 4.9 to 6.8% of all enterococcal isolates in the hospital. *E. faecium* of the *vanA*-type were most frequent, almost 80% of all VRE, followed by *vanB E. faecalis*. PFGE and *vanA* cluster analyses showed presence of three major clonal groups of *E. faecium*, of which one predominated in 1998-1999 and another in 2001-2002. Tn*1546* transposon typing confirmed the role of horizontal spread of resistance determinants among these strains and suggested several independent acquisitions of different Tn*1546* variants [76;77]. Locally and country-wide VRE rates increased in subsequent years [8]. VRE screening in samples from the general population and from poultry revealed prevalence outside the nosocomial setting, but there was no molecular evidence to support a recent exchange of strains or their resistance determinants between the animal or human commensal and the nosocomial setting [77-79].

Reliable data for VRE prevalence in Slovakia are missing [8]. Enterococci from slaughtered animals (poultry, swine, cattle) in Hungary from 2001-2004 showed a decreasing VRE prevalence after the discontinuation of avoparcin use since 1998 [80;81]. According to published reports and EARSS data VRE are rarely encountered among Hungarian hospital patients [8;82]. The limited data available to estimate VRE rates for the Baltic countries (Latvia, Lithuania, Estonia) suggest absence of any VRE cases or outbreaks [8]. Reports about VRE cases or outbreaks in hospitals in south-east European EU countries such as Romania and Bulgaria are lacking, data from EARSS show no VRE cases, but data are only provided by a few laboratories with low overall coverage of the population [8]. EARSS data for Slovenia appear comprehensive and demonstrate the country's first VRE cases in 2006.

**The Netherlands – an example of a low prevalence country**

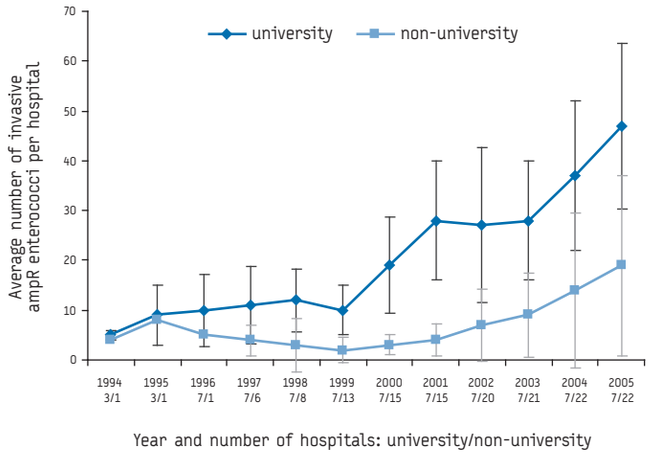
In the Netherlands, antibiotic resistance data from different bacterial species, including VRE, isolated from various clinical specimens like blood and urine are collected in the Electronic Laboratory Surveillance Program - ISIS. Furthermore, an increasing number of laboratories participated in EARSS, rising from eight in 2001 to 23 in 2006, with an estimated coverage of 69% of the Dutch population [8;25]. Despite a few major outbreaks in several hospitals in 2000 [26,83,84], the prevalence of VRE among bloodstream isolates has been consistently low (<1%) over the years, which is probably due to prudent use of antibiotics and a "search and destroy" policy in Dutch hospitals for both VRE and MRSA [25]. Although VRE prevalence rates are low, data from a recent nationwide study revealed a significant increase in invasive AREfm in the Netherlands [85]. Average annual numbers of ampicillin-resistant enterococci from normally sterile body sites

per hospital increased from 5 (standard deviation - SD 1) in 1994 to 25 (SD 21) in 2005. The increase was most pronounced in university hospitals (from 5 SD 1 in 1994 to 47 SD 17 in 2005) (Figure 2). Furthermore, among all enterococcal bacteremias, the proportion of AREfm increased from 4% in 1994 to 20% in 2005. A previous study from the University Medical Center Utrecht (UMCU) revealed that although the overall number of patients with invasive enterococcal infections decreased between 1994 and 2005, the proportion of invasive AREfm increased from 2% in 1994 to 32% in 2005, which suggests replacement of *E. faecalis* by AREfm. In the same study, monthly point-prevalence studies performed to determine the intestinal AREfm reservoir on seven hospital wards revealed carriage rates ranging from 0% in dermatology to 35% in haematology and geriatric wards. In another three-month study performed in the UMCU, ARE acquisition and environmental contamination rates were determined on two wards, haematology and a mixed gastroenterology/nephrology ward, where AREfm are endemic. This study revealed high levels of AREfm acquisition (15-39%) and environmental contamination (22%) in combination with selective antibiotic pressure [86]. In addition, a relatively high number of patients were already colonised with AREfm upon hospital admission, which was most probably due to frequent readmission [86]. Genotyping of the AREfm isolates from the different studies revealed that four genetically related AREfm types emerged nationwide, and that these were distinct from *E. faecium* belonging to the indigenous commensal flora [85;86]. The emergence of hospital-acquired AREfm will impact on the treatment of enterococcal infections. The preferred antibiotic for invasive enterococcal infections, ampicillin, must be replaced by more expensive and toxic antibiotics like vancomycin, linezolid or daptomycin.

The Dutch Working party "Infection Prevention" has developed guidelines with measures to prevent transmission of highly resistant microorganisms (HRMO), including *E. faecium* (http://www.wip.nl/).

**Figure 2**

**Average number of invasive ampicillin-resistant enterococci per hospital in university and non-university hospitals*, the Netherlands, 1994-2005**




Note: Error bars denote standard deviations.
*For each year, the numbers of hospitals that provided data are indicated.
Adapted from [85].

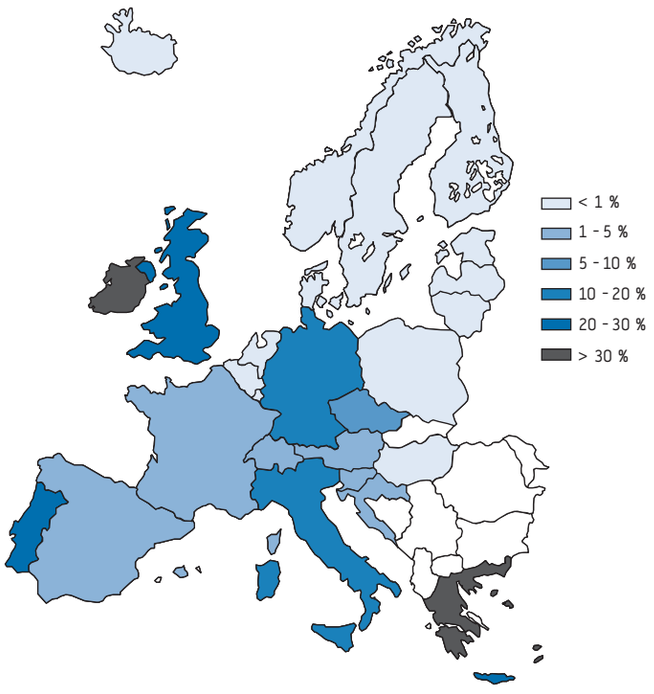
In these guidelines *E. faecium* is considered an HRMO when the strain is resistant to both ampicillin and vancomycin and isolation of patients is indicated only in those cases. Ampicillin-susceptible VREfm isolates are considered animal derived. Isolation of patients with these strains is not indicated, because these strains do not spread in hospitals and ampicillin can still be used as the first choice drug to treat these strains.

## Concluding remarks

Different types of gene clusters encoding vancomycin and partly teicoplanin resistance have been identified in enterococci; the *vanA* and to a lesser extent the *vanB* types are widely prevalent in Europe and worldwide. Both determinants are part of larger mobile genetic structures and thus are transferable via clonal dissemination and lateral gene transfer. On very few occasions, the *vanA* gene cluster has spread to *S. aureus* constituting the first seven cases of vancomycin-resistant *S. aureus* (VRSA); these cases emerged independently in northern America [87;88]. Acquired vancomycin resistance appears to be a serious and growing therapeutic challenge among enterococci all over Europe (Figure 3). Some EU countries have experienced an increasing VRE trend over time (e.g., Ireland, Germany, Greece). In other countries VRE prevalence is still low (e.g., in Nordic countries, the

**Figure 3**

**Prevalence of vancomycin resistance among clinical *Enterococcus faecium* isolates in Europe, 2007**




The estimated rates were mainly based on results of EARSS reporting resistance in invasive (bloodstream) isolates. For single countries also data from other surveillance schemes have been considered and an estimated average prevalence rate is presented. Countries with prevalence data are coloured in light blue, countries with no reliable data are shown in white. See Figure legend code I to VI for vancomycin resistance rates among *E. faecium*. The authors would like to advise using the presented data in this figure cautiously and recommend not to overestimate results for single European countries (see also critical comments stated in the EARSS annual reports (25)).

Netherlands). A few EU Member States showed decreasing VRE rates (e.g., Austria, Portugal, Italy); however, the reasons for this trend remain unclear since it could not be linked unambiguously to definite measures like stricter antibiotic usage patterns, application of alternative antibiotic policies, an activated surveillance or an improved infection control and prevention scheme including hand disinfection. Nevertheless, individual countries' experiences with VRE outbreaks and enhanced understanding of the risk factors associated with VRE acquisition, lead to a wider acceptance of active control and prevention strategies such as VRE screening for "at risk" patients [22;89]. Improvements in VRE diagnostics by extended automated systems, new manual approaches like new agar screening plates supplemented with chromogenic substrates and more reliable screening tests (for instance via real-time PCR) improve the early detection of VRE carriers and cases and thus enable rapid measures to reduce the risk of transmission within the clinical setting [90;91]. The wide distribution of (still) vancomycin-susceptible, but ampicillin-resistant hospital-acquired clonal types of *E. faecium* among hospitals European-wide is worrisome, since *vanA/B* determinants predominantly spread among *E. faecium* and experience from the US and other countries with high VRE rates show that increasing VRE rates follow several years after (vancomycin-susceptible) hospital-acquired *E. faecium* clonal types become established in the clinical environment [7;92]. Early recognition of epidemic *E. faecium* strains is critical but standardised methods for rapid diagnostics are missing. Acquired ampicillin and high-level ciprofloxacin resistance appear as good phenotypic markers of hospital-acquired *E. faecium* strains [7;10;92;93]. However, molecular markers such as the *esp* gene or the *purK1* allele (used as part of the MLST scheme) are not ubiquitous traits of hospital-acquired *E. faecium* strains and failure to detect them does not reliably indicate a strain with limited spreading or pathogenic potential [12;20;60;94]. There is an urgent need for a reliable and rapid molecular test to differentiate commensal from hospital-acquired strains; results from comparative genomic hybridisations and genome sequencing projects may come up with some promising candidate determinants [9;95].

The situation regarding VRE in Europe is diverse with prevalences ranging from <1 to >40% and many aspects of VRE acquisition and spread are still unknown. On one side we find increasing numbers of epidemic strains and mobile resistance determinants and on the other side a hospital environment with a permanently growing patient population "at risk" for acquiring multi-resistant pathogens. Increasing numbers of such multi-resistant pathogens call for prescription of increasingly more and modern antibiotics leading to a "vicious cycle" of growing resistance development. Countries, regions and hospitals with low VRE prevalence are advised implement a strict "search and destroy"-like policy – experience gained from MRSA and other hospital-acquired pathogens has taught us that multi-resistant pathogens can only be partly controlled once established in the nosocomial setting. While great efforts can be rewarded by decreases in prevalence of resistance, it is probably unlikely ever to return to 0%.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# Review articles

## Increasing prevalence of ESBL-producing Enterobacteriaceae in Europe

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Extended-spectrum beta-lactamases (ESBLs) have been increasingly reported in Europe since their first description in 1983. During the 1990s, they were described mainly as members of the TEM- and SHV-beta-lactamase families in *Klebsiella pneumoniae* causing nosocomial outbreaks. Nowadays, they are mostly found in *Escherichia coli* that cause community-acquired infections and with increasing frequency contain CTX-M enzymes. Dissemination of specific clones or clonal groups and epidemic plasmids in community and nosocomial settings has been the main reason for the increase in most of the widespread ESBLs belonging to the TEM (TEM-24, TEM-4, TEM-52), SHV (SHV-5, SHV-12) and CTX-M (CTX-M-9, CTX-M-3, CTX-M-14 or CTX-M-15) families in Europe. Co-selection with other resistances, especially to fluoroquinolones, aminoglycosides and sulfonamides, seems to have contributed to the problem. The emergence of epidemic clones harbouring several beta-lactamases simultaneously (ESBLs, metallo-beta-lactamases or cephamycinases) and of new mechanisms of resistance to fluoroquinolones and aminoglycosides warrants future surveillance studies.

### Introduction

Enterobacteriaceae have become one of the most important causes of nosocomial and community acquired infections. Beta-lactams (mainly extended-spectrum cephalosporins and carbapenems) and fluoroquinolones constitute the main therapeutic choices to treat infections caused by these microorganisms. However, resistance to these compounds has been reported more and more frequently in Europe in the past years [1-5].

Acquired resistance to beta-lactams is mainly mediated by extended-spectrum beta-lactamases (ESBLs) that confer bacterial resistance to all beta-lactams except carbapenems and cephamycins, which are inhibited by other beta-lactamase inhibitors such as clavulanic acid. A shift in the distribution of different ESBLs has recently occurred in Europe, with a dramatic increase of CTX-M enzymes over TEM and SHV variants. Other non-TEM, non-SHV enzymes, such as PER, GES, IBC or certain OXA types, have also been found in some European countries [1]. Although ESBLs still constitute the first cause of resistance to beta-lactams among Enterobacteriaceae, other "new beta-lactamases" conferring resistance to carbapenems, such as metallo-beta-lactamases

**Table 1**

**Global surveillance studies covering Europe and including ESBL-producing bacterial isolates**

| Surveillance Study | Date (Year) | Countries (no.) | Centres (no.) | Sample Origin | Overall frequency (%) | *E. coli* | *K. pneumoniae* | *K. oxytoca* | *P. mirabilis* | *Enterobacter* spp. | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SENTRY | 1997-98 | 15 | 25 | Blood, urine, respiratory tract, wounds, | 4.9 | 1.3 | 18.4 | 12.6 | 5.3 | n.a. | [3] |
| SMART | 2004 | 9 | 31 | Intra-abdominal | | 6.4 | 8.8 | n.a. | n.a. | 11.8 | [4] |
| TEST | 2004-06 | 19 | 62 | Blood, urine, respiratory tract, wounds, sterile fluids | | 7.6 | 13.3 | n.a. | n.a. | n.a. | [5] |
| MYSTIC | 2006 | 12 | 40 | Blood culture, urine, sputum, sterile fluids, wounds | 5.6 | 8.2 | 9.8 | n.a. | 1.4 | n.a. | [6] |
| EARSS | 2006 | 31 | ca. 800 | Blood | | <1-41 | 0-91 | n.a. | n.a. | n.a. | - |

ESBL: Extended-spectrum beta-lactamases; SMART: Study for Monitoring Antimicrobial Resistance Trends; TEST: Tigecycline Evaluation and Surveillance Trial; MYSTIC: Meropenem Yearly Susceptibility Test Information Collection; EARSS: European Antibiotic Resistance Surveillance System (http://www.rivm.nl./earss/).
n.a.: not available.

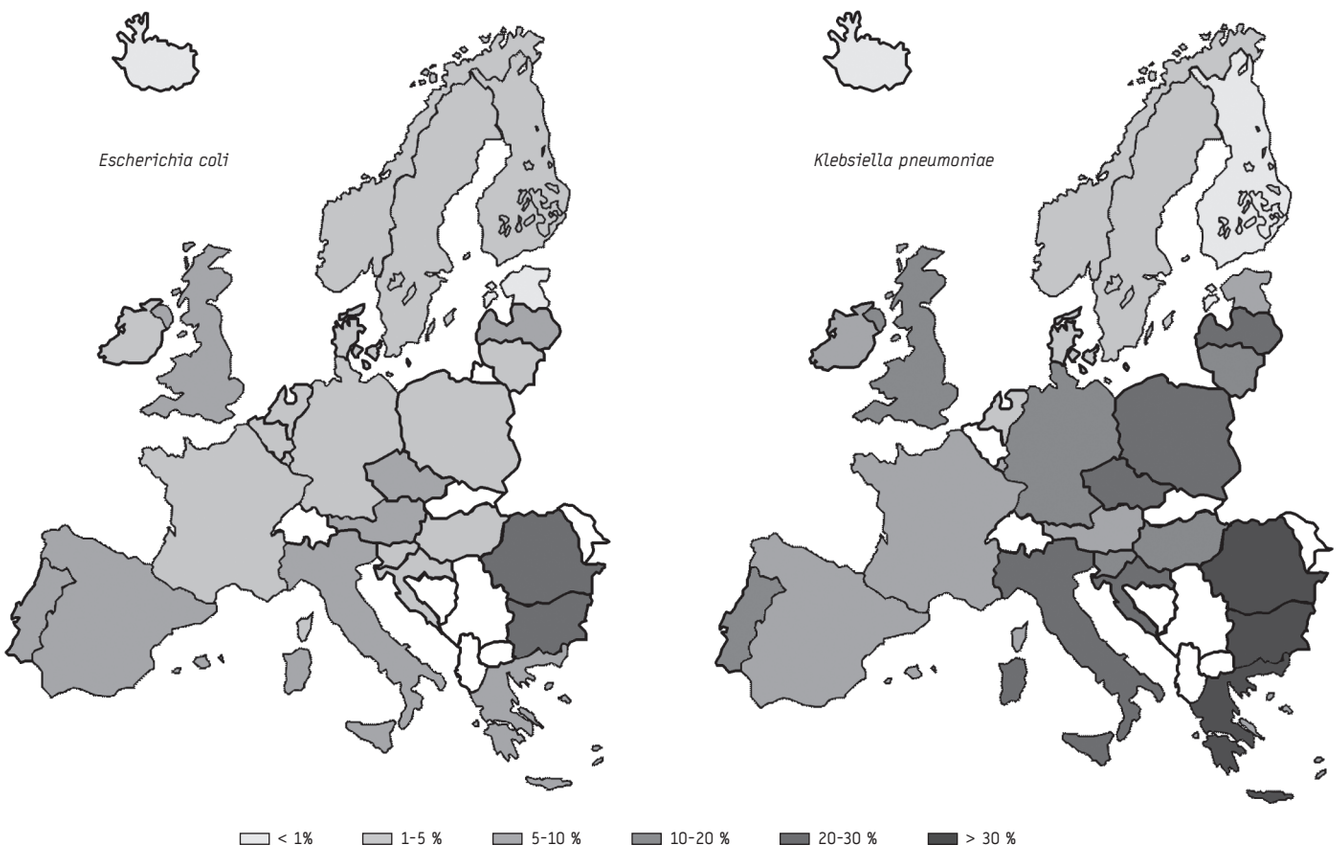
(MBL) and KPC carbapenemases, or to cephamycins, such as CMY enzymes, have more recently emerged and are often associated with ESBLs (see section *Epidemiology of ESBL in Europe*).

Overall data on resistance to third generation cephalosporins, mainly due to ESBL, in Europe have been provided by the European Antibiotic Resistance Surveillance System (EARSS; http://www.rivm.nl/earss/) and other international surveillance systems (Table 1). In addition to a large number of detailed molecular analyses on particular ESBL types, multicentre studies performed in hospitals, farms, or slaughterhouses, using different surveillance systems in each country, have contributed to a better understanding of the epidemiology of these enzymes at local, national and international level. The current increase in ESBL-producing bacteria in inpatients as well as outpatients at the time of hospital admission points towards a continent-wide rise, mainly in *Escherichia coli*, with great variations in the occurrence and distribution of different ESBLs among countries (see section *Epidemiology of ESBL in Europe*). A community-origin explaining this rise has been highlighted in many surveys, but the prevalence of ESBLs in this setting is difficult to ascertain accurately, as faecal colonisation surveys among humans without direct or indirect hospital exposure are scarce (see section *Faecal colonisation surveillance studies*).

Antibiotic overuse in humans and animals, hospital cross-infection, the food chain, trade and human migration seem to have contributed to the recent dissemination of ESBLs outside hospitals, although the role of these factors is variable and linked to particular epidemiological situations (see sections *Epidemiology of ESBL in Europe and ESBLs in non-humans hosts*). Recent studies have demonstrated the clonal expansion of certain enterobacterial clones that are able to acquire multiple ESBL plasmids (see section *Clonal expansion of ESBL-producing Enterobacteriaceae*). These successful clones seem to have favoured the expansion of ESBLs on our continent, as exemplified by the highly virulent *E. coli* O25:H4-ST131, a strain that is thought to be responsible for the pandemic dissemination of the CTX-M-15 enzyme. The origin of widespread *E. coli* clonal complexes is still unknown, although it is likely that the resistance they exhibit against trimetoprim-sulfamethoxazole or fluoroquinolones is due to a strong selection pressure prior to ESBL acquisition (see section *Clonal expansion of ESBL-producing Enterobacteriaceae*). Plasmid dissemination also plays a critical role in the wide spread of ESBL in Europe (see section *The impact of plasmid transfer on ESBL-producing Enterobacteriaceae*). The increasing description of isolates simultaneously containing ESBLs, carbapenemases, CMY or new mechanisms of resistance to fluoroquinolones and aminoglycosides is of concern (see section *Multi-resistance profiles*

**Figure 1**

**Proportion of invasive *Escherichia coli* and *Klebsiella pneumoniae* isolates resistant to third generation cephalosporins in 2006 (EARSS study)**




EARRS: European Antibiotic Resistance Surveillance System

*in ESBL producing isolates*). In this review, we summarise the more recent findings on ESBL epidemiology in Europe in order to understand the recent increase in hospitals and in the community, and to implement appropriate intervention strategies to avoid their pandemic dissemination as has happened with certain Gram-positive organisms such as methicillin-resistant *Staphylococcus aureus* or vancomycin-resistant *Enterococcus faecium*.

## Epidemiology of ESBL in Europe

### General surveillance studies

European and intercontinental surveillance studies have collected data on ESBL-producing Enterobacteriaceae in Europe, all of which consistently show a variable proportion among different geographic locations, enterobacterial species and isolates from different sources (Table 1, Figure 1). Some of them allow comparison with non-European geographic areas, such as the TEST (Tigecycline Evaluation and Surveillance Trial) or SMART (Study for Monitoring Antimicrobial Resistance Trends) [4], which showed that ESBL were far less frequent in Europe than in Latin America and Asia/Pacific regions but more common than in North America (Figure 2). However, these studies have not addressed potential differences between hospital and community isolates.

A recent multicentre European study performed in 2005 in settings with a high antibiotic selection pressure such as intensive care units (ICU) gave results similar to those collected by EARSS [7]. That study had been designed to monitor the association between specific antibiotic consumption and antimicrobial resistance, but no clear correlation was found between the two. This was probably due to differences in the prevalence of patients who were colonised with resistant pathogens at admission, and to the different efforts put in place in different ICUs to avoid cross-transmission of these bacteria.

To date, there have not been any specific European multicentre studies addressing the prevalence of ESBL among community isolates, although there have been different efforts at national and local levels. A study performed in Turkey showed a prevalence of 21% ESBL producers among *E. coli* causing community-acquired urinary tract infection (UTI) during 2004 and 2005 [8]. This percentage was higher than the 5.2% observed in a Spanish multicentre study covering 15 microbiology laboratories in 2006 [9]. Moreover, the rate of community-acquired bacteraemias caused by ESBL-producing *E. coli* was 6.5% in Spain, whereas it ranged from 12.9% to 26.8% for *K. pneumoniae* in studies performed in Spain and the United Kingdom (UK) [10-12].

### Faecal colonisation surveillance studies

There are no multicentre studies to address faecal colonisation rates with ESBL-producing isolates in Europe, although this is a common practice in the hospital setting for implementing epidemiological measures to curtail or control their spread. Nevertheless, the rate of inpatients, outpatients and healthy volunteers colonised by ESBL producers has been addressed in a few national studies and provided interesting observations. A Spanish analysis demonstrated that the frequency of faecal carriers had increased from under 1% to 5% among outpatients and from under 1% to 12% among hospitalised patients between 1991 and 2003, with a prevalence of 4% in healthy volunteers during 2004 [13]. It is of interest to note that the ESBL characterised among isolates obtained from faecal carriers was similar to the one obtained in the clinical setting in Spain at the time these studies were performed. This could prove useful for monitoring ESBL trends [14,15]. Nevertheless, these proportions are in contrast with what was found in a study performed among 322 healthy volunteers in the Paris area that did not detect any carriers of ESBLs. However, the same study frequently observed colonisation with prevalent clones that are associated with particular ESBLs but did not actually contain these enzymes [16].

Two other Spanish studies showed that the faecal carriage rate of ESBL-producing *E. coli* in community patients who had UTIs caused by this pathogen was around 70%, which is much higher than that of individuals with infections not associated with ESBLs [17,18]. Interestingly, faecal carriage in the household contacts of infected patients with ESBL-producing *E. coli* ranged from 16.7% to 27.4% in these two studies. This led to the suggestion that faecal colonisation with ESBL-producing bacteria is a risk factor for acquisition of UTI caused by these pathogens and a potential source for transmission among households.

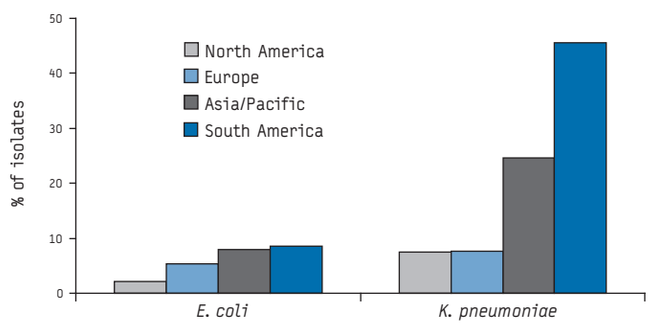
### Geographic differences and ESBL types circulating in European hospitals

The last EARSS report from 2006, covering over 800 laboratories from 31 countries, showed a continuous increase since 2000 in invasive *E. coli* and *K. pneumoniae* isolates resistant to third generation cephalosporins, with prevalences higher than 10% for half of the enrolled countries (Figure 1). In addition, it shows important geographical differences, ranging from a percentage of under 1% (Estonia) to 41% (Romania) for *E. coli* and from 0% (Iceland) to 91% (Romania) for *K. pneumoniae*. Although these proportions are generally associated with the production of ESBL, they might be somewhat overestimated due to the inclusion of isolates with a greater susceptibility to beta-lactams when EUCAST breakpoints are used, or due to isolates overproducing AmpCs which represent about 1-2% of isolates resistant to third generation cepholosporins.

All published studies have confirmed that in most northern European countries, the prevalence of ESBL isolates is still low compared to southern and eastern European countries. Unfortunately, not all publications indicate precise frequency rates, since most

**Figure 2**

**Frequency of ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* isolates reported in the TEST surveillance study (2004-2006) in different geographic areas [27]**




ESBL: extended-spectrum beta-lactamases; TEST: Tigecycline Evaluation and Surveillance Trial.

of them were designed to establish the molecular epidemiology of circulating ESBLs, but not to ascertain the prevalence of these isolates.

***Northern European countries***

In Denmark (www.danmap.org), Norway (www.antibiotikareistens.no) and Sweden (www.strama.se), yearly national surveillance and published studies show continuous rising trends of ESBLs. In the Copenhagen area of Denmark, the occurrence of ESBL producers was below 1% in isolates received at a national reference laboratory, with dominance of CTX-M and SHV enzymes [19]. In Norway, a prospective survey of clinical *E. coli* isolates with reduced susceptibility to oxyimino-cephalosporins demonstrated the dominance of CTX-M-15 (46%) and CTX-M-9-like (30%) enzymes among ESBL-positive *E. coli* and of SHV-5 (47.4%) and SHV-2 (21.0%) among ESBL-positive *K. pneumoniae* isolates [20]. This ESBL distribution is similar to that encountered in Sweden during the period from 2001 to 2006, when 92% of consecutive non-duplicate ESBL-positive *E. coli* isolates expressed a CTX-M-type enzyme, CTX-M-1 being the predominant group [21]. Similar results were found in multicenter studies performed between 2002 and 2004 in Finland [22]. More recently, clonal outbreaks caused by CTX-M-15 *K. pneumoniae* have been reported in Scandinavia [23].

***Southern countries***

The prevalence of ESBL producers in Spain and Portugal has increased over time, with a predominance of CTX-M-producing *E. coli* causing community acquired UTIs [14,24-26]. In Spain, a shift in the proportion of ESBL-producing *Klebsiella* isolates recovered from outpatients (7% to 31%) and ICU patients (41% to 25%) was observed between the periods 1989 to 2000 and 2001 to 2004 [27]. Although a high diversity of ESBLs are reported in most Spanish studies, high local prevalence of CTX-M-9, CTX-M-14, CTX-M-10 and TEM-4 enzymes is observed among inpatients, outpatients and healthy individuals [13,15,17]. In Portugal, nationwide surveys are not available. Studies of individual hospitals reflect a common spread of CTX-M-14, TEM-52, and GES [24,26]. TEM-24, CTX-M-15, CTX-M-32 and SHV-12 are frequently detected in both Spain and Portugal [15,24].

In Italy, the prevalence of ESBL producers among clinical isolates has also increased over the past ten years [28]. The most prevalent ESBL-positive species are *E. coli* among hospitalised patients and *Proteus mirabilis* among outpatients. A predominance of TEM enzymes (45.4%), SHV-12, and the emergence of non-TEM, non-SHV enzymes (CTX-M-type in *E. coli* and *K. pneumoniae*, and PER-type in *P. mirabilis*) has been described. More recent studies performed in single institutions showed the frequent recovery of CTX-M-15-producing *E. coli* and other variants from this group such as CTX-M-1 and CTX-M-32 [29-31].

In France, the prevalence of ESBL production in Enterobacteriaceae reported in different multicentre studies is under 1%, with a progressive increase in the occurrence of CTX-M enzymes linked to *E. coli* expansion [32]. The frequency of certain ESBL producers in 2005 was far lower than reported in previous years including *P. mirabilis* (3.7% versus 1.3%), *Enterobacter aerogenes* (53.5% versus 21.4%) and *K. pneumoniae* (9.4% versus 3.71%), but had increased for *E. coli* (0.2% versus 2%). In addition, ESBLs have frequently been observed in the community setting, linked to nosocomial acquisition [33]. CTX-M-variants were predominant and belonged primarily to the CTX-M-1 (85%) and CTX-M-9 (11.3%). A variety of TEM enzymes has been identified both in hospitals and in the community, although TEM-3 and TEM-24 remain the more common types, they have persistently been recovered since the late 1990s and have often been associated with clonal outbreaks [32,33].

***United Kingdom***

A recent dramatic increase in ESBL-producing organisms is being observed both in hospitals and in the community, mainly caused by the CTX-M-15 enzyme [2]. This enzyme, first reported in the UK in 2003, initially co-existed with CTX-M-9, CTX-M-14, SHV-variants (mainly SHV-12), and to a lesser extent with TEM derivatives both in the hospital and in the community. It has now become the most prevalent enzyme in both settings [2,34].

***Eastern countries***

The occurrence and distribution of ESBLs in this area differs from that in other countries. The prevalence of ESBLs is over 10% in Hungary, Poland, Romania, Russia and Turkey. *K. pneumoniae* is the most frequent ESBL-producing species in Hungary and Russia, and an increase in the percentage of ESBL producers among *K. pneumoniae* isolates has been reported from Poland, Turkey, Bulgaria, and Romania [35-40]. CTX-M-3, SHV-2 and SHV-5 are usually widely spread in eastern European countries.

In Poland, the proportion of ESBL producers in hospitals (11.1%) varied for different species from 2.5% for *E. coli*, 40.4% for *K. pneumoniae* and 70.8% for *Serratia marcescens*, the latter two having a higher prevalence due to outbreak situations. ESBL types were dominated by CTX-Ms (82%, CTX-M-3) and SHV types (17%, SHV-2, SHV-5, and SHV-12), while TEM-like enzymes (<1%, TEM-19 and TEM-48) were found only sporadically. In contrast to other countries, CTX-M-15 was rarely recovered in Poland [35]. The current scenario in Poland differs from that in the late 1990s, when there was a dominance of TEM ESBLs and spread of CTX-M-3 producers all over the country [41,42].

In Bulgaria, hospital outbreaks caused by CTX-M-3, CTX-M-15 and SHV-12 are described, often with an involvement of *S. marcescens* in addition to *K. pneumoniae* [40]. In Hungary, a recent eruptive and extensive spread of highly ciprofloxacin-resistant CTX-M-15 *K. pneumoniae* epidemic clones has been detected [36]. Nosocomial outbreaks involving SHV-2a-producing *K. pneumoniae* are also frequent [38]. In Turkey, CTX-M-15 is widely distributed [8,39], and epidemic strains of *K. pneumoniae* isolates producing the carbapenemase OXA-48 and the ESBLs SHV-12 or CTX-M-15 have emerged [43].

***Predominant ESBLs circulating in Europe***

The emergence and wide spread of the CTX-M-15 enzyme in most European countries, including those with previous low rates of ESBLs, is one of the most relevant findings associated with the current epidemiology of ESBL in Europe [8,14,23,36,44,45]. This enzyme is increasingly being associated with isolates from the community setting, including healthcare centres, as documented in studies from France, Spain, Turkey and the UK, [2,8,14,32,46, see also section *Clonal expansion of ESBL-producing Enterobacteriaceae*].

Other CTX-M variants are amplified locally, such as CTX-M-9 and -10 in Spain [15,25], CTX-M-14 in Portugal and Spain [15,24,47],

CTX-M-3 in eastern countries [35,40] and CTX-M-5 in Belarus and Russia [37]. The SHV-12 enzyme is one of the most prevalent enzymes associated with nosocomial *K. pneumoniae* isolates in Italian, Polish and Spanish hospitals and is also increasingly reported in *E. coli* isolates from community patients [13,31,48]. SHV-5, widely disseminated in Europe, is especially abundant in Bosnia and Herzegovina, Croatia, Greece, Hungary and Poland [35,38,48,49,50].

In addition, particular TEM types deserve special attention as they were traditionally associated with the ICU setting, TEM-3 and TEM-4, are associated with epidemic clones of *K. pneumoniae* in France and Spain, while TEM-24 is associated with epidemic *E. aerogenes* strains in Belgium, France, Portugal and Spain [24,32,33,51]. Nowadays, these enzymes have been also characterised in *E. coli* and *P. mirabilis* recovered in the community [24,33,51]. Finally, TEM-52, first identified in *Salmonella* spp. isolates from animal origin, is currently found among different Enterobactereriaceae species involved in human infections [24,33].

Co-production of different ESBLs is increasingly reported in European countries. Clinical isolates expressing SHV (SHV-5 or SHV-12) or TEM-24 and also other ESBL (CTX-M-9 or CTX-M-14) or carbapenemases (KPC, OXA, or VIM) have been described, sometimes associated with clonal outbreaks [43,49,52-54].

### ESBLs in non-humans hosts

ESBL-producing E.coli and non-typhoidal *Salmonella* species have been isolated from farm animals, wild animals, food, pets and from environmental samples in different European countries [55-59]. The variability in the date of emergence and in the proportion of ESBL producers among animals seem to be due to differences between European countries in cephalosporin usage, and detection method, and to the importation of resistant strains through travellers or trade [59-62].

Different national surveys performed in Italy [63], France [64], the UK [http://www.defra.gov.uk/], Denmark [60], Norway [65] and Spain [57,66] demonstrated that the resistance to broad-spectrum cephalosporins is still low among zoonotic pathogens. However, a recent study performed in Denmark showed that veterinary beta-lactams (amoxicillin, ceftiofur, cefquinome) select for indigenous ESBL-producing *E. coli* in the intestinal flora of pigs and favour the emergence of strains that acquire ESBL genes by horizontal transfer. This selective effect persists for a period longer than the withdrawal time required for these antimicrobials [67]. Although the transmission of ESBL-producing bacteria through the food

**Table 2**

**Plasmids involved in the wide dissemination of specific ESBLs in European countries**

| ESBL | Country | Year | Inc Group | Origin | Species | Reference |
|---|---|---|---|---|---|---|
| **CTX-M-1**[a] | France (10 slaughterhouses, 5 districts) | 2005 | IncI1 | Animals | *E. coli* | [64] |
| **CTX-M-2** | Belgium, France | 2000-2003 | IncHI2 | Poultry flocks, poultry meat, humans | *S. enterica* serovar.Virchow | [68, 98] |
| **CTX-M-3**[b] | Poland | 1996-2005 | IncL/M | Hospitals | *K. pneumoniae, Serratia marcescens, E. coli* | [35, 41, 99] |
| | Bulgaria, Poland, France | | IncL/M | Hospitals | *Different species* | [94] |
| **CTX-M-9** | Spain, UK[c] | 1996-2006 | IncHI2 | Hospitals | *E. coli, Salmonella* | [73, 95, 98] |
| | Spain | 1998-2003 | IncP1-α | Hospitals | *E. coli* | [86, 95] |
| | France | 2003 | IncHI2 | Poultry | *S. enterica* serovar.Virchow | [69, 98] |
| **CTX-M-14** | Spain<br>UK | 1996-2006<br>2004-2005 | IncK<br>IncK | Hospitals<br>Poultry | *E. coli*<br>*E. coli* | [47]<br>[75] |
| **CTX-M-15**[d] | Spain, Portugal, Italy, Turkey, Switzerland, France, Norway, Canada, Kuwait, India | 2000-2007 | IncFII | Hospitals | *E. coli, Klebsiella* | [30, 73, 78, 88] |
| **CTX-M-32** | Spain, Portugal, UK | 2000-2006 | IncN | Hospitals | *E. coli* | [86, 87] |
| **TEM-24** | Spain, Portugal, France, Belgium | | IncA/$C_2$ | Hospitals | *Enterobacter aerogenes, Proteus mirabilis, K.oxytoca* | [51] |
| **TEM-52**[e] | Spain, Portugal, France, The Netherlands, Belgium | 2001-05 | IncI1 | Hospitals, animals | *E. coli, Salmonella* | [65, 70, 76] |
| **SHV-5** | Poland | 1996- | IncFII | Hospitals | *E. coli* | [100] |
| | Hungary | 1998-2003 | Not determined | Hospitals | *K.pneumoniae* | [38] |
| **SHV-12** | Italy | 2005 | IncI1 | Poultry | *E. coli* | [89] |
| | Spain | 2005 | IncI1 | Humans | *E. coli, Klebsiella* | [Valverde, unpublished] |

ESBL: Extended-spectrum beta-lactamases.
([a])The $bla_{CTX-M-1}$ gene has been located on plasmids of incompatibility groups N (among *E. coli* from humans and swine in Spain and Denmark, respectively) and A/C (from Spanish inpatients) [86,98].
([b]) Relationship among these two plasmids has not been published.
([c]) Associated with travel to Spain [73].
([d]) CTX-M-15 plasmids of the group IncI1 have been described among human *Salmonella* Typhimurium isolates in the UK, although their distribution is unknown [73].
([e]) This IncI plasmid has also been associated with $bla_{TEM-20}$ in *E. coli* from Norway and *Salmonella* Paratyphi B dT from the Netherlands [65].

chain or direct contact between humans and animals has seldom been proven [66-68], animals should be considered as an important reservoir of ESBL-strains and highly transmissible plasmids.

ESBLs isolated from animals include different variants belonging to the CTX-M (-1,-2,-3,-8, -9,-13,-14,-15,-24,-28,-32), SHV (-2,-5,-12), and TEM (-52,-106,-116) families. CTX-M-1, TEM-52 and SHV-12 are the ones most commonly found to date. Their dissemination among non-human hosts seems to have been facilitated mainly by mobile conjugative elements [55; Table 2]. The epidemiology of the most prevalent variants in European countries exemplifies different transmission routes and is therefore briefly revised in this section.

The CTX-M-1-like-enzymes (CTX-M-1, -15 and -32) are widely distributed among animals from western European countries and mainly associated with epidemic plasmid spread among clonally unrelated *E. coli* [57,58,62,64,67]. CTX-M-1 is widespread among healthy and sick farm animals (poultry, swine) and pets in Belgium, Denmark, France, Italy, the Netherlands, Portugal and Spain [56-58,62,64,67,71]. It was also the most frequent ESBL in a Belgium survey, representing 27.4% of ESBL producers, some of which were also producing CMY-2 [62]. CTX-M-32 has been detected among healthy and sick animals in Greece, Portugal and Spain [57,58,72]. CTX-M-15, frequently recovered among clinical isolates, has been sporadically identified from pets and farm animals in different countries in the European Union (EU), although it is associated with different strains and plasmids than the ones that are responsible for the wide distribution of this ESBL in hospitals [73].

The CTX-M-9-like enzymes (CTX-M-9 and CTX-M-14) have been linked directly or indirectly with animals in different countries. CTX-M-9 producers have been detected among healthy and sick animals in Spain since 1997 [57,66]. In France, it was found in unrelated poultry isolates of *Salmonella enterica* serotype Virchow collected by the Agence Française de Sécurité Sanitaire des Aliments network in 2003 in a single hatchery located in the southwest of France that supplied different farms with chicks [69]. CTX-M-9 producers have also been linked to food-borne disease outbreaks or colonisation of food handlers in Spain, travellers returning to the UK from Spain and quails imported by Denmark from France [55,67,74]. CTX-M-14-producing *E. coli* or *Salmonella* on the other hand were identified from different slaughter animals in Belgium, Denmark, France, Spain and the UK. It was also linked to travellers returning to the UK from Thailand and to imported chickens in the UK [59,62,67,75].

Epidemic strains of *S. enterica* serotype Virchow producing CTX-M-2 have been isolated from poultry and poultry products in Belgium, France, and the Netherlands since 2000 [61,62,68]. The recent recovery in the UK of *E. coli* producing CTX-M-2 from imported raw chicken meat from Brazil suggests a transmission route from areas where this enzyme is endemic [59].

TEM-52-producing *E. coli* and *Salmonella* isolates have been detected in sick and healthy farm animals, pets, and beef meat food in, Belgium, Denmark, France, Greece, the Netherlands, Spain and the UK [61,70,72]. In Portugal, TEM-52 was widely disseminated among different enterobacterial species recovered from humans, pets, wild animals and livestock [56,58]. In Belgium and France, TEM-52 producers have frequently been isolated from *Salmonella* isolates of different serovars recovered from poultry and humans [70]. It is noteworthy that multidrug-resistant isolates of the serovars Agona (widely distributed in Belgian poultry) and Typhimurium phagotype DT104 (disseminated globally) have been detected which carry both SGI1 and a plasmid-borne ESBL [70]. Not only has clonal transmission involving *Salmonella* Blockey and Hadar been demonstrated within the Netherlands [61], but the joint spread of two epidemic plasmids between countries has been shown in two different studies [70,76]. Importation of animals or meat was the potential source of $bla_{\text{TEM-52}}$ in some areas in the EU [61,77].

SHV-12 producers in animals were detected in Italy during 2005 and 2006, and they were genetically related clones of *Salmonella* Livingstone, scattered on different farms in the northeast of the country, the main region for poultry production [http://www.istat.it; 63]. In Spain, the Netherlands and the UK, SHV-12-positive *Salmonella* and/or *E. coli* isolates have been identified from faecal samples from poultry and pigs [35,57,61,66]. Surprisingly, SHV-12 from animal origin has rarely been described in other European countries.

### Clonal expansion of ESBL-producing Enterobacteriaceae

One of the major factors involved in the current prevalence of ESBL-producing Enterobacteriaceae is clonal spread. The most representative example linked to ESBL-producing Enterobacteriaceae is the recent and fast global dissemination of the highly virulent ciprofloxacin-resistant clone B2-*E. coli* O25:H4-ST131 that causes UTI and is associated with the CTX-M-15 pandaemia. This clone has been detected in the majority of European countries, e.g. France, Greece, Italy, Norway, Portugal, Spain, Switzerland, Turkey, and the UK [8,22,44,45,78]. Interestingly, B2-*E. coli* ST131 is able to acquire multiple resistance mechanisms, and this strain was identified repeatedly, harbouring different CTX-Ms, AmpC or SHV-12 recovered in recent British (2004-2005) and Spanish (2004) multicentre hospital surveys [44, Oteo *et al.*, personal communication]. It was also frequently identified among quinolone-resistant non-ESBL UTI-causing *E. coli* strains in clinical isolates from 10 different countries included in the last ARESC study (2004-2005) as well as in healthy volunteers in the Paris area (2007) [16,46,79]. Other widely distributed quinolone-resistant *E. coli* clones in the EU are responsible for the spread of specific ESBLs, such as A-*E. coli* ST10 or B1-*E. coli*-ST359, ST155, which are mainly identified among CTX-M-14 producers in the central area of Spain [16,47]. These findings suggest that the acquisition of ESBL plasmids by widespread continental fluoroquinolone-resistant *E. coli* clones may have contributed to the dissemination, amplification and persistence of ESBL on our continent.

Nationwide dissemination of particular multidrug-producing *K. pneumoniae* clones has been observed in several countries. In Greece, an endemic SHV-5-producing strain that emerged in the 1990s has recently acquired plasmid-borne VIM-1. This clone is currently spread among Greek hospitals and has also been identified in France [49,80]. Clonal outbreaks caused by *K. pneumoniae* producing SHV-5 and VIM-1 have also been detected in Italy, although a possible link with the Greek clone has not been investigated [54]. A predominance of SHV-type (SHV-5 and SHV-2a)-producing *K. pneumoniae* susceptible to ciprofloxacin is responsible for major clonal outbreaks in Hungarian neonatal ICUs, but endemic or inter-hospital dissemination of these local epidemic clones has not been addressed [38]. Dissemination of

ST11, ST15 and ST147 ciprofloxacin-resistant CTX-M-15-producing *K. pneumoniae* clones has recently been reported from the ICUs of 35 hospitals in 13 counties across Hungary, representing 97% of all CTX-M producers in this country [36,38]. The ST15 *K. pneumoniae* clone has also been identified in ESBL-producing isolates from France, Poland and Portugal, although the real dissemination impact of this clone in these countries is unknown [51]. Long-term persistence (>2 years) of ESBL-producing *K. pneumoniae* has been documented in single institutions in France (TEM-24), Greece (SHV-5), Hungary (SHV-2a), Portugal (GES-1) and Spain (TEM-4, SHV-12) [27,38,81,82]. Only a few sporadic cases of international exchange of epidemic *K. pneumoniae* clones are reported in the literature [80].

Representative examples of clonal expansion in other enterobacterial species include a multidrug-resistant *E. aerogenes* strain widely disseminated in EU hospitals since the 1990s, which is responsible for the spread of TEM-24 in Belgium, France, Portugal and Spain [24,51,83]. This clone can simultaneously carry $bla_{\text{TEM-24}}$ and plasmids encoding different ESBLs ($bla_{\text{SHV-12}}$, $bla_{\text{SHV-5}}$, $bla_{\text{TEM-20}}$) and MBLs ($bla_{\text{IMP-1}}$, $bla_{\text{VIM-2}}$) [84]. An aminoglycoside-resistant *Enterobacter cloacae* clone containing a conjugative plasmid carrying the *qnrA1*, $bla_{\text{CTX-M-9}}$, and *aad*B genes has been detected in 11 of 15 Dutch hospitals and has caused outbreaks in at least four of them [85]. ESBL-producing *P. mirabilis* (TEM-24), *Shigella sonnei*, *S. marcescens* and *Klebsiella oxytoca* have caused clonal outbreaks in different EU countries, although it remains to be elucidated whether they are of more than local significance [24,51,62].

The increasingly frequent description of endemic bacterial strains that are able to acquire genes coding for ESBLs, carbapenemases (VIM, OXA), and AmpC highlights the need to identify and successfully follow up the clones occurring in Europe [43,44,49,53,80,83].

### The impact of plasmid transfer on ESBL-producing Enterobacteriaceae

Currently, the high prevalence of all blaESBL genes in different European regions is caused by horizontal transfer of plasmids among clonally unrelated clones and also among local or international epidemic clones. Plasmid transmission has played a significant role in the persistence of CTX-M-3 in Poland from the late 1990s until today [35,41], the persistence of TEM-4, CTX-M-10, CTX-M-9 and CTX-M-14 in Spanish hospitals since the first description of each enzyme [27,86], and the spread of SHV-5 in hospitals in Greece, Hungary and Poland [38]. Spread of plasmids between countries has been reported for CTX-M-2 (Belgium and France), CTX-M-15 (10 countries), CTX-M-32 (Mediterranean area), TEM-24 and TEM-52 (Belgium, France, Portugal and Spain) [51,68,70,76,78,87,88]. Plasmid-mediated horizontal transfer of $bla_{\text{CTX-M-2}}$ and $bla_{\text{CTX-M-9}}$ genes has been demonstrated between poultry and human *S. enterica* and *E. coli* strains isolated in very different geographical regions [67,68,89]. The predominant plasmids circulating in Europe in both hospitals and the community are listed in Table 2.

The emergence of epidemic strains that simultaneously carry several plasmids encoding distinct ESBLs, AmpC and MBLs is of concern and deserves further follow-up (see above, section *Clonal expansion of ESBL-producing Enterobacteriaceae*).

### Multidrug-resistance profiles in ESBL-producing isolates

ESBL producers are commonly resistant to different antibiotic families including – besides beta-lactams – fluoroquinolones, aminoglycosides and trimetoprim-sulfametoxazole, which contribute to the selection and persistence of multidrug-resistant ESBL strains and plasmids in both clinical and community settings [1,91]. The proportion of ESBL-producing isolates resistant to fluoroquinolones has increased over time, initially in *K. pneumoniae* and later also in *E. coli* [1,89,90]. This increase has apparently occurred in parallel to the increase in plasmid-mediated resistance mechanisms including *Qnr* proteins (*qnrA*, *qnrB* or *qnrS*), acetylases that can affect the action of certain fluroquinolones (*aac(6')-Ib-cr*) or systems pumping fluoroquinolones out of the bacteria (qepA) [92,93].

Very recent studies indicate that the *aac(6')-Ib-cr* gene seems to be confined to *E. coli* ST131 and thus has mainly been linked to CTX-M-15 isolates in different surveys, whereas *qnr* genes are mostly associated with enzymes from the CTX-M-9 or CTX-M-1 groups, which reflects the fact that genes coding for resistance to beta-lactams and quinolones are located on the same plasmid and thus passed on together among different enterobacterial species [79,92].

A high level of fluoroquinolone resistance is often due to additional loss of outer membrane proteins or efflux pump overexpression in clones that already contain *gyrA* and *parC* chromosomal mutations and plasmid-mediated mechanisms [79]. Genes that encode resistance to aminoglycosides (different modifying enzymes and ArmA methylase), trimetoprim or sulfonamides and are located on a wide range of genetic elements such as class 1, 2 and 3 integrons or transposable elements have been associated with different multidrug-resistant ESBL plasmids from human and animal origin [93-96; Curiao *et al.*, unpublished results].

Finally, the recent recovery of plasmids coding for ESBLs that express a low level of resistance to beta-lactams [65] or contain multiple silenced antibiotic resistance genes [97] is of particular concern, as they may serve as reservoirs of antibiotic resistance determinants in bacteria that we are unaware of and that cannot be detected by phenotype.

### Concluding remarks

Increased prevalence of Enterobacteriaceae resistant to extended spectrum beta-lactamases has been reported all over Europe, albeit with a great variability in the occurrence and distribution of ESBL enzymes among different geographic areas. Nordic European countries still show the lowest rates of ESBL prevalence in clinical isolates and have not reported any isolates in animals, while southern and eastern countries present high and increasing frequencies of ESBL-producing strains in both nosocomial and community settings. However, some general epidemiological features such as:

1. the wide representation of CTX-M enzymes, particularly among *E. coli* isolates that cause community-acquired infections,
2. the wide spread of particular successful clones and multidrug-resistant plasmids,
3. and the increasing number of Enterobacteriaceae with ESBLs that also contain MBLs or AmpCs and other new mechanisms of resistance to fluoroquinolones or aminoglycosides indicate that the recent increase of ESBL producers in Europe constitutes a complex multifactorial problem of high public health significance that deserves a deep analysis and the implementation of specific interventions at different levels.

Firstly, the use of broad spectrum cephalosporins and fluoroquinolones in humans and animals should be urgently limited to cases in which other therapeutic alternatives according to evidence-based guidelines are not possible. Limiting antimicrobial use may curtail the selection and persistence of predominant ESBL clones and the probable dissemination of conjugative plasmids among strains, thus decreasing not only the number of potential ESBL donors but also the accumulation of antibiotic resistance genes on common genetic elements.

Secondly, and in accordance with the former recommendation, methods should be improved to efficiently detect and track those bacterial clones and plasmids that constitute the major vehicles for the spread of ESBL-mediated resistance. Ideally, such methods of detection should be accessible to medium-level diagnostic microbiology laboratories, to assure the possibility of performing interventions in real time.

Thirdly, the importation of ESBL-producing bacterial strains through food animals and pets has the potential to cause the wide dissemination of antibiotic resistance among countries and their spread to humans. It highlights the need for national and supra-national public health efforts to implement surveillance, epidemiologic, environmental health, and policy-making components.

Fourth, the implementation of ecological surveillance of ESBL-producing organisms, including environmental (particularly water environments, as sewage) and faecal colonisation surveillance studies in community-based individuals and animals is urgently needed to address the "colonisation pressure" outside hospitals, to detect circulation of highly epidemic clones and to monitor ESBL trends. These ecological studies could be useful as biosensors of modifications in the ESBL landscape.

Fifth, an improvement is needed in the methods for detecting multidrug-resistant ESBL producers that express a low level of resistance to beta-lactams or might contain silenced antibiotic resistance genes not detectable by standard phenotype. Also strongly suggested is a standardisation of beta-lactam breakpoints recommended by the different agencies and committees.

Finally, the scientific and public health community should be aware that the potential interventions directed to control the world-wide spread of ESBL-producing organisms have a limited time-window for effective action. Once a number of thresholds were crossed (critical absolute number of ESBL-genes in the microbial world, critical associations of these genes with wide-spread genetic platforms, critical dissemination of ESBLs among different bacterial species and clones), the control will be simply impossible by applying the standard measures. We should act now, and be prepared for the uncertain future, by promoting innovative ways of controlling ESBL-producing organisms.

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# Review articles

## Emergence of extensively drug-resistant and pandrug-resistant Gram-negative bacilli in Europe

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International and local surveillance networks as well as numerous reports in the biomedical literature provide evidence that the prevalence of antibiotic resistant Gram-negative bacteria is escalating in many European countries. Furthermore, isolates characterised as multidrug-resistant (i.e. resistant to three or more classes of antimicrobials), extensively drug resistant (i.e. resistant to all but one or two classes) or pandrug-resistant (i.e. resistant to all available classes) are increasingly frequently isolated in hospitalised patients causing infections for which no adequate therapeutic options exist. *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* are specifically addressed in this review as the most problematic and often extensively or pandrug-resistant pathogens. According to the available multicentre surveillance studies, the proportion of imipenem-resistant *A. baumannii* strains is reported to be as high as 85% in bloodstream isolates from intensive care unit patients in Greece and 48% in clinical isolates from hospitalised patients in Spain and Turkey. Among 33 European countries participating in the European Antimicrobial Resistance Surveillance System (EARSS) in 2007, six countries reported carbapenem resistance rates of more than 25% among *P. aeruginosa* isolates, the highest rate reported from Greece (51%). According to EARSS, Greece has also the highest resistance rates among *K. pneumoniae*; 46% to carbapenems, 58% to quinolones and 63% to third generation cephalosporins. This review describes the magnitude of antimicrobial resistance in Gram-negative bacteria in Europe highlighting where the efforts of the scientific communities, the academia, the industry and the government should focus in order to confront this threat.

### Introduction

Infections caused by multidrug-resistant bacteria present daily challenges to infectious diseases physicians and their patients throughout the world. During the last decade, the efforts to combat multidrug resistant microorganisms mainly focused on Gram-positive bacteria and drug companies have developed several novel antimicrobial agents to fight these bacteria. Unfortunately, the growing problem of multidrug resistance in Gram-negative bacteria was not paralleled with the development of novel antimicrobials. As a result, there are now a growing number of reports on infections caused by Gram-negative microorganisms for which no adequate therapeutic options exist. This return to the pre-antibiotic era has become a reality in many parts of the world. The present article aims at reviewing the current state of knowledge about mechanisms that bacteria utilise to become extensively or even pandrug resistant and describing their prevalence in European countries, the risk factors for emergence and their consequences with respect to mortality, hospital length of stay and increased hospital costs. Also, currently available therapeutic options are discussed.

### Definitions

The terms "multidrug resistance (MDR)", "extensive drug resistance (XDR)" and "pandrug resistance (PDR)" are increasingly frequently used in the biomedical literature to describe various degrees of antimicrobial resistance among bacteria. Unfortunately, there are currently no internationally accepted definitions for these terms for bacteria other than *Mycobacterium tuberculosis*. As a result, these terms are used arbitrarily creating great confusion among researchers, health care professionals and the public [1]. For the purpose of this review "MDR" will be used to denote isolates resistant to representatives three or more classes of antimicrobial agents, "XDR" those resistant to all but one or two classes and "PDR" as those resistant to all classes of antimicrobial agents available and intrinsically active against the respective species.

We acknowledge that classification of microorganisms according to susceptibility may vary depending on the susceptibility breakpoints applied; there are often important differences between susceptibility breakpoints proposed by different committees so that data on the proportion of resistant isolates in different countries may not be comparable. Also, as new potent antimicrobials are added to our armamentarium, the classification of a microorganism may change from PDR to XDR, so definitions of resistance patterns need continuous update.

Another issue that has recently arisen with the emergence of metallo-beta-lactamases (MBLs) in Enterobacteriaceae is the phenotypic susceptibility of bacteria that harbour the respective antibiotic resistance determinant, i.e. a MBL gene. Currently, official recommendations on how these strains should be reported are lacking. Thus, the true incidence of resistance may be underestimated by surveillance systems that report only resistant isolates.

Finally, the European Antimicrobial Resistance Surveillance System (EARSS) as well as national or international surveillance systems very seldom report data on MDR, XDR or PDR microorganisms, probably because of lack of official definitions for these terms. Resistance to carbapenem in Gram-negative bacteria other than *Stenotrophomonas maltophilia* is probably a good marker for a MDR or even a XDR phenotype because very often it coexists

with resistance to other classes of antimicrobial agents [2]. On the other hand acquired resistance to colistin or polymyxin B in combination with resistance to tigecycline may be a good marker for a PDR phenotype [3]. For these reasons, when available, resistance rates to these antimicrobials are reported in this review.

**Acinetobacter baumannii**

***Clinical relevance***

*Acinetobacter* species are Gram-negative organisms commonly found in the environment. Although previously considered to be relatively avirulent and ignored whenever isolated from clinical specimens, the A. calcoaceticus-baumannii complex is emerging as a problematic, nosocomial pathogen with the propensity to cause outbreaks in the intensive care unit (ICU) setting [4]. It is recognised as the paradigm of MDR, XDR and lately PDR pathogen.

The incidence of severe infection caused by MDR and even XDR *A. baumannii* has been increasing worldwide as a result of: a) its ability to survive in environmental and human reservoirs, b) its aptitude to accumulate resistance mechanisms by acquisition of plasmids, transposons and integrons harbouring different antibiotic resistance genes, c) its intrinsic resistance to many antimicrobials as a result of the interplay between low outer membrane permeability and constitutive expression of efflux pumps [5] and d) intrinsic production of beta-lactamases such as an AmpC-type cephalosporinase and OXA-51/69 variant with carbapenemase properties [6]. Evidence for the "genetic plasticity" of this species was provided by the recent discovery in a French MDR isolate of a 86kb resistance island containing 45 resistance genes and transposons previously identified in Pseudomonas spp., Salmonella spp., and *Escherichia coli* [7].

*Acinetobacter* spp. has been implicated as the cause of serious infectious diseases such as ventilator-associated pneumonia (VAP), urinary tract infection, endocarditis, wound infection, nosocomial meningitis and septicaemia, involving mostly patients with impaired host defences. However, the true frequency of nosocomial infection caused by *Acinetobacter* spp. is difficult to assess because its isolation in clinical specimens may reflect colonisation rather than infection. Some clinicians believe that the recovery of *A. baumannii* in the hospitalised patient is an indicator of the severity of the underlying illness [8]. Nevertheless, according to the SENTRY antimicrobial resistance surveillance program *Acinetobacter* spp. was among the 10 most frequently isolated pathogens causing bloodstream infections in 14 European countries participating in the program from 1997-2002 [9].

A few matched case-control studies have estimated the clinical impact of carbapenem-resistant *A. baumannii* in mortality, length of hospital stay and cost. Most but not all have identified an increased mortality as compared to controls [10-13], most have found an increase in length of hospital stay [10,12,14-16] and one of them detected only increased cost [3,15]. There are currently very few reports on the clinical outcome of patients suffering from infection caused by PDR *A. baumannii*. These suggest that the mortality is high although not as high as expected given the fact that the isolates were resistant to all tested antibiotics, including polymyxins [17].

***Resistance mechanisms***

Resistance to carbapenem in *Acinetobacter* spp. is mediated mainly by class D OXA-type enzymes and less often by acquired IMP and VIM MBLS. Members of OXA-23, OXA-24 and OXA-58 groups have been increasingly isolated in Europe. Additionally, carbapenem resistance has been linked to the loss of outer membrane proteins or up-regulated efflux pumps which likely work together with beta-lactamases to confer resistance to a broad range of antimicrobial agents.

Resistance to colistin is thought to be mediated with modifications of the lipopolysaccharides of the bacterial cell membrane. Decreased susceptibility to tigecycline has been associated with the over-expression of the AdeABC multidrug efflux pump which confers resistance to various classes of antibiotics [4].

***Proportion of resistant strains***

Among *Acinetobacter* spp. derived from 30 European centres from the worldwide collection of SENTRY from 2001 to 2004, the proportion of strains resistant to imipenem, meropenem, ampicillin/sulbactam and polymyxin B was: 26.3, 29.6, 51.6 and 2.7%, respectively [18].

The MYSTIC (Meropenem Yearly Susceptibility Test Information Collection) program reported the antimicrobial susceptibility of 490 *A. baumannii* strains collected in 37 centres in 11 European countries from 1997 to 2000. Against *A. baumannii*, imipenem and meropenem were the most active agents with resistance rates of 16% and 18% respectively (Table 1) but ampicillin/sulbactam and colistin were not tested. There was important geographic variability in resistance rates in different countries. Among 11 participating countries (Belgium, Bulgaria, The Czech Republic, Germany, Italy, Poland, Russia, Sweden, Switzerland, Turkey and the United Kingdom), Turkey showed the highest resistance rates for almost all of the tested antimicrobials, followed by Italy and the UK [19]. The most recent data for 2006 from 40 centres in 12 countries participating in the MYSTIC program revealed a considerable increase in resistance rates for meropenem (43.4%) and imipenem (42.5%) (Table 1) [20].

In Greece, the proportion of imipenem-resistant *A. baumannii* isolates from patients hospitalised between 1996 and 2007 in tertiary care hospitals in several regions of the country rose from 0% to 85.1% (ICUs), 60.4% (medical wards) and 59% (surgical wards) [Greek System for Surveillance of Antimicrobial Resistance (GSSAR): http://www.mednet.gr/whonet/]. Bloodstream isolates from the same dataset exhibited even higher resistance rates [http://www.mednet.gr/whonet/]. The proportion of isolates resistant to various antibiotics in a number of other European countries revealed by local or international surveillance studies are presented in Table 1.

It is important to note that even in countries with low resistance rates the spread of MDR and even XDR or PDR isolates through transfer of patients between European countries is not an unexpected phenomenon. An outbreak of carbapenem-resistant *A. baumannii* was recently described in a burn unit of a Norwegian hospital from a transferred Spanish patient who was identified as the source [21]. A similar outbreak was also described in a Belgian hospital after transfer of two trauma patients from Greece who were colonised with the outbreak strain [22]. An unexpected outbreak of MDR (some of them also XDR) *A. baumannii* associated with casualties from the Iraq conflict was also reported in the UK. These isolates were genotypically indistinguishable from isolates derived from similar sources in the United States (US) [23].

Many smaller-scale studies also document the increase in numbers of carbapenem-resistant *Acinetobacter* spp. A report from the ICUs of a Turkish hospital revealed resistance rates of 80.3% and 71.2% for imipenem and meropenem, respectively in *A. baumannii* isolated from patients suffering from VAP in 2006 [24]. In Bulgaria, a recent report from a single centre suggested that carbapenem-resistance among clinical isolates from ICU patients was 75% [25] while in a UK medical centre a retrospective study on 399 *Acinetobacter* bacteraemias over an eight-year period identified a tremendous increase in carbapenem resistance from 0% in 1998 to 55% in 2006 [26]. An imipenem-resistant clone harbouring OXA-40 is believed to have been endemic for several years in Portuguese hospitals and to be genetically related to an imipenem-resistant clone from Spain [27]. Detailed molecular typing suggested that strains disseminated in Portugal belong to European clone II [28]. Recent reports from the Czech Republic revealed a carbapenem-resistance rate of around 15% in a collection of *A. baumannii* isolated in 2005-2006 from 19 centres. Most of the carbapenem-resistant isolates belonged to European clone II [29].

Three major epidemic European clones have been recognised to date. Clones I and II were responsible for outbreaks in hospitals of countries of north-western Europe. Clone I has also been obtained from Spain, Poland and Italy, whereas clone II has been detected in the Czech Republic Spain, Portugal, France, Greece and Turkey. Clone III was identified in France, Italy, Spain and the Netherlands. These data suggest that these clones are very fit, being virulent and MDR, causing outbreaks that are difficult to control and thus establishing endemicity in hospitals [30].

Often colistin or tigecycline are the only available treatments for XDR *A. baumannii* infections. Unfortunately, resistance to colistin has recently emerged in Europe. The European arm of the SENTRY surveillance program identified 2.7% of polymyxin B-resistant *A. baumannii* isolates collected between 2001-2004 [18]. In a recent surveillance study from Greece, among 100 *A. baumannii* strains derived from ICU patients, 3% were colistin-resistant whereas the minimum inhibitory concentration (MIC) levels of tigecycline ranged from 0.12 µg/ml to 4µg/ml [31]. Sporadic cases of infections caused by colistin-resistant isolates have been increasingly frequently reported from Greece [17,32,33]. A surveillance study performed in 34 centres across UK during 2000 reported a 2% resistance rate to colistin among 443 *A. baumannii* tested while tigecycline MICs ranged from <0.032 µg/ml to 16 µg/ml [34]. Sporadic strains exhibiting colistin resistance have also been reported in Slovakia [35].

*In vitro* activity of tigecycline against MDR strains of *A. baumannii* showed promising results [31,36] but unfortunately occasional reports of resistance emerging during treatment in this species are very disturbing [H. Giamarellou, unpublished data]. In a recent surveillance study from Germany, tigecycline resistance among 215 *A. baumannii* was 6% whereas colistin resistance was 2.8% [37]. Alarmingly high resistance rates to tigecycline (25%) have recently been reported from Turkey [24] but resistance of *Acinetobacter* to tigecycline should be interpreted and reported cautiously because it is medium- and method-dependent [38].

**Table 1**

**Proportion of *Acinetobacter baumannii* isolates exhibiting resistance to various antimicrobial agents; data from European countries**

| Country | Collection period | No of isolates tested | Ceftazidime | Cefepime | Ampicillin/ Sulbactam | Imipenem | Meropenem | Ciprofloxacin | Piperacillin/ Tazobactam | Tobramycin | Amikacin | Polymyxin B | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 11 European countries[a] | 1997-2002 | 490 | 58 | NA[b] | NA | 16 | 18 | 60 | 66 | 40 | NA | NA | 19 |
| 30 European centres | 2001-2004 | 851 | 60.3 | 56.1 | 51.6 | 26.3 | 29.6 | 61.3 | NA | NA | 45 | 2.7 | 18 |
| 12 European countries[c] | 2006 | 433 | 68.8 | NA | NA | 42.5 | 43.4 | 67.9 | 65.1 | 48.4 | 28.6 | NA | 20 |
| Sweden | 2001-2004 | 128 | 79 | NA | NA | 4 | NA | 11 | 60 | 9[d] | NA | NA | 100 |
| Spain | 2000-2003 | 92 | 41.3 | 28.3 | 28.3 | 47.8 | 44.6 | 87 | 70.7 | 56.5 | 37 | NA | 101 |
| Germany | 2004-2008 | 86 | 17.4 | 16.3 | NA | 2.3 | NA | 20[e] | 14 | NA | 7 | NA | 36 |
| Italy | 2004-2008 | 98 | 58.2 | 61.2 | NA | 26.3 | NA | 50[e] | 41.8 | NA | 37.8 | NA | 36 |
| United Kingdom | 2004-2008 | 42 | 50 | 47.6 | NA | 16.7 | NA | 45.2[e] | 45.2 | NA | 14.3 | NA | 36 |
| France | 2004-2008 | 113 | 29.2 | 31.9 | NA | 1.8 | NA | 38.1[e] | 23 | NA | 2.4 | NA | 36 |
| Turkey | 2000-2003 | 779 | 84 | 76 | NA | 48 | 42 | 79 | 82 | 57 | NA | NA | 102 |
| Greece[f] | February 2006 | * | 96.9 | 96.6 | 67.4 | 85 | NA | 97.8 | 95 | 86.6 | 87.3 | NA | GSSAR[g] |

[a] Belgium, Bulgaria, Czech Republic, Germany, Italy, Poland, Russia, Sweden, Switzerland, Turkey, United Kingdom.
[b] NA = not applicable
[c] Belgium, Croatia, Czech Republic, Finland, Germany, Greece, Poland, Russia, Spain, Sweden, Turkey, United Kingdom.
[d] Netilmicin was tested.
[e] Levofloxacin was tested.
[f] Data refers to blood isolates from intensive care unit (ICU).
[g] Greek System for Surveillance of Antimicrobial Resistance, available at: http://www.mednet.gr/whonet/
* The number of isolates submitted to susceptibility testing varied from 46 to 224 depending on the antimicrobial agent.

***Risk factors for resistance***

Risk factors for the acquisition of MDR *A. baumannii* have been studied extensively. A PubMed search comprising 20 years from September 1985 to September 2005, identified 20 case-control studies and in more than half of them antibiotic use was the most common risk factor identified in the multivariate analysis. Carbapenems and third-generation cephalosporins were the most commonly implicated antibiotics, followed by fluoroquinolones, aminoglycosides and metronidazole. The second most commonly identified risk factor in case-control studies was mechanical ventilation described in 25% of studies [39]. Other risk factors included stay in an ICU, length of ICU and hospital stay, the severity of illness, recent surgery, invasive procedures [39-43]. In 27 studies of *A. baumannii* outbreaks that did not include a case-control component, environmental contamination was found to be important in the vast majority of the outbreaks described (20/27 studies).

Implicated items included a variety of medical equipment as well as all possible objects related to patient care, furniture and surfaces in the ward. Contaminated hands of healthcare workers were found to be involved in a significant number of cases, while prior use of antibiotics (mainly carbapenems and cephalosporins) was shown to be important in 20% of the reports (5/27 studies) [39]. In a recent matched case-control study undertaken to evaluate risk factors associated with the isolation of colistin-resistant Gram-negative bacteria (*A. baumannii* or *Pseudomonas aeruginosa*) the only independent risk factor identified in the multivariate analysis was the previous use of colistin [33].

**Pseudomonas aeruginosa**

***Clinical relevance***

*P. aeruginosa* is recognised as a major cause of nosocomial infections associated with invasive devices, mechanical ventilation, burn wounds or surgery in the immunocompromised and the immunocompetent host [44]. *P. aeruginosa* has properties that make it particularly problematic to hospitals, including inherent resistance to many drug classes, the ability to acquire resistance through mutation and a high virulence potential [44-45]. The incidence of *P. aeruginosa* in bloodstream infections in Europe increased slightly from 5.5% to 6.8% between 1997 and 2002, according to the SENTRY Antimicrobial Surveillance Program (1997–2002) where 37 medical centres from 15 European countries participated [9].

Few data exist regarding the outcome of truly PDR infections due to *P. aeruginosa*. A mortality of 80% of patients with colistin-resistant Gram-negative bacilli was noted in a study in Slovakia [35]. In a report from Greece, four of five patients with PDR infections due to *P. aeruginosa* survived [46]; in a later study of the same group with three patients, two survived while the third died but not due to infection [17].

***Resistance mechanisms***

The continuously evolving resistance of *P. aeruginosa* to antibiotics has led to the emergence of clinical isolates susceptible to only one class of antimicrobial agents and eventually to PDR isolates. Extensive drug-resistance in *P. aeruginosa* isolates typically results from convergence of multiple resistance mechanisms [47]. The high intrinsic antibiotic resistance due to low outer membrane permeability, the production of an AmpC beta-lactamase, and the presence of numerous genes coding for different multidrug resistance efflux pumps as well as a high number of acquired resistance genes coding for aminoglycoside-modifying enzymes and beta-lactamases compromises every antibiotic class except the polymyxins [45]. Carbapenem resistance has been also attributed to the production of metallo-beta-lactamases (MBLs), which hydrolyse most beta-lactams except aztreonam, and usually confer high-level resistance [48]. In many European countries, mostly in the Mediterranean area, VIM-type producing *P. aeruginosa* isolates have become endemic during the past eight years [49]. Resistance to colistin in *P. aeruginosa* is rare but has been found [50]. Structural modifications of the outer cell membrane are thought to be responsible for high-level resistance of *P. aeruginosa* to colistin [51].

***Proportion of resistant strains***

According to EARSS data for 2007, *P. aeruginosa* resistance to carbapenems appears to be rather high all over Europe. Denmark, the Netherlands, Switzerland, Sweden and Finland had carbapenems resistance below 10% whereas Croatia, Turkey, Germany, Italy, Czech Republic and Greece above 25% (Table 2) [http://www.rivm.nl/earss/database].

As reported in the EARSS Annual Report for 2006 [http://www.rivm.nl/earss/result/Monitoring_reports/], 18% of *P. aeruginosa* isolates were found to be multidrug-resistant, i.e. resistant to three or more antibiotics from the EARSS protocol. In the EARSS database, the dominant phenotype (6%) in Europe in 2006 was combined resistance to all the five classes of antimicrobials recorded by EARSS (piperacillin, ceftazidime, fluoroquinolones, aminoglycosides and carbapenems). The second and third most common pattern consisted of single resistance phenotypes to either carbapenems (4%) or fluoroquinolones (4%).

In the MYSTIC 2006 results, Turner reported that among 1,012 *P. aeruginosa* isolates collected from 40 European centres, resistance to piperacillin/tazobactam was the lowest (15%), followed by meropenem (22%), amikacin (23%), ceftazidime (25%), gentamicin (29%), imipenem (32%), ciprofloxacin (33%) and tobramycin (35%) [20]. It should be pointed out that countries with the highest resistance rates to carbapenems included Greece, Czech Republic and Bulgaria, which is in line with the EARSS 2006 results.

Compared to imipenem, meropenem was more potent and was active against up to one third of imipenem-resistant strains, which indicates that a considerable percentage of these strains have lost the OprD porin, which is influential mainly against imipenem [44,52,53]. Susceptibility of *P. aeruginosa* tended to increase between 2002 and 2006 for most of the agents tested and especially in eastern Europe where the highest resistance rates were observed [44]. When comparing data for 2006 with those from 2002, there was little change in susceptibility/resistance profiles for meropenem and imipenem, but there was a notable increase in susceptibility (decrease in resistance) to piperacillin/tazobactam (84.9 vs. 79.4%), ceftazidime (75.4 vs. 69.1%), gentamicin (70.7 vs. 50.5%) and ciprofloxacin (67.4 vs. 59.5%) while there was a remarkable decrease in susceptibility (increase in resistance) to tobramycin (64.8 vs. 75.5%) [21].

According to the GSSAR data [http://www.mednet.gr/whonet/], imipenem-resistant *P. aeruginosa* isolates from patients hospitalised between 1996 and 2007 in ICUs, in tertiary care hospitals from

several regions of Greece rose from 25.8% to 54.8%, while in medical and surgical wards rose from 4.7% to 30.3% and 23.2%, respectively. Bacteraemic isolates exhibited even higher resistance rates [http://www.mednet.gr/whonet/].

Although outbreaks of MDR *P. aeruginosa* within and outside ICUs have been an increasingly frequently reported problem in hospitals [40,54,55] and MDR phenotypes have been slowly increasing in prevalence among *P. aeruginosa* [56-59], ongoing regional or national surveillance studies do not routinely report rates of MDR isolates. In many European countries, mostly in the Mediterranean area, highly carbapenem-resistant pseudomonads have become endemic during the past eight years. The most common mechanism of resistance to carbapenems identified among nosocomial *P. aeruginosa* isolates from 2001–2002 was the production of VIM-type MBLs [49]. According to the MYSTIC program conducted from 1997 to 2000, the incidence of MDR *P. aeruginosa* isolates in Europe (nosocomial infections) was 4.7% while in the ICU setting (33 European ICUs) it ranged from 50% in Turkey to ≤3% in Spain, UK, Germany, Bulgaria and Malta [60]. In the SENTRY study conducted from 1997 to 1999, 4.7% of European *P. aeruginosa* isolates were MDR, where MDR was defined as resistance to piperacillin, ceftazidime, imipenem, and gentamicin [61].

Unfortunately, currently colistin is the only available treatment for XDR *P. aeruginosa* infections. According to the SENTRY programme report for 2001–2004, in Europe *P. aeruginosa* isolates exhibited low resistance rates only for polymyxin B (1.1%) [18]. No increase in the isolation frequency of polymyxin-resistant *P. aeruginosa* was observed in the 2001–2004 period [18], despite the recent increased use of polymyxins (polymyxin B and colistin) at some of the sites monitored. In a previous SENTRY report (isolates collected in 1998), polymyxin B resistance was not observed among isolates of *P. aeruginosa* [62]. In Slovakia, an outbreak with PDR *P. aeruginosa* infections in the ICU of a cancer centre in Bratislava was reported, in which 10 patients hospitalised with post-operative peritonitis (wound infection and bacteraemia) were infected with colistin-resistant Gram-negative bacteria [35]. Six of these patients were infected with *P. aeruginosa* with a colistin MIC of ≥4 mg / L, within the context of polymicrobial bacteraemia. Five of these six patients died. All patients had been treated previously with ciprofloxacin and three of them with colistin.

**Table 2**

**Proportion of non-susceptible *Pseudomonas aeruginosa* strains isolated in 33 European countries participating in the European Antimicrobial Resistance Surveillance System (EARSS) in 2007**

| Country | Proportion (%) of strains non-susceptible to: | | | | |
|---|---|---|---|---|---|
| | Aminoglycosides[a] | Carbapenems[b] | Quinolones[c] | Ceftazidime | Piperacillins[d] |
| Austria | 11.2 | 13.7 | 17.9 | 9 | 7.1 |
| Switzerland | 4.8 | 5.4 | 7.2 | 4.2 | 5 |
| Cyprus | 25 | 21.1 | 21.2 | 15.4 | 28.8 |
| Czech Republic | 33.8 | 36 | 42.7 | 32.7 | 30 |
| Germany | 20.3 | 31.5 | 35.7 | 24.4 | 48.5 |
| Denmark | 2.4 | 3.9 | 9.1 | 4 | 4.8 |
| Spain | 23.9 | 18.4 | 27.7 | 15.2 | 8.1 |
| Finland | 8.7 | 9.4 | 10.9 | 7.7 | 7.3 |
| France | 31.1 | 18.4 | 26.3 | 18.6 | 20.5 |
| Greece | 51.9 | 50.5 | 51.9 | 44.8 | 38.4 |
| Croatia | 43.4 | 28.1 | 33 | 20.5 | 30.2 |
| Hungary | 34.4 | 21.3 | 29.5 | 15.3 | 16.8 |
| Ireland | 12.5 | 11.2 | 20.5 | 10.3 | 11.8 |
| Israel | 21.9 | 14.9 | 26.7 | 13.3 | 15.2 |
| Italy | 30.1 | 32.1 | 39.1 | 41.4 | 27.2 |
| The Netherlands | 9.8 | 5.4 | 9.4 | 5.6 | 5.2 |
| Norway | 1.9 | 14.5 | 10.7 | 6.7 | 3.1 |
| Poland | 40.3 | 22.4 | 40.3 | 22.7 | 35.8 |
| Portugal | 18.2 | 16.1 | 23 | 20.9 | 15.8 |
| Sweden | 0 | 9 | 10.3 | 9.6 | 3.1 |
| Slovenia | 13.6 | 20.4 | 18.1 | 13.6 | 12.5 |
| Turkey | 28.2 | 31 | 29.6 | 31.3 | 32.4 |
| United Kingdom | 6.6 | 17.2 | 9.6 | 14.1 | 5.4 |

Source of data: EARSS database, available at: http://www.rivm.nl/earss/database/
Reports with less than 50 isolates are not presented.
[a] Tobramycin or gentamicin was tested.
[b] Imipenem or meropenem was tested.
[c] Ciprofloxacin or ofloxacin or levofloxacin or pefloxacin or norfloxacin was tested.
[d] Piperacillin or piperacillin/tazobactam was tested.

***Risk factors for resistance***

Several studies have found that MDR strains of *P. aeruginosa* typically occur after prolonged exposure to anti-pseudomonal agents [63-65].

A high risk of emerging resistance during treatment with cefotaxime, imipenem, and piperacillin/tazobactam was reported by George et al in a study of the incidence of *P. aeruginosa* resistance to beta-lactam antibiotics in ICU patients [65]. Reported high mortality, elevated MICs and increased development of resistance to antimicrobial agents while on therapy have prompted the publication of guidelines to recommend treatment of *P. aeruginosa* with two pathogen-susceptible antibiotics, although there is limited evidence that combination therapy improves response to treatment [66].

**Enterobacteriaceae**

***Clinical relevance***

Species of the family Enterobacteriaceae are very commonly isolated pathogens from all types of clinical specimens. Among the 15 most prevalent bacterial species in ICU patients of 25 European hospitals in 1997-1998, *Escherichia coli* was the third most frequently isolated pathogen. Among bloodstream isolates, *E. coli* was the third, *Enterobacter* spp. the sixth, *Klebsiella pneumoniae* the eighth and Proteus mirabilis the tenth most frequent pathogen. Among isolates causing nosocomial pneumonia, *E. coli* was the third, *Enterobacter* spp. the fourth, *K. pneumoniae* the sixth and *Serratia* spp. the seventh most common pathogen. In urinary tract infections, *E. coli* ranked first whereas *K. pneumoniae* was the fourth, *Enterobacter* spp. the sixth and P. mirabilis the seventh most commonly found pathogen [67].

Most authors have found that mortality among patients infected by XDR Enterobacteriaceae, mostly carbapenem-resistant isolates, was high [68-71]. Nevertheless, a matched case-control study suggested that mortality of patients infected by carbapenem-resistant *K. pneumoniae* was not statistically significantly different from that of controls (patients infected by carbapenem-susceptible isolates) [72]. An interesting observation by Daikos et al. suggested that the mortality in bloodstream infections caused by VIM-1-producing *K. pneumoniae* exhibiting a MIC ≤4μg/ml was lower than that associated with isolates of MIC>4μg/ml (13.3 vs. 53.8%) but not statistically significantly different from the control group of patients infected with MBL-negative strains. In that report, resistance to carbapenems and a high Acute Physiology and Chronic Health Evaluation (APACHE) II score were independently associated with mortality [72].

Infections by PDR Enterobacteriaceae, although still rare, have been associated with a high mortality. Among 28 patients suffering from PDR infections in Greece from January 2006 to May 2007, the attributable mortality was 33.3% [17].

The isolation of PDR (MBL-positive and colistin-resistant) *K. pneumoniae* was associated with a crude mortality of 100% but with an attributable mortality of 25% in a cohort of patients from Greece [79].

***Resistance mechanisms***

Hyper-production of chromosomal AmpC beta-lactamases as well as the production of extended-spectrum beta-lactamases (ESBLs) confer a MDR phenotype in Enterobacteriaceae. Most ESBLs belong to three major groups: the TEM, the SHV and the CTX-M, with 163, 111 and 82 members, respectively, and are extensively disseminated in Europe [http://www.lahey.org/Studies/].

An XDR phenotype in Enterobacteriaceae is undoubtedly represented by carbapenem resistance which is mainly mediated by MBLs of VIM and IMP-type. The vast majority of MBL genes are carried on plasmids as gene cassettes inserted into class 1 integrons and are usually associated with aminoglycoside resistance genes [49]. Among class A beta-lactamases with carbapenemase activity, the most commonly encountered is KPC which was initially isolated from *K. pneumoniae* in the US [49]. Resistance to colistin in Enterobacteriaceae is mediated by changes in the negatively-charged lipopolysaccharides induced by the regulatory loci *pmrA* and *phoP* [74].

***Proportion of resistant strains***

Among the species belonging to the family Enterobacteriaceae, *K. pneumoniae* has been recognised during the past decade as a problematic pathogen which very often is extensively or even pandrug-resistant XDR or even PDR. According to the most recent 2007 data of EARSS [http://www.rivm.nl/earss/database/], in Enterobacteriaceae family, *K. pneumoniae* is the species with the highest rates of carbapenem resistance. Among 33 European countries, Greece has the highest proportion of this phenotype with 46% of tested isolates in 2007 being non-susceptible to carbapenems (Table 3). According to the GSSAR, in 2007 the rates of carbapenem resistance in *K. pneumoniae* from 40 participating hospitals were: 12.5% in medical wards, 21.1% in surgical wards and 48.8% in ICUs. Among blood isolates the resistance rates were even higher approaching 65% in ICUs. It seems that the current situation in Greece can be explained by the dissemination of VIM-1 producing strains of *K. pneumoniae* that have become endemic in ICUs of many tertiary care hospitals in the country [75]. A steep increase was observed in the proportion of imipenem-resistant *K. pneumoniae* from less than 1% in 2001 when MBL-producing strains first appeared to the above rates in 2007. Accordingly, resistant strains were identified in only three hospitals in 2002, while now they are isolated in at least 25 of the 40 hospitals participating in the network. Interestingly, the proportions of imipenem-resistant enteric bacteria other than *K. pneumoniae* continue to be low despite occasional reports on dissemination of $bla_{VIM}$ to other species [75]. Often the MICs of VIM-producing strains are below the resistance breakpoints obstructing the accurate detection of these strains in routine susceptibility testing. Outbreaks of VIM-1-producing Enterobacteriaceae have been reported recently from Spain [68] and Italy [69]. As was the case with *A. baumannii*, outbreaks of carbapenem-resistant *K. pneumoniae* have also occurred in countries with low-level resistance because of transfer of patients from countries where these strains are prevalent [76].

Contrary to the situation in the US where KPC enzymes prevail among Enterobacteriaceae, emergence of $bla_{KPC}$ was only recently detected in Europe, first in France from a patient transferred from a New York hospital [77] and secondly in Greece [78]. Unpublished observations suggest that in Greece the dissemination of $bla_{KPC}$ in *K. pneumoniae* involves more than one sporadic strain [H. Giamarellou, unpublished data]. Finally, in Turkey the dissemination of OXA-48 carbapenemase among *K. pneumoniae* isolates has been noted in a university hospital since May 2006 [73].

Recently, colistin-resistant and PDR *K. pneumoniae* have been reported from Greece and Slovakia in sporadic cases and multi-cluster outbreaks [35, 46, 79].

### *Risk factors for resistance*

Little has been reported regarding the risk factors for infections caused by XDR or PDR Enterobacteriaceae. In a matched case-control study multivariate analysis showed that antibiotic exposure (quinolones and antipseudomonal penicillins) was an independent risk factor for the development of infections by carbapenem-resistant isolates [80]. In a cohort of patients infected with a MBL-producing Gram-negative microorganism of the family Enterobacteriaceae, the attributable mortality was 18.8%. Sixty percent of those patients had received a carbapenem before isolation of the XDR strain and most of them were already colonised with the MBL-producing pathogen before the diagnosis of the infection [76].

In a recent case-control study by Schwaber et al., poor functional status, ICU stay and receipt of antibiotics (particularly fluoroquinolones) were identified as independent risk factors for carbapenem-resistant *K. pneumoniae* isolation. Carbapenem-resistant *K. pneumoniae* isolation was independently associated with death even after adjusting for severity of illness. In univariate analysis, carbapenem use was strongly predictive of isolation of a carbapenem-resistance pathogen [71].

In a cohort of ICU patients suffering from PDR (MBL-positive and colistin-resistant) *K. pneumoniae* infections, most patients had a long hospital stay and a significant exposure to colistin before the isolation of the PDR isolate. The emergence of colistin resistance was attributed to selection pressure from excessive colistin use in that ICU [72].

## Current therapeutic options

The armamentarium against XDR and PDR Gram-negative microorganisms has almost been exhausted. The only options left are colistin, an antibiotic introduced in the 1950s, and tigecycline, a modified minocycline [4,81]. Nowadays, parenteral colistin which is available as colistin methanesulfonate (CMS) is active *in vitro* against MDR nosocomial *P. aeruginosa*, *Acinetobacter*

**Table 3**

**Proportion of non-susceptible *Klebsiella pneumoniae* strains isolated in 33 European countries participating in the European Antimicrobial Resistance Surveillance System (EARSS) in 2007**

| Country | Proportion (%) of strains non-susceptible to: | | | |
|---|---|---|---|---|
| | Aminoglycosides[a] | Carbapenems[b] | Quinolones[c] | Third generation cephalosporins[d] |
| Austria | 7 | 0.3 | 13.2 | 8 |
| Bulgaria | 58.6 | - | - | - |
| Switzerland | 2.5 | 0 | 5 | 3.1 |
| Cyprus | 15.8 | - | - | - |
| Czech Rep. | 43.5 | 0 | 48.5 | 45.7 |
| Germany | 8.7 | 1.7 | 10.9 | 7.6 |
| Denmark | 6.3 | 0 | 17.1 | 10.8 |
| Estonia | 3.2 | - | 1.8 | 3.2 |
| Spain | 10.1 | 0 | 18.2 | 9.8 |
| Finland | 1.6 | 0 | 2.2 | 1.5 |
| France | 11.6 | 0.1 | 17.5 | 11.6 |
| Greece | 59.8 | 45.9 | 58 | 63.2 |
| Croatia | 39.8 | 0.4 | 34.7 | 40.1 |
| Hungary | 31.6 | 0 | 23.5 | 25.5 |
| Ireland | 11 | 0.6 | 18.7 | 8.9 |
| Israel | 46.4 | 21.9 | 42.6 | 43.7 |
| Italy | 27.7 | 1.7 | 28.7 | 35.2 |
| Netherlands | 8.2 | 0 | 6.5 | 7.4 |
| Norway | 0.6 | 0 | 9.7 | 3.8 |
| Portugal | 12.5 | 0 | 20.5 | 18.2 |
| Sweden | 1.1 | 0 | 10.8 | 1.7 |
| Slovenia | 24.7 | 0.7 | 30 | 28.2 |
| Turkey | 31.7 | 2.2 | 24.5 | 46 |
| United Kingdom | 8.8 | 0.3 | 13.5 | 12.8 |

Source of data: EARSS database, available at: http://www.rivm.nl/earss/database/
Reports with less than 50 isolates are not presented.
[a] Tobramycin or gentamicin was tested.
[b] Imipenem or meropenem was tested.
[c] Ciprofloxacin or ofloxacin or levofloxacin or pefloxacin or norfloxacin was tested.
[d] Cefotaxime or ceftazidime or ceftriaxone or ceftizoxime was tested.

spp., *Stenotrophomonas maltophilia*, *Enterobacter* spp. and *Klebsiella* spp., including ESBL and carbapenemase-producers [81,82]. In patients with normal renal function, CMS is usually given intravenously (i.v.) at a dose of 3,000,000 IU every 8 hours, whereas the intrathecal and the intraventricular doses range from 125,000 to 2,000,000 IU given every 8-12 hours [44,82]. Little information is available on the relationship between pharmacokinetics and pharmacodynamics of colistin in non-cystic fibrosis patients. Recent Greek data from critically ill patients in ICUs revealed a half-elimination period (T1/2) of 14.5 hours indicating the necessity of a loading dose [83]. From 1999 until 2005 in eight clinical retrospective studies CMS was given at a dose of 1-3,000,000 IU every 8 hours for 12-22 days to 335 non-cystic fibrosis patients, 78% of the total representing ICU patients and 55% of the total suffering from pneumonia, 50% of whom had a diagnosis of VAP. In almost all patients either MDR *P. aeruginosa* or MDR *A. baumannii* was isolated in relevant cultures. As a rule, colistin was given in combination with other antibiotics, mostly with a carbapenem. Clinical cure rates ranged between 57-73%, with mortality ranging from 20% to 61.9% whereas nephrotoxicity was documented in 0-37% [84-91]. The largest retrospective well-matched case-control study thus far to assess the efficacy of colistin monotherapy as compared to imipenem in VAP caused by colistin-only-susceptible (n=60) or carbapenem-susceptible (n=60) A. baumanii or *P. aeruginosa* was reported from Tunis [92]. A favorable clinical response was observed in 75% versus 71.7% (P=0.68) without difference in the time to resolution of infectious parameters between the two groups. None of the patients developed renal failure.

Despite the *in vivo* promising results with colistin most of the reported studies share common drawbacks, because: a) they are mostly retrospective without a definite protocol, b) irrespectively of the susceptibilities of the isolated pathogens, other antibiotics were given simultaneously confounding the assessment of its therapeutic efficacy, c) dosing and treatment duration varied widely, and d) resistance development during therapy was not monitored. The recent emergence of colistin-resistant *K. pneumoniae* as well as the selection of intrinsically colistin-resistant *Proteus* spp. and *Providencia* spp. in the Greek ICUs creates an alarm for the clinician who should not loose this last frontier [73]. However, it is evident that well designed, prospective studies with colistin monotherapy at various dosing schedules are urgently required.

Tigecycline is a new semisynthetic glycylcycline approved by the US Food and Drug Administration (FDA) in June 2005. It represents a modified minocycline not affected by the two major determinants of resistance to tetracyclines, that is the active efflux of drug from inside the bacterial cell and the protection of ribosomes [4]. Along with colistin, tigecycline appears to be the most potent agent *in vitro* against *A. baumannii*, and it is also very active against PDR *Klebsiella* strains [31]. However it should be pointed out that it is not active against *P. aeruginosa*. Tigecycline is available only as an i.v. formulation and is administered, after a 100 mg loading dose, at a 50 mg dose as 1-hour infusion every 12 hours. The extensive volume of distribution of tigecycline has confirmed its ability to achieve high levels in many tissue sites including the lung [4]. However, clinical experience with tigecycline is limited and the FDA has granted approval only for complicated intraabdominal and complicated skin and skin structure infections [93,94]. Only three serial studies describing the use of tigecycline, mostly in combination with other antibiotics, in patients with MDR *A. baumannii* and *K. pneumoniae* infections have been published so far with a wide range of successful results, from 50% to 84%. The obtained low levels in blood indicate the necessity of a higher dose in case of bacteraemia, particularly whenever A. baumanii is isolated [95]. The only important side effects of tigecycline are nausea and vomiting in 20-30% of treated patients [93,94].

While approaching the "end of antibiotics" a concerted action by industry, government, and academia is urgently required. In the meantime, clinicians themselves can provide some solution to the problem by the strict application of infection control measures. "Hand hygiene" is considered worldwide to be the cornerstone of nosocomial infection prevention. In a recent article from Greece it was reported that a bed-rail system of alcohol-based hand rub antiseptic improved compliance of health care workers (HCWs) from 36.4% to 51.5% [96]. The authors concluded that a multidisciplinary strategy that consists in a 'set of interventions' including continuous feedback education and motivation of HCWs is necessary to establish a constant hand hygiene practice in health care settings. At the same time infection control policies need to be always reassessed along with personal accountability for application of hand hygiene recommendations. However, antibiotic stewardship seems to be even more important. It has been shown in several studies that increased antibiotic consumption runs in parallel with increased antibiotic resistance [97]. ESAC and EARSS data have recently clearly indicated that south-eastern European countries where the use of carbapenem measured in defined daily doses (DDD) per 1,000 inhabitants and per day is excessive, share also higher rates in *P. aeruginosa* and *K. pneumoniae* resistance rates to carbapenems and subsequently to other broad spectrum beta-lactams [98]. Consequently decreasing antibiotic overconsumption resulted in decreased resistance rates of MDR Gram-negative bacteria in US and European hospitals [97,99]. It is also evident that in order to escape resistance, under-dosing should be avoided and the duration of therapy should be limited. To avoid empiricism the appropriate cultures should be taken and the relationship between pharmacokinetics and pharmacodynamics should be exploited. De-escalation of the administered antibiotics as soon as culture results are ready should remain a quality indicator. The role of the infectious diseases physician is now enhanced since (s) he is a vital resource in the implementation and promotion of the above strategies against resistant pathogens.

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# *Salmonella* Typhimurium: experiences from recent European outbreaks

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Salmonellosis is the second most common foodborne infection in the European Union (EU) with a notification rate of 34.6 cases per 100,000 population in 2006 [1]. The disease mainly causes gastrointestinal symptoms such as fever, diarrhoea, abdominal pain, nausea and vomiting but, depending on the strain and the vulnerability of the host, *Salmonella* infections can lead to septicaemia and sometimes death. Many efforts are therefore made to reduce the human burden of salmonellosis. As humans generally become infected by eating contaminated and insufficiently cooked food, the efforts are focused on EU-wide implementation of stricter control measures within the animal and food sectors. These have proven to be effective as the notification rates have been decreasing in the EU during the last years [1].

In this week's issue of Eurosurveillance, four European countries present recent outbreaks of *Salmonella* Typhimurium. *S.* Typhimurium is one of the two serotypes, the other being *S.* Enteritidis, accounting for the majority of salmonellosis cases in Europe (70-80% of the cases with known serotypes) [1]. The emergence of multidrug-resistant *S.* Typhimurium strains, like the definite phage type (DT) 104, in several EU countries is worrying. It is though debatable whether infections with these strains result in higher hospitalisation rates and/or case-fatality rates than infections with other *Salmonella* strains. In this issue, Doorduyn *et al.* [2] describes an ongoing *S.* Typimurium DT104 outbreak in the Netherlands where more than 20% of the cases were hospitalised. Also *S.* Typhimurium strains fully susceptible to antibiotics can still cause widespread outbreaks. This is presented by Schmid *et al.* [3], Grandesso *et al.* [4] and Ethelberg *et al.* [5] in this issue.

These four papers highlight the importance of molecular subtyping in outbreak investigations, which permits to compare strains within and between countries. In the investigations presented, phage typing, Pulsed Field Gel Electrophoresis (PFGE) and Multiple Loci Variable Number of Tandem Repeats Analysis (MLVA) have been used in different combinations. The results show not only that links exist between the countries, as in the outbreaks described by Switzerland [3] and France [4] and some cases in Denmark, which all seem to be caused by the same strain, but that also several outbreaks of the same serotype but different strains may be ongoing in one country simultaneously [2,3,5].

The impact of international food production and trade on infectious diseases is also worth mentioning in this respect. As shown by Schmid *et al.* [3] and Grandesso *et al.* [4] contaminated food products have the potential to cause widespread outbreaks in several countries. An even more illustrative example of that is the recent foodborne outbreak of *Salmonella* Agona linked to products intended primarily for consumption in the made-to-order sandwich trade. The outbreak resulted in over 160 salmonellosis cases in seven EU countries and had implications for additional European countries where the food product had been distributed [6,7]. In order to detect and minimise the extent of such international events, it is vital to ensure rapid communication between public health authorities in different countries and also with the food authorities. Within the human sector, the European Food- and Waterborne Diseases surveillance network (FWD), coordinated by the European Centre for Disease Prevention and Control (ECDC), has an important function as an informal network to assist in the detection of clusters or outbreaks with international dimensions. This network was used for information sharing in all four outbreaks described in this issue. Sometimes even a single case identified with the same strain in another country could be the key to finding the source, something which Doorduyn *et al.* [2] now will investigate in their case-control study.

**The impact of international food production and trade on infectious diseases is also worth mentioning in this respect.**

Articles published in this issue also present a variety of innovative outbreak investigation methods. Doorduyn *et al.* [2] used food consumption studies differentiated by age groups to support the results of the case interviews in an outbreak primarily affecting children. Grandesso *et al.* [4] used case-case comparisons to identify the food items consumed by cases with a particular strain of *S.* Typhimurium compared to cases with other *S.* Typhimurium strains. Ethelberg *et al.* [5] used an even wider array of methods, including for example focus group interviews, matched case-control studies, cohort studies in point source sub-outbreaks, shopping list analyses, case-case interviews, extensive trace-back analysis including geographical analyses etc. Despite all these efforts, the sources of these outbreaks have not yet been identified although pork products are suspected in several of them. The Danish outbreak, which is still ongoing, is by now the largest salmonellosis outbreak recorded in Denmark since the present surveillance system was put in place in 1980. This shows the difficulties that may be encountered in investigating foodborne outbreaks and pin-

pointing the source, even when the most advanced epidemiological techniques are being used. It is therefore relevant that Schmid *et al.* [3] bring the general issue of food safety legislation into this context and discuss potentials for improvement in this area based on current EU regulations.

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# Editorials

## HIV INFECTIONS AND AIDS: CONTINUOUS VIGILANCE NEEDED TO CONTAIN THE EPIDEMIC

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The 1 December, known as World AIDS Day since 1988, provides an occasion to raise awareness and take stock of the latest developments in the human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS) epidemic. Since it was first recognised in the early 1980s, this epidemic has been associated with high morbidity and mortality. UNAIDS, the Joint United Nations Programme on HIV/AIDS, estimates that in 2007 alone two million people have died of AIDS worldwide, of whom around 270,000 are children under 15 years of age [1]. An additional 33 million people are living with HIV globally; the estimated number of new HIV cases in 2007 was 2.7 million. These figures clearly demonstrate that HIV/AIDS remains a major challenge to public health; therefore measures to contain the epidemic are of paramount importance.

World AIDS Day is an opportunity to shed light on the many activities and initiatives that are being conducted at national and international levels to fight the spread of the disease. From its beginning in late 1995 until now, Eurosurveillance has focussed extensively on HIV/AIDS. The journal has closely monitored the epidemic primarily in Europe but has also reported on worldwide trends. The first publication on HIV/AIDS in March 1996 by F. Cazein et al. entitled Prevalence of HIV-2 infection in Europe [2] was followed by regular annual updates on the situation, and over the years we have covered a wide range of associated aspects of the infection and their impact on public health, such as therapeutic advances including post-exposure prophylaxis, novel testing methods, behavioural factors and prevention measures. In September 2008, a special issue was dedicated to the widespread advances made in Europe in estimating the real number of newly acquired HIV infections based on STARHS (Serological Testing Algorithms for Recent HIV Seroconversion) assays [3]. Next week's issue of the journal will include short communications providing an epidemiological update on the HIV/AIDS situation in Europe while analysing the latest figures on HIV/AIDS surveillance in the WHO European Region, highlighting the situation in intravenous drug users in Europe and reporting on the continuing HIV and other sexually transmitted infection epidemics in the United Kingdom.

**World AIDS Day is an opportunity to shed light on the many activities and initiatives that are being conducted at national and international levels to fight the spread of the disease.**

The driving forces of the HIV/AIDS epidemic are manifold and transmission patterns vary geographically. In the European Union (EU), the predominant transmission mode remains unsafe sex between men, whereas reported heterosexual transmission is in part attributed to persons from high-prevalence countries outside the EU. In eastern Europe and the Baltic States an important driver of the epidemic is intravenous drug use [4,5]. To stop the spread of the disease it is crucial to have a thorough knowledge of the transmission routes and of other factors contributing to the epidemic as well as to ensure access to testing, treatment and care for all. Furthermore, campaigns are needed to raise awareness of the risk of contracting infection and of the possible preventive measures. If such campaigns are to reach their target audiences, they must be tailored to the existing knowledge, attitudes and behaviour in the general population as well as in specific populations at risk. In this issue of Eurosurveillance an article by S.A. Cowan and J. Haff reports on the results of a survey conducted in Denmark in 2006 on HIV and risk behaviour among men who have sex with men (MSM) [6]. The results show that in this group in Denmark, the numbers of sex partners and unsafe sex practices are increasing compared to those of three earlier surveys conducted since 2000. A total of 33% of the respondents had practised unsafe sex, defined as unprotected anal intercourse with one or more partners of different or unknown HIV status. The number of partners was the strongest predictor of unsafe sex; the probability of having had unsafe sex ranged from 17% in men with one partner to 58% in men with more than 20 partners. HIV status was also a strong predictor; in a bivariate analysis, 49% of HIV-positive men had practised unsafe sex compared to 25% of HIV-negative men. The results of this survey demonstrate a clear need to respond to such ongoing risky behaviour in MSM and should be compared with the findings of similar studies in other countries in Europe and taken into consideration when designing targeted prevention campaigns in the future.

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# Editorials

## Malaria in travellers to Gambia

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Malaria incidence is reportedly declining steadily in many parts of the world, including in at least several African countries [1-3]. The incidence of imported malaria is also declining in a number of European countries [4-6]. However, incidence rates in travellers, both European tourists and the so called VFR (visiting friends and relatives) are difficult to estimate, due to problems with the numerator (many cases are not reported) and more importantly with the denominator, for which the information is generally lacking. An exception in the European Union is the United Kingdom (UK), where the International Passenger Survey provides a reliable denominator on the number of travellers to the different countries, duration of stay and reason for travel. Using this information and data on malaria notifications, British authors were recently able to show a steady decrease in the incidence rate of imported malaria from West Africa [6]. In their publication the authors comment that this trend is likely to mirror a true reduction in local malaria transmission, and argue that in some years guidelines on malaria prophylaxis might become less strict even in that part of the world, as it has already been proposed for other continents [7,8]. This time, however, has yet to come. The current issue of Eurosurveillance features two rapid communications about an unpredictable cluster of cases of falciparum malaria among European tourists returning from Gambia [9,10]. The first case reported from Denmark in November 2008, triggered a subsequent flow of notifications from other countries in Europe. Interestingly, many of these are northern European countries. Finland alone accounts for almost one quarter of the total cases. The Finnish cases are described and discussed in detail in the paper by K Valve et al. in this same issue [10]. The UK was the only country reporting more cases than Finland, which is not surprising, as many thousands of travellers from this country visit Gambia every year [6].

It is remarkable that as of 18 December, only three weeks after the first case was noted, we are able to discuss this cluster. Clearly, this would not have been possible with surveillance systems based on mandatory notifications. This emphasizes the usefulness of networks of clinicians such as TropNetEurop that can disseminate information among members very quickly; a characteristic feature that has helped to discover local epidemics of malaria and other tropical diseases in tourist resorts [11,12] before they were picked up by local reporting systems.

To date it is not entirely clear if the cluster represents a true increase in local malaria transmission in Gambia, in contrast to a very recent report [3], or rather a coincidence that cheap (last minute or similar) tourist package holidays to Gambia are offered in several European countries. In connection with such travel, the risk of the disease is often clearly underestimated which results in large numbers of people travelling with no or with ineffective prophylaxis. The Finnish paper, however, indicates that the first hypothesis may be true. The authors state that there is only one travel agency organising package trips to Gambia, and that the number of travellers has not increased. However, no information is available on the use of prophylaxis in previous years [10]. Most patients in the cluster were tourists, while in general, in recent years, VFR accounted for the majority of malaria cases in many (albeit not all) European countries [5]. Gambian coastal and tourist area is comparatively small, and therefore a cluster in tourists might represent a local rather than a countrywide increase in transmission, although the latter cannot be ruled out. I assume that this aspect will soon be elucidated after more in depth evaluation of the situation by local authorities.

**In connection with such travel, the risk of the disease is often clearly underestimated which results in large numbers of people travelling with no or with ineffective prophylaxis.**

Apparently, the Finnish travel agency reacted in a very responsible way to the first notice of an increased malaria risk [10]. Unfortunately, the experience of experts in travel medicine shows that this is not always the case. Several patients in this cluster were counselled not to take any prophylaxis [9]. Lack of prophylaxis caused at least two avoidable malaria deaths and several severe cases requiring intensive care, one case is still suffering from neurological sequelae.

The outbreak emphasizes once more the need to maintain adequate awareness of malaria, even for tourist destinations where this risk is considered to be low or very low: I believe that European countries should consider possible legal implications in cases when travel agencies provide misleading messages.

Health professionals dealing with travel medicine should be aware of the fact that local malaria epidemiology may suddenly vary in countries, and unexpected occurrences should be immediately notified. Sources like TropNetEurop, Geo Sentinel, Promed, Eurosurveillance and others provide invaluable and timely information that is freely available and should be regularly consulted by all professionals giving travel advice.

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# Distribution and antimicrobial susceptibility patterns of *Clostridium difficile* PCR ribotypes in English hospitals, 2007-8

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A surveillance study designed to provide a representative sample of the strains of *Clostridium difficile* causing infections in hospitals in England was in operation from April 2007 to the end of March 2008. Six hundred and seventy-seven isolates were obtained from 186 hospitals in the nine geographical regions of England as recognised by the Health Protection Agency's Regional Microbiology Network. Typing studies revealed that PCR ribotype 027 is now the most common strain isolated from symptomatic patients, accounting for over 41.3% of isolates in English hospitals. Type 106 was the second most common strain (20.2%) and Type 001, which was once the most common strain associated with hospital outbreaks, has now been reduced to only 7.8% of the total. A mixture of 44 other PCR ribotypes accounted for the remaining 28.9% of isolates. This represents a changing distribution of strains when compared to a previous study performed two years earlier which showed roughly equal proportions of types 106, 001 and 027. Antimicrobial susceptibility testing by the E test method revealed significantly lower susceptibility to metronidazole in the more common strains when compared to the less common ribotypes, although none were classified as clinically resistant. Similarly, no resistance to vancomycin was detected. However, common PCR ribotypes were more resistant to moxifloxacin and erythromycin than the less common strains, which may indicate a selective advantage for resistance to these agents, and combined resistance to these two agents was a good indicator of a common ribotype.

## Introduction

Hospital-acquired infections due to *Clostridium difficile* are a major cause of morbidity and mortality in many European countries. The problem is quite acute in the United Kingdom (UK) and the UK government's Department of Health has launched a variety of programmes aimed at tackling the rising number of such in England. One such initiative is an ongoing surveillance scheme to monitor those strains that actually cause disease and to determine their antimicrobial susceptibility patterns. This scheme is run under the auspices of the Regional Microbiology Network of the Health Protection Agency (HPA) in England and the Anaerobe Reference Laboratory (ARL) of the National Public Health Service for Wales.

The first study performed in 2005 showed that three PCR ribotypes known as Types 106, 027 and 001, in roughly equal proportions, were responsible for approximately 75% of all cases of *C. difficile* infection [1]. This second study was designed to identify whether the distribution of strains was changing, or if it was stable.

## Materials and methods

The nine HPA regions took part in the programme that covered the whole of England but did not include Scotland, Wales or Northern Ireland; these run their own surveillance schemes. To collect a statistically valid number of isolates, a sampling framework was drawn up to obtain *C. difficile* isolates from toxin-positive stools from acute hospitals identified within each region that had active cases of *C. difficile* infection. Each of the 52 participating hospitals was allocated one week for sampling within the 12-month study period. The hospitals sampled a maximum of ten toxin-positive stools and submitted them to a Regional HPA laboratory for culture. Sometimes hospitals detected fewer than ten or no cases in their allotted week. No patient data were required and there was no working hypothesis. Putative isolates of *C. difficile* were then referred to the ARL at the University Hospital of Wales in Cardiff for confirmation, PCR ribotyping and susceptibility testing.

The acute hospitals selected to take part in the study by the Regional HPA network tested stool samples for toxins of *C. difficile* by their own chosen methods. Toxin-positive samples were then sent to the nearest Regional HPA laboratory for *C. difficile* culture using a national HPA Standard Operating Procedure [2]. Putative isolates of *C. difficile* were submitted without patient details but with reference numbers identifying both the originating and regional laboratories in batches to the ARL in Cardiff.

Isolates were confirmed as *C. difficile* by a combination of their characteristic odour, colonial fluorescence under long wave ultra-violet light and agglutination of a latex antibody reagent to somatic antigens of *C. difficile* (Microscreen Ltd) [3]. Isolates confirmed as *C. difficile* were then typed by the PCR ribotyping method developed

in Cardiff [4] and compared to the library of ribotypes held by the ARL which currently stands at around 200 types [5].

For the convenience of the methodology involved and to permit testing of many small batches of the isolates as they were received, susceptibility to eight antibiotics was determined using the E test method with an inoculum of McFarland standard 5.0 on Fastidious Anaerobe Agar (Oxoid Ltd) incubated for 48 hours. The antibiotics tested were: metronidazole, vancomycin, erythromycin, imipenem, moxifloxacin, co-amoxyclavulanic acid, penicillin and piperacillin-tazobactam. Minimum inhibitory concentrations of each antibiotic were recorded for each isolate and the minimum inhibitory concentration ($MIC_{50}$, the antibiotic concentration to which 50% of the tested strains are susceptible, and $MIC_{90}$, 90% susceptible) values calculated for each combination of drug and PCR ribotype. Differences in MIC between common and less common types were assessed for statistical significance by Student's unpaired t test

## Results

The figure shows the national distribution of PCR ribotypes identified amongst the 677 isolates obtained in the study. Compared to the results in 2005 there was a 15.4% increase in the percentage of cases due to Type 027, taking it to just over 41%. The percentage of Type 001 cases had fallen by 17.3% to 7.8% and of Type 106 by 6% to 20.2%. Forty-four less common strains accounted for 28.9% of the total, an increase of 6.7% on the figures from 2005.

**Figure**

**National distribution of PCR ribotypes in England, 2007-08 (n=677)**

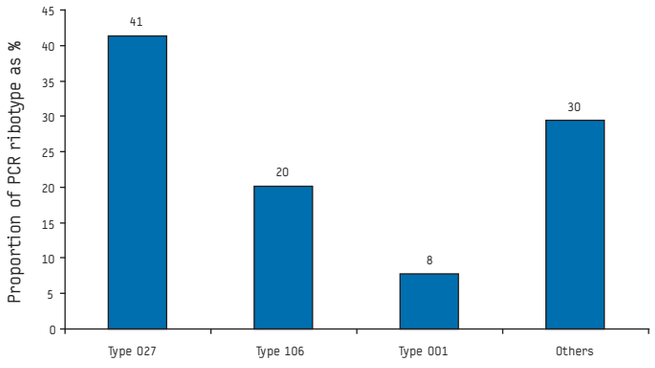


**Table 1**

**$MIC_{50/90}$ results for all *C.difficile* isolates in England, 2007-08 (n=677)**

| | $MIC_{50}$ [mg/l] | $MIC_{90}$ [mg/l] | RANGE [mg/l] |
|---|---|---|---|
| Metronidazole (16mg/l) | 0.38 | 1.0 | 0.064 - 4.0 |
| Vancomycin (4mg/l) | 0.5 | 1.0 | 0.19 -3.0 |
| Erythromycin (4mg/l) | >256 | >256 | 0.032 - >256 |
| Imipenem (16mg/l) | >32 | >32 | 0.75 - >32 |
| Moxifloxacin (4mg/l) | >32 | >32 | 0.5 - >32 |
| Co-amoxyclav (16mg/l) | 0.38 | 0.75 | 0.094 - 3.0 |
| Penicillin (2 mg/l) | 1.0 | 4.0 | 0.38 - >32 |
| Piperacillin-tazobactam (128mg/l) | 4.0 | 8.0 | 0.5 - 32.0 |

**Table 2**

**MICs of the five most common PCR ribotypes of *C. difficile*, England, 2007-08 (n= number of each PCR ribotype tested)**

| Metronidazole | $MIC_{50}$ [mg/l] | $MIC_{90}$ [mg/l] | RANGE [mg/l] | n |
|---|---|---|---|---|
| **Metronidazole** | | | | |
| Type 001 | 0.38 | 0.75 | 0.094 – 2.0 | 53 |
| Type 027 | 0.5 | 1.0 | 0.094 - 4.0 | 280 |
| Type 106 | 0.5 | 1.0 | 0.064 – 3.0 | 137 |
| Type 002 | 0.125 | 0.25 | 0.032 – 0.25 | 27 |
| Type 015 | 0.19 | 0.25 | 0.032 – 0.25 | 25 |
| Others (range only) | | | 0.047 – 0.5 | 155 |
| **Vancomycin** | | | | |
| Type 001 | 0.75 | 2.0 | 0.38 – 3.0 | 53 |
| Type 027 | 0.5 | 0.75 | 0.19 – 2.0 | 280 |
| Type 106 | 0.5 | 1.0 | 0.25 – 2.0 | 137 |
| Type 002 | 0.5 | 1.0 | 0.38 – 1.0 | 27 |
| Type 015 | 0.75 | 0.75 | 0.032 – 1.0 | 25 |
| Others (range only) | | | 0.19 - 1.0 | 155 |
| **Erythromycin** | | | | |
| Type 001 | >256 | >256 | 0.75 - >256 | 53 |
| Type 027 | >256 | >256 | 1.5 - >256 | 280 |
| Type 106 | >256 | >256 | 256 - >256 | 137 |
| Type 002 | 1.5 | 2.0 | 0.5 – 3.0 | 27 |
| Type 015 | 2.0 | 2.0 | 0.19 - >256 | 25 |
| Others (range only) | | | 0.75 - >256 | 155 |
| **Imipenem** | | | | |
| Type 001 | >32 | >32 | 6.0 - >32 | 53 |
| Type 027 | >32 | >32 | 2.0 ->32 | 280 |
| Type 106 | >32 | >32 | 2.0 - >32 | 137 |
| Type 002 | >32 | >32 | 4.0 - >32 | 27 |
| Type 015 | >32 | >32 | 2.0 - >32 | 25 |
| Others (range only) | | | 0.75 - >32 | 155 |
| **Moxifloxacin** | | | | |
| Type 001 | >32 | >32 | 0.75 - >32 | 53 |
| Type 027 | >32 | >32 | 1.0 - >32 | 280 |
| Type 106 | >32 | >32 | 4.0 - >32 | 53 |
| Type 002 | 1.0 | 2.0 | 0.75 – 3.0 | 27 |
| Type 015 | 1.0 | 2.0 | 0.5 – 12.0 | 25 |
| Others (range only) | | | 0.75 - >32 | 155 |
| **Co- amoxyclav** | | | | |
| Type 001 | 0.25 | 0.38 | 0.125 – 0.5 | 53 |
| Type 027 | 0.5 | 0.75 | 0.19 – 1.5 | 280 |
| Type 106 | 0.38 | 0.75 | 0.19 – 3.0 | 137 |
| Type 002 | 0.25 | 0.5 | 0.125 – 0.75 | 27 |
| Type 015 | 0.38 | 0.5 | 0.19 – 0.75 | 25 |
| Others (range only) | | | 0.094 – 1.0 | 155 |
| **Penicillin** | | | | |
| Type 001 | 1.0 | 1.5 | 0.5 – 3.0 | 53 |
| Type 027 | 2.0 | 4.0 | 0.5 - >32 | 280 |
| Type 106 | 0.75 | 4.0 | 0.38 - >32 | 137 |
| Type 002 | 0.75 | 1.0 | 0.38 – 1.5 | 27 |
| Type 015 | 1.0 | 1.5 | 0.5 – 3.0 | 25 |
| Others (range only) | | | 0.38 – 1.0 | 155 |
| **Piperacillin-Tazobactam** | | | | |
| Type 001 | 3.0 | 4.0 | 0.5 – 8.0 | 53 |
| Type 027 | 6.0 | 8.0 | 1.0 – 24.0 | 280 |
| Type 106 | 4.0 | 8.0 | 1.0 – 32.0 | 137 |
| Type 002 | 4.0 | 6.0 | 1.5 – 8.0 | 27 |
| Type 015 | 4.0 | 6.0 | 1.5 – 12.0 | 25 |
| Others (range only) | | | 0.75 – 12.0 | 155 |

Table 1 lists the range of MICs and the MIC50/90 values obtained for the eight antimicrobials tested and (in brackets) the susceptibility breakpoints chosen for each antibiotic. There was no clinical resistance to the drugs of choice for treatment (metronidazole and vancomycin) but high levels of resistance to macrolide, fluoroquinolone and carbapenem agents.

Table 2 lists the MIC values of the five most common PCR ribotypes for each of the eight antibiotics. A control strain of C. perfringens (NCTC 11209) was used to control each drug in each batch of E tests, and the MIC results for this organism never varied by more than one dilution for any of the drugs.

High levels of resistance to erythromycin and moxifloxacin were noted among the common *C. difficile* types (027, 106, 001). Imipenem shows poor activity against all types, whilst co-amoxyclav is highly active against all types.

When analysing the MIC results for metronidazole it was noticed that the MIC values for the three most common *C. difficile* strains, namely Types 027, 106 and 001, appeared higher than those for other PCR ribotypes. The median and mean MIC values of metronidazole were calculated for each of the top ten most common strains and are listed in Table 3.

The difference in mean MICs of metronidazole for the most common PCR ribotypes 027, 106 and 001, compared to types 002, 005, 014, 015, 020, 023, 078, was 0.410 mg/l. This difference between common and uncommon types was statistically significant ($p < 0.0001$) (95% confidence interval (CI) 0.333-0.488) in the unpaired t test statistical analysis.

## Discussion

Compared to a previous study analysing 881 isolates from England obtained in a similar manner in 2005 to 2006 [1], the same three strains of *C. difficile* are predominant but their proportions have changed. The most noticeable change was a drop of over 17% in the prevalence of Type 001, which decreased from 25.1% to 7.8 %. The incidence of Type 027 rose from 25.9% to 41.3%, an increase of 15.4% and Type 106 decreased by 6% from 26.2% to 20.2%. The incidence of other PCR ribotypes rose to 29.5% These results are broadly in agreement with a previous unstructured sampling of ribotypes in English hospitals [6].

**Table 3**

**Median and mean MIC values of PCR ribotypes to metronidazole (Mz), England, 2007-08**

| | Mean Mz MIC [mg/l] | Median Mz MIC [mg/l] |
|---|---|---|
| Type 001 (n=53) | 0.5 | 0.38 |
| Type 027 (n=280) | 0.61 | 0.5 |
| Type 106 (n=137) | 0.58 | 0.5 |
| Type 002 (n=27) | 0.14 | 0.125 |
| Type 005 (n=14) | 0.16 | 0.19 |
| Type 014 (n=20) | 0.18 | 0.19 |
| Type 015 (n=25) | 0.18 | 0.19 |
| Type 020 (n=17) | 0.20 | 0.19 |
| Type 023 (n=13) | 0.09 | 0.094 |
| Type 078 (n=15) | 0.13 | 0.125 |

The emergence and spread of Type 027 in England may be an indication of what may happen in other countries where this strain has been detected since it was first reported in North America and soon after emerged in Stoke Mandeville Hospital in England in 2004. Eurosurveillance has published a number of articles tracking its incidence in outbreaks across Europe [6-8], but to date nationwide surveillance has been conducted only in England to reveal the accurate distribution of this and other ribotypes across the nation. Looking at reports from other European countries [6] it is of interest to note that Type 106 is virtually unique to the UK, although the reason for this is unknown. In England, some regional variation in the distribution of strains has been noticed. For example, Type 001 was the most common isolate in the North East Region of England, but was not found in the East Midlands Region, whereas theYorkshire and Humberside Region showed a greater variety of different ribotypes than any other region.

The breakpoints listed for erythromycin and moxifloxacin (see antibiogram in Table 2) showed widespread resistance amongst the common ribotypes. Importantly, the MIC levels for the antibiotics of choice for treatment (metronidazole and vancomycin) were not indicative of clinical resistance. However, the $MIC_{50}$ and $MIC_{90}$ levels for metronidazole for the common PCR ribotypes 027, 106 and 001 were several dilutions higher and their MIC ranges much larger than those for the less common strains.

The mean and median MIC values to metronidazole for the ten most common PCR ribotypes listed in Table 3. suggest that metronidazole MICs are increasing in common *C. difficile* PCR ribotypes, and this should be closely monitored by further surveillance studies. A recent report by Kuijper et al. on decreased effectiveness of metronidazole treatment [10] is another warning to this effect. Baines et al. suggested that Type 001 in particular had higher MICs than the other common strains, although a different testing methodology was used [11].

There was no evidence of similar elevated MICs for vancomycin among common or non-epidemic ribotypes. Vancomycin MICs for all types ranged from 0.19 to 3.0mg/l. Common PCR ribotypes exhibited much higher MICs to moxifloxacin and erythromycin than the less common strains, which may indicate a selective advantage for resistance to fluoroquinolone and macrolide agents. Combined resistance to these agents is a good indicator of a common ribotype. Imipenem has little activity across all ribotypes, both common and uncommon, and it is probably of little value to continue testing this agent since resistance is so widespread. Co-amoxyclav had a high degree of activity against all types, with MICs ranging from 0.094 to 3.0mg/l. MICs for penicillin ranged from 0.38 to over 32mg/l, but resistance to penicillin did not appear to be related to type. Piperacillin-tazobactam MICs ranged from 0.5 to 32mg/L and the highest values were seen in Type 106.

A limitation of this study is the omission of clindamycin susceptibility data that would have been of interest to compare the susceptibility of Type 027 isolates in the UK with data from other countries. This agent was excluded because it is rarely used in the UK. Nor was it possible to determine seasonal variations since each hospital was allocated only one week to collect toxin-positive stools during the 12-month study period.

The above data fulfil the primary objectives of the study, which were to establish the distribution of the types of *C. difficile*

causing infections in English hospitals and to obtain data on their antimicrobial susceptibilities. These data are of value in our understanding of which strains are dominant in English hospitals, which antimicrobial agents are important in terms of treatment, and which of them may be important in applying antibiotic selective pressure.

A third one-year study funded by the UK Department of Health has just begun testing the same set of antibiotics, and it will be of interest to see if the distribution pattern of PCR ribotypes will change yet again.


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# Tuberculosis in a shopping centre, Portugal, 2004-5

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Genotyping enables to confirm or exclude a tuberculosis (TB) cluster. Excluding the link between cases is particularly important in countries with intermediate/high incidence of TB where the emergence of several TB cases in a particular location in space or time (higher than the expected) could be explained by chance alone.

During 2004 and 2005, five TB cases occurred in five shops of a Portuguese shopping centre which employed a total of about 1000 workers. After an epidemiological survey, 52 close contacts were identified and screened. Latent tuberculosis infection was diagnosed in 10 contacts (eight family members and two work colleagues of cases). Genotyping of the *Mycobacterium tuberculosis* isolates revealed no link between the cases. For this reason no screening of all staff of the shopping centre was carried out. However, close contacts (52) and all fellow workers (1000) were kept under surveillance for two years, and no additional cases were diagnosed.

The present analysis demonstrates that the exclusion of a chain of ongoing transmission by genotyping for the investigation of a cluster is cost-effective from the perspective of the public health service, because it allows to avoid unnecessary large scale screening operation and instead to direct resources to more effective measures of TB control.

## Introduction

Tuberculosis (TB) remains a serious problem worldwide. In Portugal, the incidence of TB is 29.4 per 100,000 inhabitants per year [1], higher than the European Union average of 17 per 100,000 [2]. Contact screening is mostly aimed at identifying family, social and work contacts of cases [3,4]. It is often difficult to decide how far to proceed with screening, particularly if several cases coincide in time and space, which in intermediate/high incidence countries can be due to chance only. A good understanding of the factors affecting the transmission of the disease in the community may result in avoiding the diagnosis of false clusters and directing the resources to more effective measures of TB control.

Molecular typing can help clinicians and public health practitioners to identify or exclude clusters of recently acquired tuberculosis [5,6]. *Mycobacterium tuberculosis* isolates from space or time clusters are expected to show identical or very closely related genotypic patterns [7]. IS6110 restriction fragment length polymorphism (RFLP) has been used as the "gold standard" method for more than a decade [7]. This method provides the highest discriminatory power among *M. tuberculosis* typing techniques, showing sufficient variability to distinguish unrelated strains.

This paper is based on a retrospective review of the investigation of a suspected time–space cluster of cases of TB. During 2004 and 2005, five cases of active tuberculosis were identified among employees of a shopping centre with 172 shops and a total of approximately 1000 workers in Vila Nova de Gaia, south of Porto in north-west of Portugal. The TB diagnosis was based on culture and identification of *M. tuberculosis*. The isolates were confirmed to be fully sensitive. The patients were voluntarily tested for human immunodeficiency virus (HIV) infection and all were negative. All five patients were started on directly observed therapy short-course (DOTS) and all have completed the treatment.

A number of common features were found in the five patients. They all lived in neighbouring districts near the shopping centre and frequented the same food and leisure places. This raised the question of what size of population should be subject to screening.

## Material and methods

An epidemiological survey was performed in order to identify the daily activities of all TB patients. Home, transport, workplace and social settings of the TB cases were described (size of place, ventilation, time and length of exposure, etc.) and contacts were identified. This information was put together to disclose all possible links between the five cases, taking into account the presumed infectious period of the cases, known contacts between the cases, residence, transport used, spatial distribution of the shopping centre and social activities.

Close contacts were defined as household members, co-workers (of the same shop), and persons who had spent more than a cumulative contact time of eight hours in a confined environment with the case during the symptomatic phase (before the diagnosis and the beginning of treatment). All close contacts of TB patients were offered the screening programme, including symptom questionnaire, tuberculin skin testing (TST) and chest X-ray (CXR).

Distant contacts were defined as employees of other shops of the same shopping centre with no known contact with the TB patients or other persons who had only had sporadic contact lasting less than 8 hours with the cases during the symptomatic phase.

All identified contacts (both close and distant) were followed for a two-year period and special attention was given to the identification

of all new cases in the area covering the districts of residence of the cases and the shopping centre to discard possible links with these five cases. The follow-up included clinical examination of all the close contacts screened (52 close contacts) and surveillance of all employees of the shopping centre, including those not considered to be close contacts and therefore not screened. The period of follow-up was two years, as it is known that 10% of cases of latent TB infection develop active tuberculosis, and 5% do so two years after infection [7].

In order to identify the link between the cases and thus provide evidence for further public health decisions, genotyping techniques were used to analyse the clinical strains. Molecular strain typing was performed using the standard method IS6110 RFLP [7]. We used a combination of external and internal standards as positive controls, including a reference strain of *M. tuberculosis* named Mt14323. The latter gives 10 approximately evenly spaced bands of known size. This combination of markers allows extremely precise band molecular size determinations and permits computerised comparisons between strains.

The costs of the screening programme were calculated based on the values published in the Portuguese official journal of legal acts – Diário da República (Republic Diary) 113 1ª série/B published 12 June 2006. All costs are reported in euros and presented in Table 1. TST licensed for Portugal is PPD RT 23, 2 T.U. from the Danish Statens Serum Institute. The delivered price for 10 glass vials, each containing 1.5 ml RT 23, is 149.99 euros. From the 1.5 ml vials, we withdraw 10 test doses of 2 T.U. The charge for the RFLP analysis made by the reference laboratory (Instituto Nacional de Saúde, Laboratório de Tuberculose, Porto) is 149.64 euros.

**Table 1**

**Base-case estimates used in cost analysis of the investigation of tuberculosis cases in a shopping centre in Vila Nova de Gaia, Portugal, 2004-5**

| Public health service (PHS) procedures and other costs (in euros) | Base-case cost estimate (in euros) | Source |
|---|---|---|
| Tuberculin skin test | 15 | PHS* |
| Chest radiography | 9.80 | PHS* |
| Medical consultation (doctor, 25 minutes) | 30 | PHS* |
| Restriction fragment length polymorphism (RFLP) | 149.64 | Laboratory provider |

PHS* Costs of public health service procedures are per test/procedure as listed in Diário da República (Republic Diary) 113 1ª série/B published 12 June 2006

## Results

The five cases' mean age was 31 years (range 28 to 36 years). They all had pulmonary active tuberculosis, based on microbiologic identification. The median time between the onset of symptoms and diagnosis was four months (range 2-9 months). In practice, there was no delay between diagnosis and treatment initiation (diagnoses took one day).

The cases lived in neighbouring boroughs around the shopping centre but did not have any social contact outside the workplace, not even in public transport. They all worked in different shops within the shopping centre but two pairs of cases worked on the same floor. Three cases frequented the same restaurant regularly and four cases went to the same leisure place once a week, but they never met on those occasions.

Fifty-two close contacts (mean 10 per case, range 7-16 contacts) were identified and examined. Close contacts were identified among family (20), close friends (15) and work colleagues (17). Latent tuberculosis infection was diagnosed in 10 contacts (eight among family members and two among work colleagues) and treatment was provided (OR=2.2, 95% CI: 0.4-22, p=0.466). No additional cases of TB infection were diagnosed.

All cultures obtained from cases were regrown on Lowenstein Jensen culture medium slants but only four out of five produced colonies, resulting in the loss of one strain. When growth was considered to have attained an optimal biomass, cells were harvested and inactivated. IS6110 RFLP results revealed that at least four of the five TB cases were caused by strains with different hybridisation patterns thus discarding the possibility of transmission of the disease inside the shopping centre. The fingerprints of the four *M. tuberculosis* isolates investigated are shown in the Figure. Taking into consideration the definition of cluster as two or more strains sharing the same IS6110 RFLP pattern, none of the strains included in this study were clustered, because all patterns were different as demonstrated in Figure.

Therefore, we concluded that the TB cases were not linked and decided not to extend the screening programme beyond the close contacts.

The cost of TB screening in our public health service is 54.8 euros per patient. The total cost of screening of the 17 close contacts identified among the shopping centre employees was 931.6 euros. The additional cost of genotyping of the four *M. tuberculosis* isolates was 598.56 euros. Had we screened all workers of the shopping centre (about 1000 people) we would have spent 54,800 euros.

No additional cases of TB were diagnosed during the two years of follow-up, either among the close contacts who had been screened or other employees of the shopping centre.

**Table 2**

**Characteristics of cases of tuberculosis identified among employees of a shopping centre in Vila Nova de Gaia, Portugal, 2004-5 (n=5)**

| Case | Date of diagnosis | Age at time of diagnosis(years) | Sex | TB site | AFB sputum smears | PCR result | Symptoms |
|---|---|---|---|---|---|---|---|
| 1 | May 2004 | 29 | Female | Pulmonary | Positive | Positive | Yes |
| 2 | July 2004 | 33 | Female | Pulmonary | Positive | Positive | Yes |
| 3 | Sep 2005 | 28 | Female | Pulmonary | Positive | Positive | Yes |
| 4 | Oct 2005 | 36 | Female | Pulmonary | Positive | Positive | Yes |
| 5 | Dec 2005 | 31 | Male | Pulmonary | Positive | Positive | Yes |

## Discussion

A disease cluster is a local anomaly in the data where the observed incidence for a particular location in space and/or particular time interval appears to be different (higher) from the expected, based on the assumption of a uniform disease distribution among persons at risk, irrespective of time or location [9].

When the possibility of a TB cluster arises, particularly in a public space, screening procedures must be extended to a larger population. However, when making decisions about the extent of contact tracing and screening we need to weigh the chance of missing potentially exposed individuals against causing unnecessary anxiety in a large number of people involved.

Of the 17 work colleagues screened, two (12%) had latent TB infection (LTBI). Among family and close friends, LTBI was detected in eight out of 35 contacts (23%). In the general Portuguese population, LTBI prevalence has been estimated at 15% [1], which is higher than the rate observed in the co-workers but lower than that found among family and close friends. Thus the number of LTBI cases detected among work colleagues was not higher than expected in the general population. Family contacts, on the other hand, were found to be at increased risk for LTBI.

Extending the screening procedures beyond close contacts raises questions regarding the efficacy and the real benefit especially in a population with intermediate/high incidence of tuberculosis. Universal screening is no longer advised. We must therefore direct our efforts at identifying the individuals at risk and understanding the local mechanisms of tuberculosis transmission in order to define the best strategy to control the disease.

The investigation described in this paper benefited from evolving technologic solutions in the field of genotyping. IS6110 RFLP revealed that none of the four isolates obtained shared the same genotype, which ruled out the hypothesis of an outbreak.

With the exclusion of a link between the cases and a prevalence of LTBI lower than expected among the other workers of the same shop, we decided not to extend the screening procedures to the rest of the staff. Thus we managed to save about 1000 screenings.

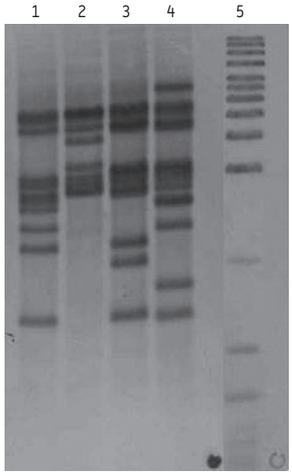
The follow-up provided evidence that our decision was right because no other TB case was diagnosed among the employees of the shopping centre or residents of the neighbouring area.

A thorough assessment based on clinical and laboratory diagnosis combined with genotyping of all *M. tuberculosis* isolates is recommended for the confirmation or exclusion of an outbreak. Usually, the literature describes the usefulness of genotyping techniques in confirming a cluster, but it is also very important when it can exclude a link between the cases. In countries with intermediate/high prevalence of TB, resources must be directed towards the optimisation of active TB treatment and the screening of contacts at risk.

The present analysis demonstrates that the combination of genotyping with the traditional TB screening procedures in the investigation of a cluster, from the perspective of the public health service, allows to save financial and human resources. In the situation presented, it allowed to exclude a link between the different cases and concentrate the resources on the individuals who were really at risk. We would therefore suggest that in countries with intermediate/high TB incidence genotyping should be performed whenever there is a suspicion of a cluster to confirm or exclude a chain of ongoing transmission and thus decide on the size of population to be screened.

**FIGURE**

**IS6110 restriction fragment length polymorphism (RFLP) result of *M. tuberculosis* isolates obtained from four cases of tuberculosis in a shopping centre in Vila Nova de Gaia, Portugal, 2004-5**




Legend: Southern blot hybridisation
Lanes 1-4: Four *M. tuberculosis* isolates.
Lane 5: molecular markers: 16.2 kb, 14.2 kb, 12.3 kb, 10.1 kb, 8.1 kb, 7.0 kb, 6.0 kb, 5.0 kb, 4.0 kb, 3.0 kb, 766 kb, 500 kb, 350 kb.

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# Serologic and virologic surveillance of avian influenza in Nigeria, 2006-7

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Since January 2006, H5N1 avian influenza has affected Nigeria's poultry population causing enormous loss of resources. The current circulating virus is a potential candidate for pandemic influenza which may severely affect the human and animal population worldwide especially in the resource-poor countries. In this study, we report on our field and laboratory surveillance efforts in Nigeria. A total of 1,821 tissue samples, 8,638 tracheal swabs, 7,976 cloacal swabs and 7,328 avian sera were analysed over a period of two years, with 312 positive results.* We recovered 299 isolates of highly pathogenic avian influenza virus H5N1 mainly from the diagnostic samples of poultry kept in backyard, small scale and free range farms. This finding emphasised the role played by these farming systems in the dissemination of avian influenza in Nigeria and highlights the need for a continued surveillance in humans since human-animal interaction is a key feature in Africa. Furthermore, there is a need for the strengthening of border controls. Since October 2007, there has been no reported and confirmed outbreak of avian influenza in Nigeria.

## Introduction

In late 1996, a farm in Guangdong, China was affected by infections with highly pathogenic avian influenza (HPAI) virus H5N1 [1,2]. Since the time of these reports, several countries in Asia (n=17), Europe (n=27), the Middle East (n=7) and Africa (n=11) have reported infection or re-infection of poultry flocks and/or wild and migratory birds [3].

In parts of the continents that reported infection, with the exception of Europe, it has been documented that the virus is becoming entrenched in the poultry populations and many clades and sub-clades are emerging [4]. Several hundred human infections (n=372) including 235 fatalities have similarly been confirmed [5], and most of these human infections have been linked to exposure to domestic poultry [6].

The expanding geography (infection of new locations), biology (acquisition of new biological properties) and ecology (adaptation to new host range) of H5 influenza viruses necessitated that every country should actively search for H5 avian influenza viruses within its territories. Nigeria, a country with an estimated human population of over 140 million, first reported infection in poultry in January 2006 [7], and in humans in January 2007 [5], and since that time, efforts to carry out active surveillance for the influenza viruses have been intensified by the national authority. Poultry production is a key economic activity in Nigeria. It contributes significantly to the family income, especially in peri-urban and poor rural communities [8]. The effect of growing urbanisation the rural, peri-urban and urban poultry production and on human-animal interaction has previously been reported [9]. Backyard poultry production thrives in view of the level of poverty and the economic return associated with the venture. Free-range systems of poultry production are also widespread in various parts of the country [10].

Due to H5N1 avian influenza infection in Nigeria, millions of poultry have been destroyed and one human death has occurred. A recent serological survey in humans in those administrative regions in Nigeria that were most heavily affected by HPAI H5N1 showed that, despite the widespread infection in the poultry population, human infection is rare [11]. In this report, we describe our surveillance efforts in Nigeria and discuss the role of poultry and backyard flocks and their implications for humans vis-à-vis our laboratory findings.

## Materials and methods

### Poultry surveillance on farms and live bird markets

*System 1 (October to December 2007).* Based on available records, a stratified sampling with cluster sampling within each strata was adopted that included locations around previously infected farm premises and live bird markets as well as locations with suspected outbreaks and dense poultry populations. Each state of Nigeria was visited three times at intervals of two weeks, and samples were taken at two new locations during every visit. At each location, cloacal, tracheal and serum samples were taken from 29 birds, and six moribund, clinically ill or dead birds were purchased. All samples were transported in appropriate media and the cold chain was maintained throughout the activities.

*System 2 (May to July 2008).* The national active surveillance covered all 36 Nigerian states and the Federal Capital Territory (FCT), irrespective of whether or not HPAI H5N1 infections had been reported from the area, but was carried out in two parts: Part A of the targeted live bird market surveillance covered only the states with infections (25 states and FCT), while part B covered the 11 states without infections. This targeted surveillance programme is still ongoing.

*System 3 (February 2006 to December 2007).* While these activities were going on, additional routine diagnostic samples

(mostly tissue samples) were submitted to the National Veterinary Research Institute (NVRI) or collected in the field by the NVRI staff.

**National surveillance programmes and team**

In response to the outbreak of H5N1 influenza in the poultry population in 2006, the Nigerian government set up an inter-ministerial committee comprising health (Federal Ministry of Health), veterinary/agricultural (Federal Ministry of Agriculture and Rural Development) and information personnel (Federal Ministry of Information) to tackle the growing problem. Several routine surveillance efforts were jointly carried out at various times by the national teams in collaboration with representatives from the Food and Agricultural Organisation of the United Nations (FAO), the United States Centers for Disease Control and Prevention (US CDC), the World Organisation for Animal Health (OIE), the Istituto Zooprofilattico Sperimentale delle Venezie (IZSVE) and others. Teams were regularly dispatched to suspected farms nationwide to collect samples and identify infected birds, advise on compensations and carry out cullings.

**Sample collection, virus isolation and serology from avian species**

Following sample collection, post mortem examinations were conducted on birds acquired moribund, dead or freshly killed, and on tracheas, lungs, livers, spleens, brains, hearts, intestines as well as intestinal contents were collected in sterile containers.

Virus isolation was done in 9-11-day-old embryonated chicken eggs according to standard protocols [12]. The eggs were candled daily to determine viability and dead eggs were removed and kept at +4oC. All eggs were opened aseptically and the allantoic fluids (ALF) were spot-tested by haemagglutination test. The chorio-allantoic membranes (CAM) of positive eggs were tested by agar-gel immunodiffusion (AGID) to detect influenza A virus group antigen.

Haemagglutination-inhibition (HI) test was conducted to determine the virus subtype. All negative ALF were further passaged in a second set of embryonated chicken eggs. Any samples negative after the second passage were declared negative. As of May 2008, no isolates of influenza A virus have been obtained from the second passage**.

Serological assays including AGID test using the H5 antigen and HI test using standardized H5, H7 and H9 panels of antigens (OIE reference laboratory for Newcastle disease virus and avian influenza, Padova) were conducted on all sera submitted to the laboratory.

**Molecular analysis**

Viral RNA extraction and reverse transcription-polymerase chain reaction (RT-PCR) were carried out. A cascade-type analysis was performed starting with the gene for the viral matrix protein (M). Every positive result was subjected to an RT-PCR for the haemagglutinin gene (HA) of subtype H5 and an additional RT-PCR for the N1 gene was done for all HA-positive cases. The following oligonucleotide primers were used: M forward: 5'-AGA TGA GTC TTC TAA CCG AGG TCG-3'; M reverse: 5'-TGC AAA AAC ATC TTC AAG TCT CTG-3'; H5 forward: 5'-CCT CCA GAR TAT GCM TAY AAA ATT GTC-3'; H5 reverse: 5'-TAC CAA CCG TCT ACC ATK CCY-3'.

Conventional RT-PCR has been shown to detect titre as low as 3 EID50 (Fifty percent egg infectious dose). In addition, our results were confirmed by the OIE reference laboratory for avian influenza and Newcastle disease, Padova, Italy.

**Human sero-epidemiological surveillance**

Several locations (poultry farms, live bird markets) with suspected or confirmed HPAI H5N1 infections were visited (between 21 March and 3 April 2007) following the compilation of a list of affected areas by the Federal Ministry of Agriculture and Rural Development in Nigeria. Specifically, a total of 295 poultry workers (76% farm workers, 15% market workers, 5% poultry cullers and 4% veterinarians), from 83 farms and four live bird markets in Kano state, and 25 laboratory workers were included in the surveillance.

In addition, surveillance in humans had been carried out by Ortiz et al. between 21 March and 3 April 2006 [11]. In that study, human sera had been collected with the informed consent of participating individuals. In addition, serum samples had been collected from people potentially exposed to the HPAI H5N1 virus, including laboratory workers, veterinarians and culling staff that agreed to participate in the sero-survey. The blood samples had been transported on ice to the laboratory (Institute of Human Virology, Abuja). Sera had been prepared in the Human Virology Laboratory, Abuja, and split in two aliquots, one of which was kept for the Federal Ministry of Health while the other one was sent to the US CDC for H5N1 serologic testing. The human sera had been tested by microneutralisation assay and a modified horse red blood cell haemagglutination-inhibition (HRBC H-I) assay. For details see Ortiz *et al*. [11]*.

## Results

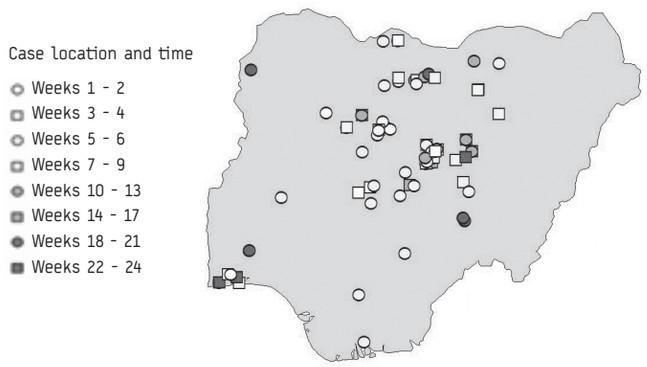
**Poultry sero-surveillance**

In the period between 2006 and 2007, farms located in 25 Nigerian states and the FCT reported poultry infections with HPAI H5N1 virus. The geographical distribution of the positive cases is shown in Figure 1.

Details of the results are shown in the tables below. During the two-year study period from January 2006 to December 2007, a total of 1,205 suspected routine diagnostic samples, 8,638 cloacal swabs, 7,976 tracheal swabs, 7,328 sera and 616 carcasses were received either from the field staff or directly from the farmers.

**Figure 1**

**Temporal and geographical distribution of highly pathogenic avian influenza H5N1 poultry cases, Nigeria, January-June 2006 (n=113)**




The surveillance in birds was carried out in the whole country with particular attention paid to the infected locations and live bird markets around them. Further 186 cases occurred between June and December 2007, bringing it to a total of 299 cases, but the overall geographical distribution did not change, with additional infections happening only in already affected locations.

The samples submitted as part of routine surveillance (system 3) yielded 300 positive results (Table 1).

Table 2 shows the results of the national surveillance using stratified a sampling procedure covering farms and live bird markets in the 36 Nigerian states and FCT (system 1).To date, all of these 10,961 samples have been negative.

For the targeted live bird market surveillance (system 2), results are available for part A covering only the 25 infected states and the FCT (Table 3). A total of 13,597 samples were analysed, of which 12 were found to be positive. The targeted live bird market surveillance for the 11 states without report of avian influenza infections (part B) is ongoing.

In the period from January 2006 to December 2007, 299 isolates of HPAI H5N1 were obtained and characterised. The haemagglutinin genes of 52 isolates have been sequenced and deposited in the GenBank and EMBL databases [13]. All of the positive isolates that were characterised belonged to clade 2.2. Efforts to genetically characterise more of the remaining isolates are currently underway.

**Table 1**

**Avian diagnostic samples tested in Nigeria between 2006 and 2007**

| | Suspected total number | Positive samples |
|---|---|---|
| Diagnostic samples (tissues/swabs) tested in 2006 | 619 | 145 |
| Diagnostic samples (tissues/swabs) tested in 2007 | 586 | 154 + 1* |
| Total | 1,205 | 299 + 1* |

Note: 52 isolates have been fully sequenced and are published [13]. A large majority (98%) of the isolates originated from farms.
1* represents a sample from Benin republic diagnosed in Nigeria.

**Table 2**

**National active surveillance covering the 36 states and the Federal Capital Territory, Nigeria, October 2007 to July 2008 (n=10,961 samples)**

| Samples Collected | Number analysed | Number positive |
|---|---|---|
| Tracheal swabs | 4,253 | 0 |
| Cloacal swabs | 3,608 | 0 |
| Sera | 3,100 | 0 |

**Table 3**

**Targeted live bird market surveillance covering 25 states and the Federal Capital Territory, Nigeria, October-November 2007 (n=13,597 samples)**

| Samples Collected | Number analysed | Number positive |
|---|---|---|
| Tracheal swabs | 4,385 | 3 |
| Cloacal swabs | 4,368 | 0 |
| Sera | 4,228 | 6 |
| Carcasses and moribund birds | 616 | 3 |

Tables 1-3 reveal a certain pattern in that H5N1 influenza virus isolates were obtained mainly from routinely submitted diagnostic samples and live bird markets. Following infection of farms, farmers promptly report outbreaks to the NVRI or other appropriate government agencies since this will ensure payment of compensation. However, we are aware that the level of education may affect reporting in certain circumstances and our systems may have inadvertently missed some outbreak situations. It is also very likely that viruses that escape detection at the farm level will get to the live bird market and can be detected there. These two locations (farms and live bird markets) are important in the epidemiology of avian influenza viruses in Africa.

Figure 2 gives an overview on HPAI H5N1 outbreaks in the years 2006 and 2007 as determined by the routine diagnostic poultry surveillance.

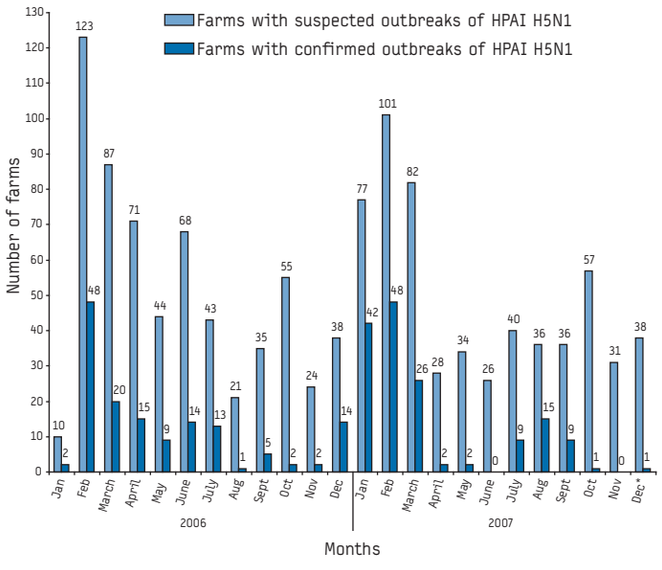
The overall rate of confirmed outbreaks was 24.8%. The peaks of infection around January and February in both years may be linked to poultry movement which is usually on the increase around festive periods (December/January). The peaks in the June/July 2006 and July/August 2007 period similarly represent the times when seasonal guineafowl eggs are available. The same period is accompanied by sale of commercial poultry due to a surplus in the egg market caused by the cheaper guineafowl eggs.

### Human sero-surveillance

As previously reported, none of the 320 human serum samples tested was positive for H5N1 avian influenza by micro-neutralisation assay or HRBC H-I test despite the degree of possible exposure to H5N1 influenza virus [11]*.

**Figure 2**

**Suspected and confirmed outbreaks of HPAI H5N1 from routine diagnostic samples of avian species in Nigeria, 2006-2007 (n=1,205 reports and 299 confirmations)**




2007 Dec*: The only isolate in that month originated from Benin Republic.
HPAI: Highly pathogenic avian influenza.

## Discussion

Before the first occurrence of avian influenza in Nigeria, active surveillance on wild fowl and migrating birds was conducted between September and November 2005 (results not shown) at the Nguru-Hadejia wetlands covering an area of about 4,125 km2. Similar surveillance was done in the same period in the high risk agroecological/farming areas and live poultry markets, but failed to detect H5 or H7 avian influenza virus.

However, after the first avian influenza outbreak in Nigeria in January 2006, surveillance efforts in the period between January, 2006 and December, 2007 yielded a total of 299 Nigerian isolates of HPAI H5N1. Mutations at antigenic sites were identified in the haemagglutinin genes of the viruses, the significance of which need to be confirmed by further analyses. The implications of these mutations for human and animal health is yet unknown [13]. Although the H5N1 virus has not yet adapted to effectively infect humans, there remains a potential pandemic threat in view of continuous infections on farms in the West African sub-region. Furthermore, there is a need to carry out routine surveillance for other influenza viruses in human and animals, since a recent report using animal models indicated that the H9N2 influenza virus showed increasing pandemic potential [14].

We are aware that our surveillance systems are subject to certain limitations. Firstly, the systems were limited and not all locations within each state were considered. In addition, some bias may be caused by the fact that the surveillance of birds may not be possible in difficult terrains. However, we made every effort to give priority to locations that serve as points of aggregation of poultry products from many locations.

We may have underdetected some cases in view of the availability of more robust and sensitive analytic systems like real time RT-PCR, and are currently making an effort to put in place such an analytic system. It was also difficult to get paired serum samples in most locations since farmers were free to dispose of their birds without regards to the on-going surveillance.

Despite these limitations, we think that this nationwide effort is critical and important since Sub Saharan Africa faces many challenges of controlling and eradicating H5N1 in poultry and implementing a good surveillance system for H5N1 in humans.

The human sero-epidemiological survey reported by JR Ortiz *et al.* did not detect any human H5N1 infections in Nigeria [11]*. This result is similar to the data recorded in previous studies in Cambodia (0/351) and Guangdong, China (1/110) [15,16]. This probably confirms that the virus has not yet adapted to effectively infect humans.

Although the human serosurveillance was negative, human H5N1 infections in Nigeria cannot be excluded. It is common practice in the northern part of the country, for reasons of culture, religion and poverty, to bury a deceased person within 24 hours of death, sometimes without ascertaining the cause of death through post mortem and detailed laboratory examinations. The only human case in Nigeria, which was officially reported by the World Health Organization on 3 February 2007, was diagnosed following a thorough investigation of a fever complicated by respiratory distress which finally led to death. It is important to ensure in the future that at least diagnostic specimens are collected before burial for proper retrospective analysis. Since it is beyond the mandate of NVRI to do a nation-wide serosurveillance in humans, the Nigerian Federal Ministry of Health, human medical practitioners, virologists and immunologists are encouraged to carry out a similar study in humans in Nigeria and parts of the West African sub-region.

Globalisation can affect animal and human health and change the disease ecology especially in those countries that presently claim to be free from HPAI infection in humans and animals [17], and risk assessment studies have shown that the European Union and parts of North America are at high risk of infection with animal diseases, in particular those originating from Africa [18-21]. These countries will need to strengthen their borders with respect to animal disease controls.

To date, the majority of the HPAI H5N1 cases in Europe has been introduced through wild birds. The source of contamination as well as the movement pattern of these wild and migratory birds needs to be studied more critically in order to exclude cross-continent infection of a potentially pandemic influenza virus.

Since October 2007, there has been no confirmed outbreak in Nigeria despite the on-going intensive surveillance. This situation has helped to stabilise the Nigerian poultry industry and has had a positive psychological effect on consumers. However, the continued absence of HPAI H5N1 will depend on sustained surveillance of poultry farms and live-bird markets, changed agricultural practices and a heightened biosecurity system entrenched in the farming system in Nigeria. Cross-continent collaborative research is encouraged and a network of funding systems, especially from the rich countries, to support research and diagnosis in developing economies like Nigeria will be greatly valued.

** Note added in proof: Since the time of submission of this report, the FAO laboratory has recently (June and July, 2008) isolated and molecularly characterised new HPAI virus isolates obtained from live bird markets and from outbreaks in farms in a total of four Nigerian states. While the viruses from two states, Kano and Katsina, (isolated from farms) belonged to the old clade (2.2) circulating in Nigeria, the isolates from two other states, Gombe and Kebbi, belonged to a new sublineage of clade 2.2, EMA3, that is novel to the African continent. This sublineage was previously circulating in Europe (Italy), Asia (Afghanistan) and the Middle East (Iran) in 2006.

* Erratum: The following amendments were made to correct the fact that supporting data on sero-surveillance in humans had mistakenly not clearly been labelled as cited from a previous publication: The sentence "Limited human sero-surveillance involving 320 individuals was also carried out but yielded no positive results" was removed from the abstract. The paragraph "Surveillance in humans was carried out between 21 March and 3 April 2006 [11]. Human sera were collected with the informed consent of participating individuals. In addition, serum samples were collected from people potentially exposed to the HPAI H5N1 virus including laboratory workers, veterinarians and culling staff that agreed to participate in the sero-survey. The blood samples were transported on ice to the laboratory (Institute of Human Virology, Abuja). Sera were prepared in the Human Virology Laboratory, Abuja, and split in two aliquots, one of which was kept for the Federal Ministry of Health while the other one was sent to the US CDC for H5N1 serologic testing. The human sera were tested by microneutralisation assay and a modified horse red blood cell haemagglutination-inhibition (HRBC H-I) assay. Details of the tests have been reported comprehensively in another paper [11]." was changed to "In addition, surveillance in humans had been carried out by Ortiz et al. between 21 March and 3 April 2006 [11]. In that study, human sera had been collected with the informed consent of participating individuals. In addition, serum samples had been collected from people potentially exposed to the HPAI H5N1 virus, including laboratory workers, veterinarians and culling staff that agreed to participate in the sero-survey. The blood samples had been transported on ice to the laboratory (Institute of Human Virology, Abuja). Sera had been prepared in the Human Virology Laboratory, Abuja, and split in two aliquots, one of which was kept for the Federal Ministry of Health while the other one was sent to the US CDC for H5N1 serologic testing. The human sera had been tested by microneutralisation assay

and a modified horse red blood cell haemagglutination-inhibition (HRBC H-I) assay. For details see Ortiz et al. [11].“ The sentence “None of the 320 human serum samples tested was positive for H5N1 avian influenza by micro-neutralisation assay or HRBC H-I test ...” was changed to “As previously reported, none of the 320 human serum samples tested was positive for H5N1 avian influenza by micro-neutralisation assay or HRBC H-I test ... [11].” The sentence “The human sero-epidemiological survey reported in this study did not detect any human H5N1 infections in Nigeria.” was changed to “The human sero-epidemiological survey reported by JR Ortiz et al. did not detect any human H5N1 infections in Nigeria [11].”

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# A swimming pool-associated outbreak of cryptosporidiosis in Staffordshire, England, October to December 2007

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In October 2007 an increase in laboratory-confirmed cryptosporidiosis cases in Staffordshire, England prompted an outbreak investigation. Case ascertainment included interviewing suspected cases and contacts and obtaining faecal specimens from those with diarrhoea for laboratory identification. Over a three-month period we identified 57 cases of cryptosporidiosis (39 confirmed) distributed across 36 households. The majority of cases (69%) were younger than 20 years. The most plausible exposure was multiple swimming episodes (56% of cases) in 13 local public swimming pools. One large swimming pool was most frequently visited by swimmers and considered a significant contributor to transmission because of substandard filtration and maintenance systems. Control measures focused on inspecting and improving operating standards at swimming pools, hygiene information to swimmers, and early detection and exclusion of cases. The rapid case investigation described in this paper provided adequate information for the early detection and control of a typical seasonal swimming pool related cryptosporidiosis outbreak. Ensuring adequate filtration standards at public swimming pools particularly before the high use periods of late summer and autumn remains a priority.

## Introduction

The improvement of water treatment systems in England and Wales has resulted in fewer drinking water-related cryptosporidiosis outbreaks in recent years [1]. By contrast, swimming pool-associated outbreaks continue to occur, with incidence peaking in late summer and autumn when swimming pool use is highest [2]. Outbreaks linked to interactive water features have also increased in prominence [3].

In November 2007 laboratory surveillance indicated a fourfold increase of cryptosporidiosis cases in northern Staffordshire, England, compared to 2006 data (16 vs. 4 cases). Routine questioning of cases by environmental health officers revealed all had recent public swimming pool exposures. We undertook a rapid case investigation aimed at targeting timely and appropriate control measures.

## Methods

The University Hospital North Staffordshire microbiology laboratory serves the northern Staffordshire population consisting of approximately 500,000 residents.

A confirmed case of cryptosporidiosis was defined as any northern Staffordshire resident with diarrhoea confirmed by the detection of *Cryptosporidium* oocysts in a stool sample by microscopic examination, from 15 October to 24 December 2007.

A probable case was defined as any household or close contact of a confirmed case presenting with watery diarrhoea or diarrhoea plus abdominal cramps with nausea and/or vomiting from 15 October to 24 December 2007.

An outbreak management team consisting of public health investigators, microbiologists, environmental health officers, and a media officer was convened to oversee the investigation and the implementation of control measures. We alerted local general practitioners and acute care hospital practitioners to be vigilant and encourage confirmatory testing of suspected cases, and to give patients appropriate hygiene and exclusion advice. Public health officers used a standardised questionnaire to interview the cases in person or over the telephone. Children were interviewed with an adult family member present. Exposure data included sources of drinking water, recreational water exposure including swimming, food consumption, animal contact and recent travel. Cases and their close contacts were given detailed advice on hygiene measures, exclusion from work or school if indicated, and exclusion from swimming until 14 days after last symptoms [4].

Further probable cases were identified through the investigation of family members and close contacts of cases, and encouraging those with symptoms to submit faecal samples.

Laboratory and interview data were captured anonymously in a line listing and analysed descriptively using EPIData statistical software (Version 2) [5].

Swimming pools identified during questioning of cases were inspected by environmental health officers against the standards laid down by the Pool Water Treatment Advisory Group (PWTAG) [6]. Water samples were not taken from individual pools for *Cryptosporidium* testing, as control interventions were implemented without delay based on pool inspection results.

Primary laboratory diagnosis was based on the demonstration of *Cryptosporidium* oocysts in stool specimen, using the modified Ziehl-Neelsen stain [7]. A number of samples positive for oocysts

were submitted for confirmation and species identification at the UK Cryptosporidium Reference Unit. Oocysts separated from faecal debris by saturated salt flotation were disrupted at 100 ºC for 60 minutes, digested with proteinase K in lysis buffer at 56 ºC and deoxyribonucleic acid (DNA) extracted by spin-column filtration (QiaAMP DNA mini kit, Qiagen) [8]. DNA was routinely subjected to polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) of the *Cryptosporidium* oocyst wall protein (COWP) gene in the first instance and a subset confirmed by PCR-RFLP using nested primer sets for the small subunit ribosomal ribonucleic acid (SSU rRNA) gene [9,10].

## Results

Thirty nine confirmed cases were identified. Their median age was 13 years with a male: female ratio of 1.2. Twenty seven (69%) cases were younger than 20 years with males (18/27) predominating (Figure 1). The distribution of symptom onset dates for confirmed cases is given in Figure 2. Confirmed cases were distributed across 36 households. An additional 18 probable cases were identified in these 36 households yielding a total of 57 cases. Three households each had a second confirmed case representing likely transmission between siblings.

Six confirmed cases (three of them less than 15 years old) were admitted to hospital for treatment.

With the help of the two water companies supplying domestic water to northern Staffordshire we were able to confirm that cases were distributed over several separate raw water supply and quality zones. Domestic water could therefore be excluded as a potential source of infection at the outset of the investigation. Confirmed cases reported no swimming in open water sources (rivers, ponds). Cases had minimal exposure to other non-swimming pool potential sources of infection: three had visited a zoo or livestock farm; three had contact with a sick pet; and seven reported recent travel abroad.

Twenty two (56%) confirmed cases reported recent swimming at one or more of the 13 local swimming pools prior to onset of symptoms. The median time from exposure to swimming pool water to onset of symptoms was seven days (range 1 to 25 days), representing an approximate incubation period. These 22 swimming pool-exposed cases were distributed across 20 households, with two households each having a second case. Eight cases confirmed swimming in more than one swimming pool, others frequented one place only. Pool A (a large and very busy water theme park) was visited on at least one occasion by 14 cases. Eight of these 14 cases swam exclusively at Pool A. Thirteen of the cases with pool A exposure attended weekend swimming disco or Halloween parties organised at the pool between 26 October and 17 November 2007.

Faecal samples from 22 cases (including nine with swimming pool exposure) were submitted for species identification. Four were *Cryptosporidium parvum* (of which one case had swimming pool exposure but not at pool A); and 18 were *Cryptosporidium hominis* (of which eight had swimming pool exposure – four exclusively at pool A and four at other pools). Three of the cases with no swimming exposure in which C. hominis was identified were pre-school siblings of cases with the same infection and swimming exposure at pool A.

In 17 cases (13 reporting swimming pool exposure) species identification was not requested and a meaningful analysis of species type association with individual swimming pools could not be undertaken.

Pool A was inspected during the last week of November 2007 and found not to have adequate sand filtration depth, continuous coagulant dosing, flow rate monitoring, and backwashing routines. Policies for pool evacuation and decontamination following faecal incidents were not in place. Urgent remedial measures were instigated and hygiene conditions improved. Subsequently, all other public and school swimming pools were also inspected and found to have adequate filtration standards but variable faecal incident policy standards and uptake. Bacteriological standards at all 13 swimming pools were within acceptable limits.

## Discussion and conclusion

This outbreak of 39 confirmed and 18 probable cases of cryptosporidiosis had a significant impact on the affected population and households in terms of hospitalisation and absence from schooling or work. We believe that swimming parties at pool A, coupled with ineffective filtration systems and large numbers of visitors, contributed to the early part (first 5 weeks) of the outbreak.

**Figure 1**

**Age and sex distribution of confirmed cryptosporidiosis cases, Staffordshire, England, October to December 2007 (n = 39)**

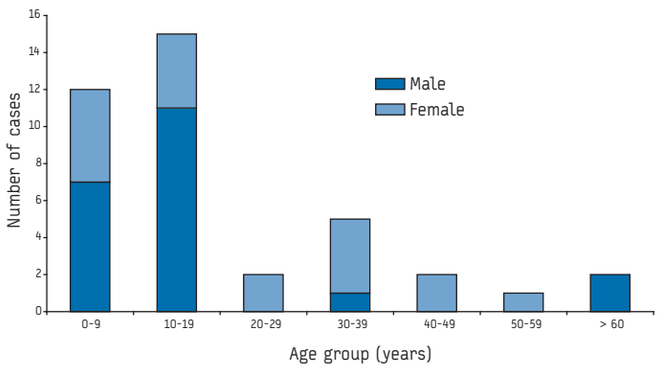
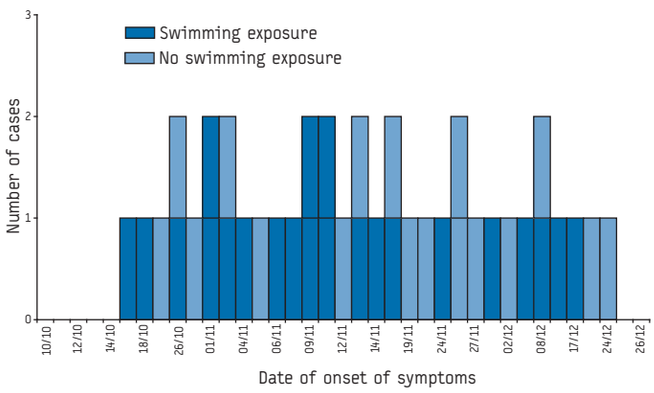



**Figure 2**

**Confirmed cryptosporidiosis cases, by swimming pool exposure and date of onset of symptoms, Staffordshire, England, October to December 2007 (n = 39)**



Not surprisingly, the incidence of infection was highest in younger age groups who swam often and at a variety of different swimming pools. Although difficult to verify due to sampling limitations, C. hominis was likely to be most associated with swimming pool exposure during the initial stages of the outbreak. Secondary household transmission contributed to the size of the outbreak and was probably underreported. The role of travel exposure appeared to be limited but had been an important factor at the onset of other similar outbreaks [11].

Outbreaks associated with several swimming pools are often prolonged and difficult to investigate due to multiple exposures and incomplete case ascertainment [11,12]. We were limited in our ability to fully investigate the contribution of other exposures, such as private swimming pools and common food sources, that could have accounted for some cases. It is likely that more severe cases were overrepresented in this outbreak. Despite this limitation, the laboratory based surveillance system proved reliable in detecting the outbreak. Coupled with rapid case investigation, we were able to identify public swimming pool exposure as the most likely cause of the outbreak and implement control measures. Improved hygiene measures at Pool A could not be implemented early enough in the outbreak to impact on disease incidence, but are in place for the next season.

Developing a pre-emptive approach to seasonal swimming pool-associated *Cryptosporidium* outbreaks is clearly feasible and important. The means for detection, prevention and control are readily available although often not implemented in time [11]. The existing guidance published by PWTAG should be followed and audited by swimming pool operators and local authorities to ensure adequate filtration systems, maintenance standards, and hygiene policies are in place well before the summer months [6,13]. One example of an auditing framework is that provided by the Institute of Sport and Recreation Management National Pool Safety Award [14]. Public health units are in a strong position to closely monitor *Cryptosporidium* incidence in anticipation of the seasonal swimming-related peak, and to rapidly communicate advice to clinicians and appropriate health messages to schools and the public.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# Parvovirus outbreak in a kindergarten in a municipality in the north of Portugal, April-June 2008

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In response to an alert raised due to epidemic exanthematous rashes in children in a kindergarten, an outbreak investigation was carried out in a municipality in the north of Portugal in late spring 2008. The intention was to establish an aetiological diagnosis and take corrective measures if necessary. The warden at the kindergarten was interviewed, and a self-administered questionnaire was given to parents and staff. Blood samples from seven children with facial erythema were collected for serological investigation. Seventeen cases of erythema infectiosum, due to infection with parvovirus B19, were identified and classified as "confirmed". No cases occurred among the eight adult staff members. An overall attack rate of 38% was observed among the 45 children (born in 2002 and 2003). All cases were mild and without fever. This parvovirus B19 outbreak made it possible to estimate the basic reproduction number ($R_0$) at between 6 and 8 (or above). Staff members, parents and local clinicians were informed that the infection could pose a risk when caught by people with special clinical conditions. All children had received one dose of measles-mumps-rubella vaccine and 60% had received two doses. The seven children with serologically confirmed parvovirus B19 infection were immune to measles and rubella. All seven were negative for measles- or rubella-specific IgM.

## Introduction

The Portuguese vaccination programme includes two routine doses of the combined vaccine against measles, mumps and rubella (MMR), at the recommended ages of 15 months and five or six years [1]. Coverage with the first and second dose of MMR vaccine has reached high and sustained levels in the north of Portugal for years [2]; this also applies to the municipality where this outbreak occurred.

Epidemic exanthematous rashes can have different aetiological causes, and differential diagnosis may be needed in the context of measles elimination programmes in Europe [3]. Previous outbreaks caused by parvovirus B19 have been studied in Portugal [4]. Measles and rubella are statutory reportable diseases in Portugal, and guidelines to study cases of measles were issued in the context of a catch-up vaccination programme in 1998/9 [5]. The Health Ministry has recently issued warnings to all services and health professionals about the possibility of importation of measles due to the international epidemiological situation, and emphasised the need to sustain high vaccination coverage [6]. This is the setting for the alert and response described here.

### Alert

In the morning of 16 April 2008, the local health authority (LHA) was contacted by telephone by a nurse working in the school health programme team. She reported that several children in a kindergarten presented spots on the face. The kindergarten warden suspected that the nearby plane trees were causing an allergic reaction to several young children.

### Preliminary assessment

In the afternoon of 16 April, two members of the LHA visited the kindergarten premises and spoke with the warden. The team examined six children with the spots. The appearance was strikingly similar to pictures published in the literature describing cases of erythema infectiosum, with the typical "slapped face appearance". All children were in a good physical condition, none had fever or other symptoms, and only one presented a rash in the abdominal region.

It was decided to conduct an outbreak investigation with the main objectives of:

- Testing the hypothesis that it was not a measles or rubella outbreak;
- Establishing an aetiological diagnosis;
- Providing information to the kindergarten community and clinicians on appropriate measures;
- Collecting data on MMR vaccination and taking corrective action if necessary.

## Methods

### Collection of clinical information

The warden was asked to provide a list with names and birth dates of all members of the kindergarten community (staff and children). Staff members and parents were asked to fill a questionnaire which was collected in the last week of June, a few days before the kindergarten would close for the summer holidays. Just before the holidays, a phone call was made to confirm that no further cases had occurred.

A case was defined as "probable" if erythema on face, extremities or trunk, was observed in members of the kindergarten community between 5 April and 19 June 2008. A case was classified as "confirmed" if in addition to the "probable" case definition it was laboratory-confirmed or had an epidemiological link with a confirmed case.

Written vaccination data from all children and adults were checked by a nurse.

### Laboratory study

A nurse visited the kindergarten on 9 May 2008, to collect blood samples from seven of the children who had presented facial erythema and whose disease onset had been 11 to 34 days before. Enzyme immunoassays (EIA) for specific IgG and IgM antibodies levels against measles, parvovirus B19 and rubella were done by two local general practitioners (GPs), who had been treating the children and previously asked informed consent from the parents.

## Results

### Kindergarten community

The 45 children attending the kindergarten (29 boys and 16 girls) were born between January 2002 and December 2003. There were two groups of children, 20 in class room 1 (13 boys and seven girls) and 25 in class room 2 (16 boys and nine girls). The eight adult staff members were all women, born between September 1954 and August 1972.

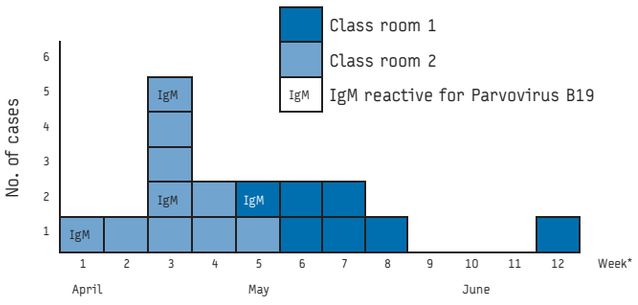
None of the staff members had ever been vaccinated against measles but, following the Portuguese guidelines for their age group, only one was young enough (born in 1972) to have received one dose of that vaccine. All children had received one dose of MMR vaccine at between 15 and 20 complete months of age (mean age at vaccination = 15.9 months). Twenty-seven children (60%) had received a second dose between 60 and 73 complete months of age (mean age at vaccination = 63.9 months). Among the children that had received only one dose, nine had not yet completed six years of age, and the remaining nine had not yet completed the age of seven years.

### Epidemiology

In total, 17 cases were observed among the 45 children and none among the eight staff members. The date of onset of the first known case was on 5 April 2008 and the date of onset of the last case on 19 June 2008. The peak of the outbreak was in the third week, when five cases occurred (Figure 1). The attack rate (AR) among the children was 38% (17/45), 35% among the group in class room 1 and 40% among the group in class room 2 (Figure 1; difference not statistically significant: p=0.73). The AR

**Figure 1**

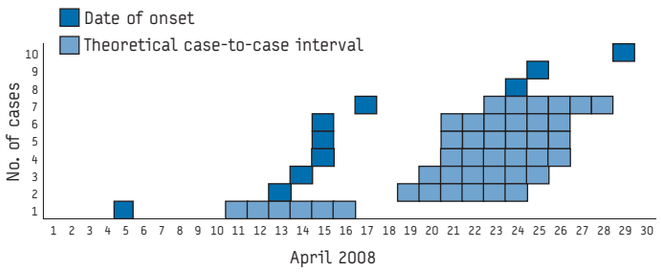
**Epidemic curve of the outbreak of erithema infectiosum in a kindergarten; disease onset by week and class room, Portugal, April-June 2008 (n=17)**




* First day of first week = 31 March 2008

**Figure 2**

**Erithema infectiosum outbreak in class room 2 by date of disease onset, Portugal, April-June 2008 (n=10), with the theoretical case-to-case interval of 6-11 days as proposed by Heegaard and Brown, 2002 [7]**




**Table**

**Laboratory results of the serological study of kindergarten children with clinical manifestations typical of erythema infectiosum, Portugal, April-June 2008 (n=7)**

| Case | Ig Class | Measles | | Rubella | | Parvovirus B19 | |
|---|---|---|---|---|---|---|---|
| Age / Sex<br>MMR doses | | Concentration | Interpretation | Concentration | Interpretation | Assay index | Interpretation |
| 4y / F<br>1 | IgG<br>IgM | 3,974 mIU/ml<br>- | Immune<br>Negative | 160 IU/ml<br>- | Immune<br>Negative | 6.22<br><1.00 | Reactive<br>Not reactive |
| 4y / F<br>1 | IgG<br>IgM | 4,012 mIU/ml<br>- | Immune<br>Negative | 68 IU/ml<br>- | Immune<br>Negative | 5.18<br><1.00 | Reactive<br>Not reactive |
| 5y / F<br>2 | IgG<br>IgM | 4,093 mIU/ml<br>- | Immune<br>Negative | 164 IU/ml<br>- | Immune<br>Negative | 6.32<br>1.32 | Reactive<br>Reactive |
| 5y / M<br>2 | IgG<br>IgM | 5,090 mIU/ml<br>- | Immune<br>Negative | 125 IU/ml<br>- | Immune<br>Negative | 6.27<br><1.00 | Reactive<br>Not reactive |
| 6y / M<br>2 | IgG<br>IgM | 5,227 mIU/ml<br>- | Immune<br>Negative | 75 IU/ml<br>- | Immune<br>Negative | 6.67<br>2.06 | Reactive<br>Reactive |
| 6y / F<br>2 | IgG<br>IgM | 6,361 mIU/ml<br>- | Immune<br>Negative | 340 IU/ml<br>- | Immune<br>Negative | 5.66<br>1.22 | Reactive<br>Reactive |
| 6y / F<br>2 | IgG<br>IgM | 538 mIU/ml<br>- | Immune<br>Negative | 42 IU/ml<br>- | Immune<br>Negative | 6.16<br>1.47 | Reactive<br>Reactive |

Note: concentration and interpretation of the results as proposed by the assay manufacturer.

was higher among females (41.7%) than among males (24.1%) but the difference was not statistically significant (p=0.29). Five additional cases were reported among the household contacts of the 17 kindergarten cases: four siblings and one parent. None of the staff members became ill.

The days of onset of the 10 cases from class room 2 are graphically represented in Figure 2. If the case-to-case interval is six to 11 days [7], then it is very likely that the first case on 5 April was the primary case, infected outside the kindergarten, while the following six cases were secondary cases, probably infected by the first case. Cases 8 to 10 were a third generation, infected by one or more of the secondary cases (Figure 2). Thus, provided that all children were susceptible before this outbreak and taking into account the definition of the basic reproduction number ($R_0$) [8], the estimated value of $R_0$ in this outbreak was 6. However, if 25% or more of infections were asymptomatic [9], the $R_0$ for this outbreak is likely to have had a value of up to 8 or more.

***Laboratory study***

Blood samples had been collected from seven of the 17 cases that had occurred before the nurse visited the kindergarten on 9 May. The specific IgM antibody tests for measles and rubella were negative for all seven children tested. Measles IgG concentrations varied from 538 to 6,361 mIU/ml, and all children were classified as "immune". Rubella IgG concentrations varied from 42 to 340 IU/ml and all children were classified as "immune". Regarding parvovirus B19-specific antibodies, all seven children were "reactive" for IgG, but only four were also "reactive" for IgM (Table).

***Clinical manifestations***

The seventeen erythema episodes were classified as "confirmed cases" of erythema infectiosum. All other members of the kindergarten were classified as "non-cases", while there were no situations compatible with the definition of "probable case".

The 17 cases presented facial erythema, lasting between two and five days (in 16 children) and 10 days in one child. Eight patients had only facial erythema while the remaining nine also had the rash on the trunk and/or extremities. Itching was reported by two children and none of the cases were febrile. All cases were very mild and no clinical complications were observed.

**Control and prevention measures**

The premises were inspected and the procedures were verified; they complied with the Portuguese legal requirements.

The kindergarten staff was informed about the benign nature of erithema infectiosum and the possible risk for pregnant women and those with anaemia and immunodeficiencies. It was recommended to exclude children form the kindergarten if they developed fever. Strict handwashing procedures after contact with patients were recommended. The same information was issued by letter to all parents.

The medical coordinator of the local National Health Service (NHS) unit was informed about the outbreak, the data to be collected and the measures to be taken. An email explaining the situation and the clinical conditions under which parvovirus B19 infection poses a particular risk was sent to all GPs working at the local NHS unit.

## Discussion and conclusion

It was confirmed that the described outbreak was due to infection by parvovirus B19. All seventeen cases unequivocally met the case definition criteria. The three cases that were not reactive for parvovirus-specific IgM (see Table) had very typical clinical symptoms, and the blood samples had been collected 15, 25 and 26 days, respectively, after the onset of symptoms. We are not sure about the reasons for these negative laboratory results, but we think that low sensitivity of the laboratory method cannot be excluded because the levels of parvovirus B19-specific IgM were generally very low, even in the reactive samples. Although it is arguable whether effective preventive measures can be taken [4,8], the usual recommendations were issued.

Several parvovirus outbreaks had been detected and studied in a neighbouring municipality in 2004 [4]. Should there be a connection between these outbreaks and the one described in this paper, it would be consistent with the reported periodicity of between three and seven years for parvovirus B 19 epidemics in a given community [9]. In 2004, the children described here had not been exposed to the infection because they were attending any kindergarten and didn't have much contact with other children. Moreover, seroprevalence data in 2001-02 showed that the infection was rare in young age groups [10]. We therefore believe that our estimated range for $R_0$ is likely to be valid. Should there be immune children, then the reported $R_0$ values would be an underestimate.

No cases were observed among staff members, probably because they were all immune. Recent Portuguese seroprevalence data [10] have shown a high proportion of immune individuals in the age groups of the staff members of the described kindergarten. Furthermore, we believe that their professional activity is associated with increased exposure to parvovirus, compared with the general population.

The virus seems to have entered the kindergarten with the first case and spread first into class room 2 and then into class room 1 (Figure 1). For class room 2, we can identify a likely transmission chain (Figure 2). However, this is more difficult for class room 1, where one or more cases seem to be missing in the period from 20 May to 18 June 2008. This may have been the result of a recall bias by parents and staff or of an unidentified transmission chain outside the kindergarten.

We did not recommend vaccination against measles for adult staff members because previous studies have shown that Portuguese women in those age groups are not only immune to measles but have measles-specific IgG levels well above protective levels [11].

We were able to prove that the outbreak was not measles or rubella. Furthermore, all children had received one dose of MMR vaccine and the levels of measles- and rubella-specific IgG among the seven studied children were well above the protection thresholds. Those children who had not received the second MMR dose were still within the age range recommended for that vaccination. Such high coverage values are consistent with what has been observed in the north of Portugal [2] and in the annual internal evaluations in our municipality (unpublished data).

After the described outbreak investigation, a report on imported cases of measles in Portugal was published [12]. Two importation episodes (in 2005 and in 2008) were identified and reported. The measles cases imported in 2005, affecting migrant Romanian communities, were studied by community physicians (see

Acknowledgements) in two neighbouring municipalities, including the one where the present parvovirus outbreak was observed. These experiences have been helpful in the current parvovirus investigations. Once again, our local public health unit was able to quickly respond to an alert due to an eruptive epidemic disease, and would have detected a measles (or rubella) outbreak, if that had been the aetiology of the cases.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# Surveillance of listeriosis in Navarre, Spain, 1995-2005 – epidemiological patterns and characterisation of clinical and food isolates

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We monitored the incidence of human listeriosis in Navarre, a region in north of Spain between 1995 and 2005, and carried out the characterisation of *Listeria monocytogenes* isolates obtained from clinical samples and ready-to-eat products (sliced cooked meat, smoked salmon and liver pate). The active surveillance requesting hospitals to notify all listeriosis cases (n=40) yielded higher incidence rates (average annual rate 0.65/100,000 inhabitants, range 0.18-1.18/100,000 inhabitants) than expected. Pregnant women were the largest group affected (n=13, 32.5% of the cases), with a peak in incidence during the last three years of the study period. From the 40 human cases we obtained 33 *Listeria* isolates. Serological and molecular characterisation by PFGE identified 20 different pulsotypes, which on three occasions enabled us to link sporadic cases into clusters. Although we could not identify the incriminated food product we found two clinical pulsotypes among smoked salmon and cooked meat isolates. Surveillance of listeriosis in Spain should be improved and coordinated with other European Union Member States in order to better estimate the burden of disease and to prevent foodborne outbreaks.

## Introduction

*Listeria monocytogenes* has been recognised as a serious foodborne pathogen, with a case-fatality rate between 20% and 50% [1-3]. However, the important impact that this disease has on public health is not always recognised, particularly since listeriosis is a relatively rare disease compared with other common foodborne infections such as salmonellosis. Listeriosis is likely to be underreported due to its status as a non-notifiable disease in many countries, including Spain, and because of the absence of adequate surveillance programs. The Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents, Antimicrobial resistance and Foodborne outbreaks in the European Union produced jointly by the European Food Safety Authority (EFSA) [4] reported an incidence between 0 and 10 cases per million in 2006 among the 24 countries that submitted listeriosis information. It is significant that incidence rates above 5 cases per million were reported in countries where listeriosis is statutorily notifiable, such as France, Germany, Finland and Switzerland. However, even in countries with obligatory notification of listeriosis and efficient surveillance systems, such as PulseNet in the United States [5], the number of cases could be greater than reported due to the occurrence of many sporadic cases and spontaneous miscarriages which are not investigated.

As pointed out by Kiss et al. and MacKenzie et al. [6,7], integrated food chain surveillance is necessary for all national and international authorities in order to achieve adequate information regarding the true impact of listeriosis in the population. Serotyping of *L. monocytogenes* has a low discriminatory power for subtype differentiation [8], however, when combined with molecular methods based on DNA macrorestriction pattern analysis [9] it becomes a useful tool in epidemiological investigation. Among the molecular typing methods, pulsed-field gel electrophoresis (PFGE) has been one of the most frequently used in epidemiological investigation of listeriosis because of its excellent discriminatory power and reproducibility [10-15].

In Spain, the current surveillance of listeriosis is based on voluntary reporting of cases to the Microbiological Information System of the National Reference Laboratory. The present study set out to evaluate the incidence of listeriosis in Navarre, a region in northern Spain, over a period of 11 years from 1995 to 2005, by implementing active surveillance in this geographical area. The objectives of this study were:

1) to obtain epidemiological data on cases of listeriosis reported by the three main hospitals of Navarre;
2) to compare *L. monocytogenes* isolates recovered from food products and human cases of listeriosis in Navarre over the same period of time, by serological and PFGE characterisation.

## Methods

### Listeriosis surveillance and epidemiological data

In order to determine the incidence of listeriosis, we asked the three main hospitals in Navarre to report cases of listeriosis. The case definition was based on the isolation of *L. monocytogenes* from a hospitalised patient with a clinically compatible illness. A case was considered perinatal in the following cases: infected pregnant woman, miscarriage, stillbirth or newborn less than one month old. When the pathogen was isolated from both the pregnant woman and her newborn child, this was considered to represent a single case. Information regarding sex, age, clinical symptoms, immunosuppressive treatment or underlying disease, and death or recovery of the patients, was reported when available.

In addition, patients diagnosed in 2005 were interviewed about their consumption habits with regard to high risk foodstuffs. The questionnaire (available upon request from the corresponding author) covered different aspects relating to the consumption of ready-to-eat (RTE) products (type, brand and store where purchased) during the two months preceding disease onset. Specific questions regarding high-risk RTE products sampled in the study were also included.

### *Collection of L. monocytogenes strains*

A total of 87 *L. monocytogenes* isolates were obtained from food samples in a study which we performed in 2003-2005 [16]. Of these, 45 were isolated from a market sampling pool of 783 RTE high-risk food products that included sliced cooked meat products (pork, chicken and turkey), sliced smoked fish products (salmon and trout) and liver pate. Isolation and identification of *L. monocytogenes* was carried out using aseptic techniques following the NF EN ISO 11290-1 [17]. The remaining 42 food strains were obtained as a result of our earlier study on the occurrence of *L. monocytogenes* in the same type of RTE food products carried out in Navarre between 1995 and 2002 [18].

With respect to clinical strains, from the 40 human cases of listeriosis reported between 1995 and 2005, we were able to obtain only 33 isolates. They were isolated in the hospital microbiology laboratories and most of them originated from either blood or cerebral spinal fluid or placenta, while stool cultures for *Listeria* were not available. These isolates were subsequently submitted to our laboratory at the University of Navarre, where identification of strains was carried out by biochemical and serological methods.

All strains were stored at -80ºC in sterilised skimmed milk.

### Serological characterisation

Serotyping was carried out using commercial specific antisera (Denka Seiken Co., Ltd., Tokyo, Japan) following the manufacturer's instructions. Both polyclonal anti-O antisera (O-I/II, O-V/VI, O-I, O-II, O-VI, OVII, O-VIII, Y O-IX) and anti-H (H-A, H-AB, H-C, H-D) were used in the determination of somatic and flagellar antigen, respectively. Interpretation of the results was carried out according to the serotyping scheme established by Seeliger and Höhne [19].

### Molecular characterisation by pulsed-field gel electrophoresis (PFGE)

PFGE was performed according to Graves and Swaminathan [8], with minor modifications. Before performing PFGE, strains were revitalised by plating onto blood agar (Biomerieux, Marcy L'Etoile, France) and incubated at 37ºC for 18 h. DNA from a single *Listeria* colony was digested with ApaI (Roche Diagnostics, Barcelona, Spain) and separated at 6 V/cm for 19.5 h on a CHEF-DR II PFGE apparatus (Bio-Rad, Hercules, California, US) with switch time from 4 to 40 seconds at 14ºC. Staphylococcus aureus ATCC 29213 was used as a control for digestion. The obtained images were digitised and analysed using Gel Compar II® software (Applied Maths, Kortrijk, Belgium). Restriction patterns were analysed using the criteria described by Tenover et al. [20]. Similarity values of the patterns were calculated using the Dice correlation coefficient with a 1.0% band position tolerance and unweighted pair group method using arithmetic average (UPGMA). Clinical and food isolates were compared, and pulsotypes were numbered consecutively.

### Statistical analysis

The statistical package used was SPSS v13.0. The contingency table analysis was based on the chi square distribution (Pearson's chi square test).

## Results

### Clinical and epidemiological data

A total of 40 cases of listeriosis were documented in Navarre during the 11-year surveillance study period (Figure 1). Between 1995 and 2005, the mean annual incidence was 0.65/100,000 inhabitants, ranging from 0.18/100,000 in 1998 (n=1) to 1.18 /100,000 in 2005 (n=7). Table 1 shows the epidemiological data of reported cases. From the available information, 26 cases (65%) correspond to non-perinatal infections, while perinatal infections (pregnant women and newborns) were described in 13 cases.

In total, 21 deaths were reported resulting in the average case fatality rate of 52.5%. These included eight foetal deaths in

**FIGURE 1**

**Cases of listeriosis reported in Navarre, Spain, from 1995 to 2005 (n=40)**

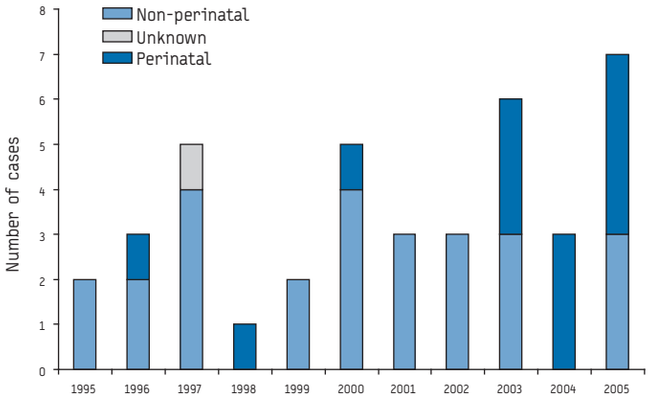



**TABLE 1**

**Principal characteristics of listeriosis cases reported in Navarre, Spain, from 1995 to 2005 (n = 40)**

| Epidemiological data[a] | Number of cases (% of the total) | Number of deaths | Case-fatality rate (%) by group |
|---|---|---|---|
| Clinical form | | | |
| Perinatal | 13 (32.5) | 8[b] | 61.5[b] |
| Non-perinatal | 26 (65.0) | 13 | 50.0 |
| Risk factor (non-perinatal) | | | |
| No detectable pathology | 5 (12.5) | 1 | 20.0 |
| Known risk[c] | 15 (37.5) | 8 | 53.3 |
| Other conditions[d] | 6 (15.0) | 4 | 66.7 |
| Age group (in years) | | | |
| <1 | 1 | 1 | 100 |
| 1-19 | 2 (5.0) | 1 | 50.0 |
| 20-39 | 12 (32.5) | 0 | 0 |
| 40-59 | 8 (20.0) | 2 | 25.0 |
| >60 | 12 (30.0) | 8 | 66.7 |

[a] Information not available in all cases
[b] Only foetal death (all women recovered)
[c] Aged >60 or immunocompromised patients (cancer, HIV, organ transplantation).
[d] Chronic diseases

perinatal cases (all women recovered) and 13 deaths among the non-perinatal cases (case-fatality rate of 50%). Among this latter group, 15 patients (37.5% of the total) had an underlying listeriosis risk factor defined as age >60 years and/or immunosuppressive conditions such as cancer, HIV or organ transplantation. Further six cases had underlying chronic conditions, such as diabetes (n=1), addiction to alcohol (n=2) and other (n=3). The remaining five non-perinatal cases (12.5% of the total) were healthy people aged between 2 and 59 years.

Clinical symptoms most frequently reported were septicaemia (37.5%) and meningitis (15.0%) (Table 2). No cases of acute gastroenteritis caused by listeriosis were detected in the course of the study.

The most affected group at risk of listeriosis was pregnant women (n=13; two of them with underlying diseases). The number of perinatal cases was significantly higher in the years 2003-2005 compared with the previous period of 1995-2002 (10 vs. 3) (Figure 2). The clinical information available showed that all of the infected mothers recovered (one of them was diagnosed with meningitis). However, 61.5% of all pregnancy-associated cases resulted in miscarriage (n=5), stillbirth (n=2) or infant death within 24-48 hours of birth (n=1) (Table 2).

### Serotyping results

Four serovars were determined among the 33 clinical isolates as shown in Table 3. In all of the different risk groups most of the isolates belonged to serogroup 4 (78.8%). Serotype 4b was the predominant (75.8%, n=25), followed by serotype 1/2a (18.2%, n = 6). With respect to the food isolates, the predominance was for serogroup 1 (77.0%). In contrast with the results obtained from clinical strains, serotype 1/2a was the most common (51.7%, n=45), followed by serotype 4b (23.0%, n=20), serotype 1/2c

**Table 2**

**Clinical symptoms of listeriosis patients reported in Navarre, Spain, 1995-2005 (n=40)**

| Clinical symtoms[a] | Number of cases | Proportion of total (%) |
|---|---|---|
| Septicaemia[b] | 15 | 37.5 |
| Meningitis[c] | 6 | 15.0 |
| Miscarriage | 5 | 12.5 |
| Stillbirth | 2 | 5.0 |
| Premature | 1 | 2.5 |
| Endocarditis | 2 | 5.0 |
| Other[d] | 5 | 12.5 |
| Unkown | 5 | 12.5 |

[a] Multiple responses were possible
[b] One case also with encephalitis
[c] Classified as meningitis (n = 4), meningoencephalitis (n = 1) and cerebral abscess (n = 1)
[d] Hormonal disorders, arthritis, osteomyelitis, kidney failure, heart attack

**Figure 2**

**Incidence of listeriosis in Navarre, Spain in 1995-2005 and pregnancy-associated cases per 1,000 births in the same time period**


**Table 3**

**Serotype distribution of *Listeria monocytogenes* isolated from ready-to-eat (RTE) food and clinical cases of listeriosis in Navarre, Spain, during the period 1995-2005**

| Source | Proportion of serogroup 1 (%) | Proportion of serogroup 4 (%) | Number of isolates of each serotype | | | | | Total number of isolates |
|---|---|---|---|---|---|---|---|---|
| | | | 1/2a | 1/2b | 1/2c | 4b | 3b | |
| Clinical isolates | 21.2 | 78.8 | 6 | 1 | -[a] | 25 | 1 | 33 |
| Non condition associated with risk of listeriosis | 20.0 | 80.0 | 1 | - | - | 4 | - | 5 |
| Pregnancy | 20.0 | 80.0 | 2 | - | - | 8 | | 10 |
| Transplantation | 28.6 | 71.4 | 1 | 1 | - | 4 | 1 | 7 |
| Cirrhosis/Alcoholism | 0 | 100 | - | - | - | 4 | - | 4 |
| Cancer | 33.3 | 66.7 | 1 | - | - | 2 | - | 3 |
| Others | 25.0 | 75.0 | 1 | - | - | 3 | | 4 |
| RTE foods | 77.0 | 23.0 | 45 | 3 | 19 | 20 | - | 87 |
| Sliced cooked meat | 94.6 | 5.4 | 32 | 3 | 18 | 3 | - | 56 |
| Sliced smoked fish | 43.3 | 56.7 | 13 | - | - | 17 | - | 30 |
| Pate | 100[b] | 0 | - | - | 1 | - | - | 1 |

[a] No clinical isolates of this serotype detected
[b] Only one isolate obtained

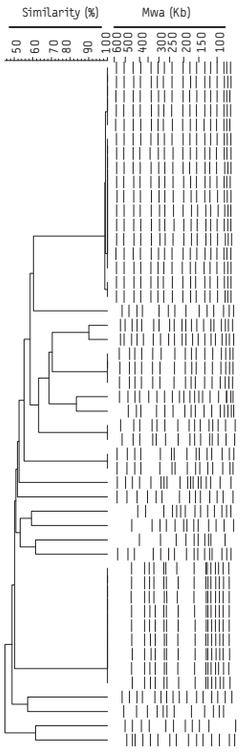
(21.8%, n=19) and finally by serotype 1/2b (3.5%, n=3). When food categories were examined according to serotype, we found that serotype 4b was the predominant in smoked fish (56.7%, n=17), while serotype 1/2a was the most frequent among sliced meat products (57.1%, n=32). The unique strain isolated in liver pate belonged to serotype 1/2c.

### PFGE results

PFGE revealed a total of 20 different pulsotypes among clinical isolates, distinguished by one or more band differences ranging in size from 50 to 500 kb (Figure 3). Pulsotypes 1, 5, 8 and 9 contained two or more strains which remained indistinguishable from each other. While strains of pulsotype 5 (n=3) were recovered from different years, strains of pulsotype 1 (n=10) and pulsotype 9 (n=2) were related in time and geographical distribution, showing that possible outbreaks could have occurred. Among isolates with pulsotype 1, three strains corresponded to listeriosis cases diagnosed between November and December 2003, and four were isolated from four pregnant women affected between November and December 2005. However, the oral interviews about food intake in these patients did not give us information about a possible common food source. With respect to the 87 food isolates, we found 19 different pulsotypes (data not shown), but only two of them were similar to the previous clinical pulsotypes characterised. We found a cluster of seven strains isolated from smoked salmon showing pulsotype 1. Two of these were isolated in October 2003, 1-2 months before the isolation of three clinical strains with identical pulsotype. In addition, pulsotype 16 was shared by a clinical strain and seven food isolates (three isolates from sliced meat and four from smoked salmon).

**FIGURE 3**

**Dendrogram for *Listeria monocytogenes* pulsotypes of all 33 isolates obtained from clinical cases and some isolates from food samples, Navarre, Spain, 1995-2005**




| Strain | Serotype | Source | Month-year | Pulsotype |
|---|---|---|---|---|
| 533 | 4b | Clinical case | 09-2002 | 1 |
| 612 | 4b | Smoked salmon | 02-2003 | 1 |
| 605 | 4b | Smoked salmon | 10-2003 | 1 |
| 608 | 4b | Smoked salmon | 10-2003 | 1 |
| 539 | 4b | Clinical case | 11-2003 | 1 |
| 540 | 4b | Clinical case | 11-2003 | 1 |
| 541 | 4b | Clinical case | 12-2003 | 1 |
| 610 | 4b | Smoked salmon | 01-2004 | 1 |
| 611 | 4b | Smoked salmon | 01-2004 | 1 |
| 614 | 4b | Smoked salmon | 01-2004 | 1 |
| 615 | 4b | Smoked salmon | 02-2004 | 1 |
| 552 | 4b | Clinical case | 06-2004 | 1 |
| 554 | 4b | Clinical case | 02-2005 | 1 |
| 556 | 4b | Clinical case | 11-2005 | 1 |
| 557 | 4b | Clinical case | 11-2005 | 1 |
| 558 | 4b | Clinical case | 11-2005 | 1 |
| 559 | 4b | Clinical case | 12-2005 | 1 |
| 72 | 4b | Clinical case | 09-1996 | 2 |
| 520 | 4b | Clinical case | 05-1999 | 3 |
| 103 | 4b | Clinical case | 06-1997 | 4 |
| 112 | 4b | Clinical case | 07-1997 | 5 |
| 522 | 4b | Clinical case | 02-2000 | 5 |
| 521 | 4b | Clinical case | 09-1999 | 5 |
| 73 | 4b | Clinical case | 08-1996 | 6 |
| 86 | 4b | Clinical case | 01-1997 | 7 |
| 528 | 1/2 a | Clinical case | 06-2001 | 8 |
| 529 | 1/2 a | Clinical case | 10-2001 | 8 |
| 531 | 4b | Clinical case | 05-2002 | 9 |
| 532 | 4b | Clinical case | 06-2002 | 9 |
| 553 | 4b | Clinical case | 11-2004 | 10 |
| 537 | 4b | Clinical case | 07-2003 | 11 |
| 1 | 3 b | Clinical case | 02-1995 | 12 |
| 535 | 4b | Clinical case | 07-2003 | 13 |
| 2 | 1/2 b | Clinical case | 04-1995 | 14 |
| 530 | 4b | Clinical case | 12-2001 | 15 |
| 71 | 1/2 a | Clinical case | 09-1996 | 16 |
| 199 | 1/2 a | Sliced meat | 03-1998 | 16 |
| 200 | 1/2 a | Sliced meat | 08-1998 | 16 |
| 273 | 1/2 a | Sliced meat | 08-1998 | 16 |
| 53 | 1/2 a | Smoked salmon | 11-1995 | 16 |
| 54 | 1/2 a | Smoked salmon | 11-1995 | 16 |
| 55 | 1/2 a | Smoked salmon | 11-1995 | 16 |
| 56 | 1/2 a | Smoked salmon | 11-1995 | 16 |
| 57 | 1/2 a | Smoked salmon | 11-1995 | 16 |
| 113 | 4b | Clinical case | 07-1997 | 17 |
| 538 | 1/2 a | Clinical case | 11-2003 | 18 |
| 555 | 1/2 a | Clinical case | 04-2005 | 19 |
| 551 | 1/2 a | Clinical case | 06-2004 | 20 |

## Discussion

A collaborative surveillance study of *Listeria* infections in Europe led by the French Institut de Veille Sanitaire and Institut Pasteur [21] described an incidence of 0.15 cases per 100,000 inhabitants in the year 2000 for Spain (based on information from voluntary reporting), whilst for that same year the incidence detected for Navarre in our study was 0.91 (five cases reported in our active surveillance). Similarly, for 2004, the EFSA report [22] described an incidence of 0.20 in Spain whereas the rate obtained in our study was 0.51 (three cases). Considering that the likelihood of listeriosis in Navarre was similar to the whole of Spain (similar proportion of people at risk and food intake habits), we think that the active surveillance carried out in our study was the reason for the higher incidence obtained in Navarre compared to that reported for the whole of Spain in the mentioned publications. Similar incidence rates to those we found in Navarre were described in countries with mandatory notification of listeriosis, such as Denmark, where Gerner-Smidt et al. [23] reported incidences ranging from 0.45 to 0.75 cases per 100,000 between 1994 and 2003, and increasing to 0.8. during 2004 [22]. It is interesting to note that the sensitivity of the Danish system for *Listeria* infection is thought to be almost 100% and is based on immediate notification to the National Surveillance Centre (Staten Serum Institute) of all patients from whom *Listeria* has been isolated. This corresponds to the methods used in our study. Likewise, active surveillance of *L. monocytogenes* infections in the Netherlands revealed an increase in incidence from 0.26 cases per 100,000 inhabitants in 2001 to 0.62 per 100,000 in 2005 [24].

It should be noted that the 52% case-fatality rate (foetal death included) obtained in our study is similar to that described in other Spanish reports [25] but higher than the average reported over recent years in the EFSA publication [4]. However, the EFSA report admits that the lower than expected reported case-fatality rate might be due to a lack of data on patient outcomes after the initial notification, which indicates the importance of clinical data recovery to assess the impact of listeriosis.

With respect to listeriosis associated with pregnancy, several authors have reported high incidences with case-fatality rates above 45% [6,26]. The high numbers of listeriosis cases we detected among this group in Navarre is due, in part, to the fact that we included investigation of tissue samples obtained from hospitalised women after miscarriage. However, it should be noted that testing for *L. monocytogenes* was performed in a similar way during the entire study period, without a systematic analysis of all miscarriage tissues. So, the increase of the number of pregnancy-associated cases in recent years can not be considered a surveillance artefact. Taking into account that bacterial cultures are not routinely obtained from spontaneously aborted foetus or stillborn neonates in a wide range of Spanish hospitals, we believe that the true incidence of listeriosis in this risk group may still be underestimated. In order to assess the rates more accurately, we recommend the routine investigation of *L. monocytogenes* in tissue after miscarriage and stillbirth in the whole of Spain and also at European level.

To assess the real impact of listeriosis in Spain and in the whole of EU, better harmonisation of data collection systems at national

level is required. Validated clinical and food questionnaires would be valuable in all diagnosed or suspected cases of listeriosis, providing more precise and complete information about symptoms, outcomes and food consumption habits of affected people. This way we would be able to conduct epidemiological studies (useful for outbreak investigations) and to provide dietary advice to high-risk individuals in avoiding specific foods. In our study, suspecting a possible outbreak in December 2005 after four cases had been detected in a short period of time, we decided to interview the patients using a food questionnaire but this oral survey failed to identify a possible common food source. Nevertheless, this does not exclude the possibility of a common source but rather reflects the limitations of this preliminary survey and the need for validation. Active surveillance in Italy [27] which involved distributing clinical and food questionnaires to the hospitals and the characterisation of all strains resulted in a higher number of cases of listeriosis than reported by mandatory notifications.

In addition to the accurate recovery of epidemiological information, there is a need to isolate and characterise *L. monocytogenes* clinical strains. Although most cases of listeriosis occur sporadically [28], serological and molecular analysis could help us to relate sporadic cases that are geographically and time-related, allowing the detection of possible outbreaks that perhaps go unnoticed if few people are involved. Serological characterisation is useful for rapid screening of strains during suspected outbreaks. In line with the findings of several authors [6,11,15,25,29] our results confirm the fact that most cases of human listeriosis are caused by serotypes 4b (75.8%). However, recent studies observed a variation of this classical distribution with an increase of serotype 1/2a [27,30].

The combination of serology and PFGE has provided us with the opportunity to link sporadic cases on three different occasions during the period 2002-2005. It should be noted that pulsotype 1 was the most frequent among the clinical strains isolated in the study (10 out of 33), and also one of the predominant profiles in the food isolates (seven isolates from smoked salmon). The profile of three patient isolates in November-December 2003 (pulsotype 1) was indistinguishable from that of two isolates obtained from smoked salmon a month before, but we had no available information about the food intake of these patients as the food questionnaire was not carried out at that time. The repeated isolation of pulsotype 1 in smoked salmon (2003-2004), and pulsotype 16 in smoked salmon and sliced cooked meat (1995-1998) suggests the persistence of specific subtypes in food processing plants. Considering that food products at risk of containing *Listeria* are often commercially available over a wide area, characterisation of food and clinical strains should be managed at a national level in order to trace probable sources of infection and to detect related cases occurring in other regions of Spain.

In conclusion, the present study shows that harmonised and active surveillance of listeriosis is needed in Spain in order to increase knowledge about real impact of this serious health problem. Accurate national surveillance should be based on the obligatory notification of listeriosis, the collection of epidemiological information by the application of a standardised food and clinical questionnaire and the sending of isolated strains to a reference laboratory for a serological and molecular characterisation. This active surveillance could be extended at European level, improving the available information to detect compatible cases and to trace probable sources of infection.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# Syphilis epidemiology in Sweden: re-emergence since 2000 primarily due to spread among men who have sex with men

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Syphilis has re-emerged in western Europe since 2000. Changes in sexual behaviour have facilitated the spread of syphilis especially among men who have sex with men (MSM) and improved surveillance systems and case detection have lead to an increase in the reported numbers of cases. This report describes recent trends (2000-2007) of syphilis in Sweden, where the spread among MSM, particularly in the big cities, has been a major contributor to an increase in cases. Estimated syphilis incidence among MSM was up to twenty-eight times higher than in the general Swedish male population. The most affected age group among males was 25-44 years of age. The majority of infections in men and women through heterosexual contacts were acquired abroad whereas the majority of infections attributed to sex between men were acquired in Sweden. Appropriate prevention activities are needed to reach vulnerable populations in Sweden.

## Introduction

Syphilis is a bacterial sexually transmitted infection which has several stages. Primary, or early syphilis lasts on average about three months. During this stage syphilis is very infectious. The symptoms may vary, but the first symptom of primary syphilis is often a small, painless sore (a chancre) on the genitals. It is easy to overlook the chancre and not to seek the specialist help and receive treatment. In such cases the sore heals and the infection progresses to the secondary stage which often starts with a rash that may last several weeks or even months as the most common symptom. Transmission can occur during this stage as well, especially if there is contact with mucous membranes or skin. The symptoms of secondary syphilis resolve with or without treatment. If treatment is not received, the infection progresses to the latent and late stages of syphilis. If in the late latent stage of syphilis the patient still does not receive treatment, the tertiary stage of syphilis starts, during which almost all organs can be affected. Neurosyphilis and cardiovascular syphilis are possible sequelae [1].

The World Health Organization (WHO) estimates 12 million new cases worldwide annually, of which 140,000 are estimated to occur in western Europe [2]. Syphilis can be successfully controlled by early detection and effective treatment of cases, contact tracing and effective preventive measures such as condom use [3]. The successful control of syphilis in adults also prevents congenital syphilis, which is a serious neonatal disorder leading to deformities and delayed development of a newborn if untreated [4].

Between the 1980s up to the 1990s, the incidence of sexually transmitted infections (STIs) such as syphilis and gonorrhoea decreased substantially in the western part of the World Health Organisation (WHO) European Region [5]. Increased high risk sexual behaviour and migration have contributed to the rise in the incidence of STIs since 2000; furthermore, improvements in surveillance systems and case detection have lead to higher number of reported cases [5,6]. In western Europe the resurgence of syphilis was mostly due to outbreaks among men who have sex with men (MSM) with increasing risk sexual behaviour and novel sexual networks [6-8]. This pattern was also observed in Sweden [9] where, after a decrease in reported syphilis incidence from 5.8 cases per 100,000 population in 1982 to 0.4 cases per 100,000 population in 1999, the number of reported cases increased in 2000 to 1.1 cases per 100,000 population and the rise has continued since then. This paper presents syphilis trends in Sweden from 1970 and provides a descriptive epidemiology for the years 2000-2007.

## Methods

### Surveillance system

Reporting of syphilis cases has a long history in Sweden. The first law (Lex veneris) in Sweden requiring mandatory reporting of venereal diseases, contact tracing and treatment of cases and their contacts came into force on 1 January 1919 [10]. Today the Communicable Diseases Act requires mandatory notification of syphilis cases by any health care professional e.g., general practitioner, gynaecologist, STI specialist, etc. when diagnosing an STI [11]. Contact tracing is also a mandatory activity [11]. Health care professionals electronically report notifications of STIs to the national surveillance database SmiNet (www.sminet.se), which is maintained by the Swedish Institute for Infectious Disease Control (Smittskyddsinstitutet - SMI). In the national population-based surveillance system the notification of syphilis cases (as any other notifiable STI in Sweden) includes case-based clinical notification from the health care professional and a case-based laboratory notification from the diagnostic laboratory. All notifications for STIs in the surveillance system are coded and therefore do not contain the patient's name or address. The coding is based on social security number (*personnummer*) which is a unique key for merging clinical and laboratory notification in the surveillance system. This unique code prevents double reporting of the same

**Figure 1**

**Incidence of syphilis, notified cases in Sweden, 1970-2007**

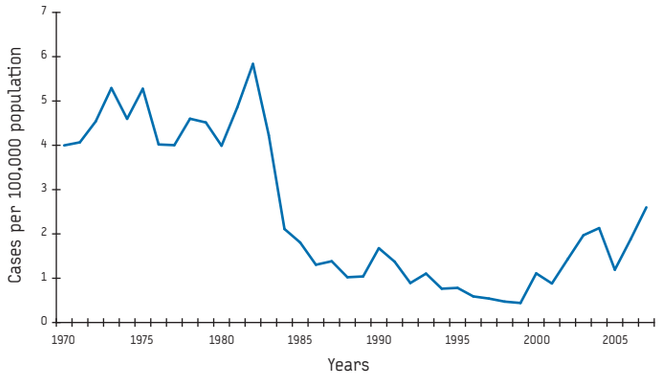



**Figure 2**

**Incidence of syphilis by sex and male-to-female-ratio, notified cases in Sweden, 1990-2007**

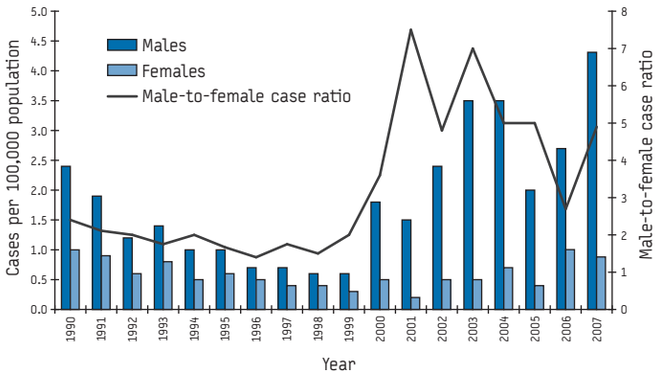
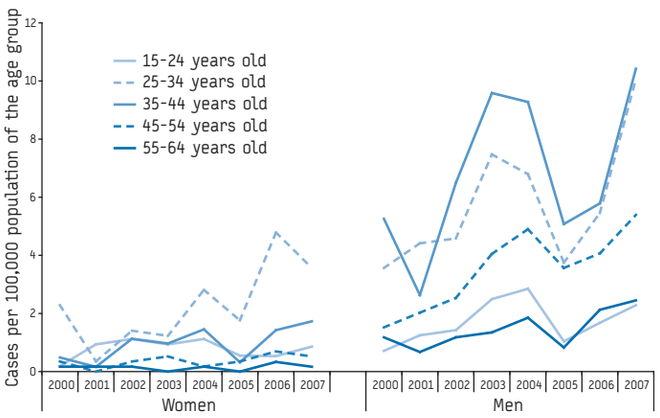



**Figure 3**

**Syphilis incidence in females and males by age group, notified cases in Sweden, 2000-2007**




case. Additional check for double reporting is done at the regional level where the staff has access to the personal data of the patient in the clinics and from laboratories.

### Syphilis notifications and incidence

Notifications are based on the following syphilis case definition used since 1997: laboratory confirmed case with clinical picture corresponding to infectious syphilis (primary, secondary and early latent syphilis with less than two years after infection) [12]. Tertiary syphilis and late latent syphilis with more than two years after infection are not notifiable in Sweden.

Forms for notifications of clinical syphilis cases contain information on age, sex, reporting county of Sweden, possible country of acquisition, as indicated through a consistent incubation period and the patient's history, type of infection (symptomatic or asymptomatic), reason for diagnosis (having symptoms, routine diagnostic, contact tracing, etc.), stage of infection (primary, secondary, early latent syphilis, late syphilis, tertiary, unknown). Information on non-notifiable late latent and tertiary syphilis is included in order to know the stage of the infection reported by the clinicians. If cases of non-notifiable stages (late latent and tertiary) of syphilis are reported, the county medical officers are in charge of eliminating them, thus only notifiable stages of syphilis are kept in the surveillance system. Also information on route of transmission is collected. If the "route of transmission", according to the information from the patient, is sexual, the patient is then asked if this was through a heterosexual or a homosexual contact. Other options such as vertical transmission from mother to child or transmission via blood products are available. Laboratory notification forms contain information on age, sex, reporting county of Sweden, type of specimen (urine, anal or pharyngeal swabs, etc.), diagnostic method used and test results.

We calculated incidence using all reported syphilis cases per 100,000 population (total, female or male, age group-specific). Data on population in Sweden and counties, for the respective years, were taken from Statistics Sweden (www.scb.se).

### Data analysis

We performed descriptive analysis by:

- person (sex and age),
- place (reporting county of Sweden and country where the infection was acquired),
- time (between 2000-2007),
- and behavioural aspects (reported route of transmission).

All analyses (proportions, incidence, male-to-female ratio) were calculated in Excel.

The incidence of syphilis among MSM in Sweden was calculated based on estimates of the proportion of men reporting sex with men from studies in the United Kingdom and Sweden, which were 2.5% [13,14].The mentioned estimates were applied for 16-44 years old male population in UK. We used the above mentioned estimate for the rough estimate of MSM population among all male age groups in Sweden.

## Results

### Overall trends

Syphilis incidence decreased significantly in the 1980s, from a high of six per 100,000 population in 1982, and continued to decline during 1990s to 0.4 cases per 100,000 (38 cases) in 1999 (Figure 1). From 2000, the incidence of syphilis began to

rise, culminating in 2.1 cases per 100,000 population (99 cases) in 2004. In 2007, an incidence of 2.6 was reported, an increase of 136% compared with the year 2000 (1.1/100,000).

**Sex**

Syphilis incidence was three to seven times higher among males than females during 2000-2007. The male-to-female ratio increased to 7.5 in 2001, being the highest since the 1990s (Figure 2). Between 2000 and 2007, 80-88% of syphilis cases were in men.

**Age**

During 2000-2007 the median age for females was on average 33 years (median age range between 2000-2007: 31–35 years), for men infected through heterosexual contacts it was 38 years (median age range between 2000-2007: 32–43 years) and for men infected through sex between men the median age was 39 years (median age range between 2000-2007: 37–41 years). Among females, age-specific syphilis incidence was highest and increased most in the age group 25-34 years (from 0.4 in 2001 to 4.8 cases per 100,000 women in 2006); a substantial increase was also seen in the age group 35-44 years in 2006 (from 0.3 in 2005 to 1.7 in 2007) (Figure 3).

Among men, during 2000-2007 the age-specific syphilis incidence was highest and increased most in the age groups 35-44 years (from 2.6 in 2001 to 10.4 in 2007), 25-34 years (from 3.6 in 2000 to 10.1 in 2007) and 45-54 years (from 1.5 in 2000 to 5.4 in 2007) (Figure 3).

**Reported route of transmission**

During 2000-2007, 51-70% of males acquired syphilis through sex between men (Figure 4). In 2006 the proportion of males who acquired syphilis via sex between men decreased to 51%, whereas the proportion of males with unknown route of transmission increased.

Two counties in Sweden (Skåne and Stockholm County) with two big cities (Malmö and Stockholm) reported the majority of syphilis cases among MSM during 2000-2007. Estimated syphilis incidence among MSM in Sweden was 20-28 times higher than that of the Swedish male population (Figure 5).

**Figure 4**

**Reported route of transmission (in percent) most likely associated with acquisition of syphilis in men, notified cases in Sweden, 2000-2007 (n=974)**

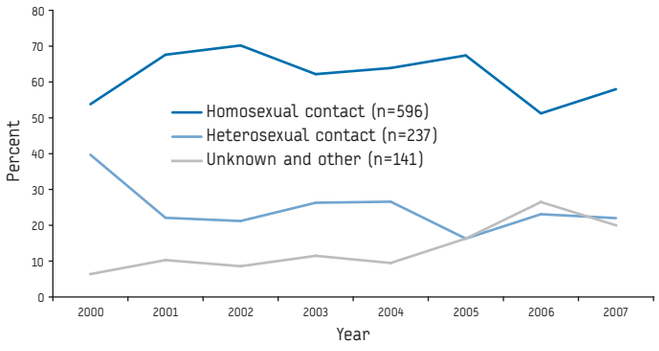
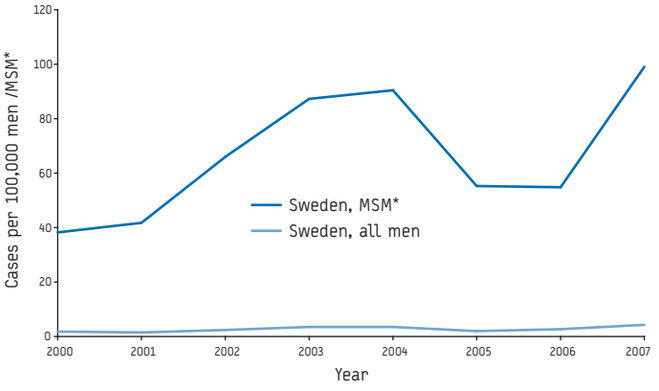



**Figure 5**

**Estimated syphilis incidence among men who have sex with men (MSM) and observed syphilis incidence among all males, notified cases in Sweden, 2000-2007**




**Table**

**Reported country of acquisition (%) of syphilis by sex and type of sexual contact 2000-2007 (n=1,047)**

| | | 2000 | 2001 | 2002 | 2003 | 2004 | 2005 | 2006 | 2007 |
|---|---|---|---|---|---|---|---|---|---|
| Females (all sexual contacts) | Sweden | 33.3 | 20.0 | 20.8 | 23.8 | 26.5 | 23.5 | 15.2 | 34.1 |
| | Abroad | 57.1 | 70.0 | 66.7 | 57.1 | 61.8 | 64.7 | 45.7 | 56.1 |
| | Unknown | 9.5 | 10.0 | 12.5 | 19.0 | 11.8 | 11.8 | 39.1 | 9.8 |
| | **Total number** | **21** | **10** | **24** | **21** | **34** | **17** | **46** | **41** |
| Males with heterosexual contacts | Sweden | 12.9 | 26.7 | 27.3 | 31.7 | 21.4 | 6.7 | 29.6 | 38.6 |
| | Abroad | 71.0 | 73.3 | 72.7 | 58.5 | 76.2 | 80.0 | 66.7 | 56.8 |
| | Unknown | 16.1 | 0 | 0 | 9.8 | 2.4 | 13.3 | 3.7 | 4.5 |
| | **Total number** | **31** | **15** | **22** | **41** | **42** | **15** | **27** | **44** |
| Males with homosexual contacts (MSM) | Sweden | 66.7 | 60.9 | 68.5 | 76.3 | 64.4 | 61.3 | 56.5 | 78.8 |
| | Abroad | 26.2 | 39.1 | 27.4 | 16.5 | 27.7 | 32.3 | 37.1 | 19.5 |
| | Unknown | 7.1 | 0 | 4.1 | 7.2 | 7.9 | 6.5 | 6.5 | 1.8 |
| | **Total number** | **42** | **46** | **73** | **97** | **101** | **62** | **62** | **113** |

### Geographic spread

Syphilis incidence varied by county and year with a constantly higher incidence in Stockholm county. During 2000-2007, on average 46% of all syphilis cases were reported from Stockholm county which accounts for 21% of Sweden's population. Other counties with large cities are Västra Götaland with Göteborg and Skåne with Malmö. On average 10% of all syphilis cases were reported from Västra Götaland county (17% of Sweden's population) and on average 14% of cases were reported from Skåne county (13% of Sweden's population). Some counties, such as Värmland, Västerbotten and Gotland, did not report any cases of syphilis for several years.

### Country of acquisition of the infection

During 2000-2007, the majority of females and heterosexual males acquired syphilis abroad while the majority of MSM acquired the infection in Sweden (Table). However, in some years data on country of acquisition were lacking.

## Discussion

Syphilis clearly re-emerged in Sweden between 2000 and 2007. During the same period there has also been an increase in other STIs in Sweden and worldwide [6,7,9,15,16]. Data from routine surveillance systems provide important information which is used for public health purposes. The Swedish Surveillance system of syphilis is population-based. All health care specialists have the responsibility to notify diagnosed syphilis (and other notifiable STIs) directly to the national surveillance system SmiNet. Also all laboratories diagnosing syphilis have the responsibility to notify to the SmiNet. This dual notification from clinicians and laboratory minimises the chance of underreporting of syphilis cases and data on syphilis incidence in Sweden are thus largely reliable. Some delays may occur, however, since assessment of the timeliness of notifications for syphilis and other STIs has not been performed in Sweden so far and therefore exact data on delay are not available. Some problems in the surveillance system may arise from duplicates due to coded and anonymous notification of syphilis cases. However, the medical officers in the counties in Sweden have the duty to check for duplicates and erase them from the system as they are able to obtain access to the patient's full identity from the notified clinic and laboratory. As a result we believe that duplicate reporting did not affect the presented syphilis incidence. Some other difficulties are seen in syphilis surveillance, such as, the difficulty in establishing the stage of syphilis (e.g. differentiating between early and late latent syphilis). This might affect the quality of reported cases. The health care professionals have to use their best judgement and follow the case definition (only early infectious syphilis with laboratory confirmation should be reported) to ensure the reported data provide a realistic picture of syphilis epidemiology in Sweden. Data quality in terms of data completeness varies for some variables (e.g. reported country of acquisition) and some conclusions need to be drawn with caution.

From the data obtained through the surveillance system we conclude that the major contributor to the recent rise in syphilis cases in Sweden is infections among MSM. Recently described outbreaks of syphilis and gonorrhoea among MSM showed that unsafe sex practices were more widespread among this population group in many countries and included a growing number of casual sexual partners, non-use of condoms and contact with anonymous sexual partners [6-9,17,18]. Adopted risk reduction strategies against human immunodeficiency virus (HIV) transmission referred to as "safe(r) sex" such as oral sex and choosing a partner with the same HIV status does not necessarily protect against syphilis. Among MSM who acquired a syphilis infection in Sweden in recent years, the majority acquired it in big cities, especially Stockholm (up to 96% of all reported cases among MSM). According to our estimate syphilis incidence among MSM was 20-28 times that of males in general in Sweden. It can be assumed that MSM to a larger extent choose to live in big cities since they assure more anonymity and less stigmatisation for MSM. Big cities also supply a meeting ground for sexual networks that facilitate the spread of STIs, as has been reported from other European Union countries [17-20]. A study in Sweden on risk factors for syphilis among MSM showed that current syphilis patients are 7.8 times more likely to have had syphilis in the last five years than MSM without current syphilis [21]. Also MSM with current syphilis are 3.8 times more likely to have had more than ten sex-partners in the past 12 months than MSM without current syphilis. The change in sexual behaviour with more risk-taking practices is likely to have contributed to the recent increase in STIs in Europe [9,19,20,22]. This supports the need for improved preventive work with adapted health prevention messages and education for MSM.

Another group of concern is women. Syphilis cases in females are reported mostly from the counties with big cities, such as Stockholm county and Skåne (Malmö). The increased number of infected women is worrying and suggests either novel sexual networks or a change in sexual behaviour or increased sexual contacts with bisexual men. The latter can link MSM sexual networks with heterosexual females and introduce syphilis and other less common STIs into these sexual networks as it was reported in USA [16]. Closer analysis of such behaviours and sexual networks is needed to gain a thorough insight into the matter.

The constant high number of heterosexual males who acquire syphilis abroad suggests that this is an additional group of the population which requires targeted prevention activities.

## Conclusions

The described syphilis trends in Sweden over the past eight years give insight into key features of the syphilis epidemiology: the most affected population groups are MSM and heterosexual men (especially when travelling abroad). However, reported numbers in women are rising and a cause for concern. Overall the increased incidence of syphilis and other STIs points to insufficient use of condoms and more risk-taking behaviour and possible lack of knowledge about STIs and their transmission. The findings presented in our study should guide public health professionals in planning targeted preventive campaigns.

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# Reported cases of congenital syphilis in the French national hospital database

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In France, the resurgence of syphilis infection since the beginning of the 2000s, with cases reported among women of reproductive age is a reason for concern considering the possible occurrence of congenital syphilis (CS). Using the French national private and public hospital database, we investigated the number of children with a diagnosis of CS born in France in 2004. Six cases less than one year old were identified as probable CS in the database. Two of these cases were adopted children from outside Europe, whereas the other four were born in France. The mothers of these last four infants tested positive for syphilis during the third trimester of pregnancy, two of them during premature delivery. Three of the four mothers were born abroad. Specific socio-cultural conditions may have been responsible for a lack of antenatal care responsible for the disease. Since CS is a preventable disease and the treatment of syphilis infection is cost-effective, we conclude that surveillance of CS cases and assessment of syphilis screening practises during pregnancy should be performed to prevent the occurrence of CS cases in France.

## Introduction

Because of the availability of cost-effective treatment and better prevention of sexually transmitted infections (STI) following the emergence of HIV in the 1980s, syphilis infection had almost disappeared in western European countries by the end of the 90s [1-5]. However, since 2000, France as well as other western European countries has been facing an increasing number of syphilis cases especially among men who have sex with men (MSM), although heterosexual transmissions affecting an increasing number of women are reported too [6,7]. Neither syphilis infection nor congenital syphilis (CS) is notifiable in France. The surveillance of syphilis is based on voluntary notifications by clinicians working in STI health centres, and the burden of the disease in the general population is by consequence underestimated.

Syphilis infection among women of reproductive age can lead to CS in their children. In France, syphilis screening is mandatory and free of charge during the first trimester of pregnancy. However, in specific circumstances, pregnant women may miss antenatal care and by consequence pose a higher risk of CS for the infant due to the lack of appropriate treatment during pregnancy [8,9].

We used the national public and private hospital database, which includes information on all hospitalisations with a specific diagnosis associated to each stay, to assess the number of children with a diagnosis of CS born in France in 2004. We also attempted to identify maternal characteristics associated with the risk of CS.

## Methods

### Study design and population

We performed a retrospective cross sectional study of CS cases recorded in 2004 in the French national private and public hospital healthcare information system [Programme de Médicalisation des Systèmes d'Information (PMSI) implemented since 1999]. This database includes information on all the diagnoses made for all the patients admitted to French hospitals. Medical doctors register all hospital stays with a principal and associated diagnosis coded according to the 10th edition of the International Classification of Diseases (ICD-10) [10]. All data contained in the database are anonymous and protected by professional confidentiality.

In our study, information on hospital stays in 2004 of patients less than one year old with a principal or associated diagnosis of CS (Table 1) were extracted from the PMSI database. As some patients may have stayed in hospital several times, a unique identification number per patient in each hospital allowed us to identify all the stays related to the same patient.

### Data collection

For each diagnosis of CS identified in the PMSI database, we contacted the hospital where the patient had stayed and asked the head of the medical informatics department to collect the following information from the patient's medical records: sex, age, date of stay, clinical symptoms, serology of syphilis and place of birth of the infant and term of the pregnancy, screening of the

**Table 1**

**Codes based on the 10th edition of International Classification of Diseases (ICD-10) used for initial extraction of data on cases with diagnosis of congenital syphilis from the national hospital information system, France, 2004**

| Diagnosis | Code |
|---|---|
| Early congenital syphilis, symptomatic | A50.0 |
| Early congenital syphilis, latent | A50.1 |
| Early congenital syphilis, unspecified | A50.2 |
| Late congenital syphilis oculopathy | A50.3 |
| Late congenital neurosyphilis (juvenile neurosyphilis) | A50.4 |
| Other late congenital syphilis, symptomatic | A50.5 |
| Late congenital syphilis, latent | A50.6 |
| Late congenital syphilis, unspecified | A50.7 |
| Congenital syphilis, unspecified | A50.9 |

mother for syphilis during pregnancy, date of screening, antenatal care and treatment received for syphilis during pregnancy, and mother's place of birth. Based on these data, cases were confirmed as definite or probable early CS according to the case definition.

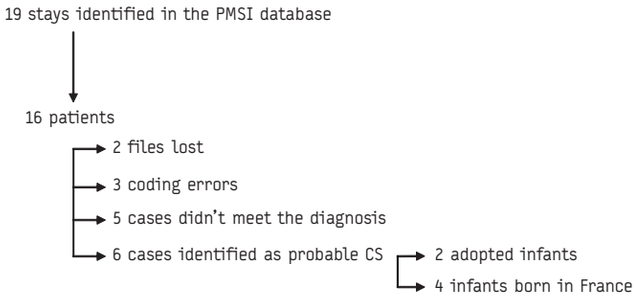
## Results

A total of 1,811 hospital stays were recorded with a diagnosis of syphilis in the PMSI database in 2004. In 113 of these CS appeared among the diagnoses coded, but only 19 hospital stays had the principal or associated diagnosis of CS. These 19 stays corresponded to 16 infants, as four stays were linked to the same patient (Figure). No medical record could be located for two patients. In three cases coding error occurred; the principal diagnosis coded was different than the principal diagnosis mentioned in the medical records. Five patients did not fulfil the case definition and were excluded.

Among the remaining six infants classified as probable cases of CS, two were adopted babies born in North Africa. The remaining four cases were born in France, two of them prematurely, including one with foetal hepatomegaly (Table 2). The mothers of these four cases were screened positive for syphilis during their third trimester of pregnancy two of them at the premature delivery. They had not received proper treatment for syphilis infection: two were not treated at all, one received only two doses of extencillin (benzathine benzyl penicillin), and the last one received three doses but starting less than 30 days before delivery. No information on syphilis screening during the first trimester of pregnancy was available for any of these four women. Additional information on social integration difficulties and undesired pregnancy was mentioned in the medical documentation of two mothers.

**FIGURE**

**Congenital syphilis (CS) cases identified in the PMSI database, France, 2004**




## Discussion

This original study is the first documentation of CS occurrence in France. We identified six probable early CS cases less than one year of age according to the CDC definition. None of them were confirmed CS that would have required laboratory confirmation with evidence of Treponema pallidum [11]. Among these six probable cases, two adopted infants were born outside Europe, in North Africa. Only four probable CS cases born in France were identified in the PMSI database in 2004. The CS diagnosis was made at birth for all of them. Syphilis infection among the mothers was detected or treated too late to prevent the infection in the infant.

This number may have been underestimated. Indeed, CS can occur with non-specific symptoms, or the infection can be asymptomatic at birth and the diagnosis is by consequence delayed. Among the nine children with CS reported between 1994 and 1997 in the United Kingdom [12], only three had clinical abnormalities. In our study, one of the four cases born in France had obvious clinical symptoms. Our study focused on early CS in children less than one year of age but the diagnosis of CS can be made in older children ("early" CS defined as affecting children less than two years old and "late" CS diagnosed in children older than two years). In the PMSI database, a two-year-old child and eight children aged between two and 15 years were reported with a principal or associated diagnosis of CS in 2004. These cases were not

**TABLE 2**

**Probable cases of congenital syphilis (CS) less than one year of age born in France identified in the PMSI database in 2004, France**

| Cases | Age | Sex | Congenital syphilis diagnosis: serologic tests and others investigations | Syphilis screening during pregnancy | Treatment of the mother during pregnancy | Place of birth of the mother |
|---|---|---|---|---|---|---|
| 1 | Newborn (reached term) | Male | +TPHA/VDRL** | +TPHA/VDRL | Two doses of extencillin given at one week intervals before delivery (at term) | East Europe |
| 2 | Premature (33 WA*) | Male | +TPHA/VDRL; +Fta-IgM | First prenatal consultation at 29 WA* with +TPHA/VDRL; undesired pregnancy | Three doses of extencillin given at one week intervals | South Europe |
| 3 | Premature (32 WA*) | Female | Foetal hepatomegaly; +TPHA/VDRL | Threat of premature delivery with admission to hospital and +TPHA/VDRL during the third trimester; pregnancy followed up by a general practitioner; no information on syphilis screening during the first trimester | Never treated | Outside Europe |
| 4 | Diagnosis made at birth (unknown week of amenorrhea) | Male | No clinical symptoms; two reactive +TPHA/VDRL at one week intervals; second titration higher than the first | +TPHA/VDRL at delivery; difficulties with social integration | Refused treatment | Overseas French district in America |

* Week of amenorrhea
**Treponemal test: *Treponema pallidum* hemagglutination test (TPHA) / venereal disease research laboratory (VDRL)

investigated. Finally, coding errors may have occurred, and cases who had never been hospitalised were missed. Also, miscarriages and cases of stillbirth due to CS were not included in the study.

Previous studies showed that CS occurs more frequently in absence of or inappropriate antenatal care [13,14]. The consequence is a late or no syphilis screening and inappropriate treatment of the mothers which fail to prevent foetal contamination [15,16]. In France, a pregnant woman should attend seven antenatal consultations, and syphilis screening must be performed during the first trimester (Décret n°92-143) [17]. Antenatal care is free of charge, the syphilis screening included. However, despite this regulation, different factors such as language and cultural barriers or an illegal administrative status can hinder pregnant women from getting appropriate medical care [8,9]. In our study, specific socio-cultural conditions may have been responsible for the lack of antenatal care: three mothers were migrants and "social integration difficulties" (but no details) were reported for the fourth one. The fact that one mother refused the treatment and one had an undesired pregnancy may be indicative of further psychosocial problems [18].

In France, like in the other western European countries, the re-emergence of syphilis especially affects MSM [6]. However, the number of infected women also increased from 11 (of the total of 207 cases of syphilis reported) in 2001 to 30 (total of 455 cases) in 2006 [6]. The contagiousness of syphilis as well as the high percentage of heterosexuals who engage in high risk sexual behaviours, such as not using condoms for oral sex (92% heterosexuals in France), may be responsible for an increasing number of syphilis cases among women. Immigrant women were identified to be at a higher risk of syphilis infection during pregnancy [19]. In France, among the 19 women with a diagnosis of syphilis notified in 2004, 13 were born in foreign countries and four were born in France (information unavailable for the remaining two women). In this context, health care practitioners should consider the option of syphilis screening at each pregnancy consultation [20,21], especially in the presence of risk factors identified above. Indeed, the French National Authority for Health (Haute Autorité de Santé - HAS) recommends another syphilis screening performed before 28 weeks of gestation when the woman or her partner has high risk sexual behaviour. Finally, a lack of syphilis screening during pregnancy should result in performing serology at birth to avoid late diagnosis of CS [21].

Despite their limits, data from PMSI can be used to describe the annual trend of CS cases, but not to perform a prospective follow up as the data are available with a minimum delay of three years. In France, the very low number of CS cases suggests that the antenatal care system is efficient. However, because of the overall increasing incidence of syphilis infection the French gynaecologists, obstetricians and paediatricians should be aware of the risk of CS, especially among women belonging to specific under-privileged groups, and should double check at delivery whether and how the screening was performed during pregnancy. A prospective notification of CS cases by maternity or paediatric wards with an investigation of each case should allow to better characterise the circumstances of syphilis infection before or during pregnancy and the performance or the absence of syphilis screening. These information may improve the prevention and the treatment of CS in the population at risk. This strategy is necessary taking into consideration the severity and the burden of CS avoidable with a simple cost effective treatment.

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# An international outbreak of Shiga toxin-producing *Escherichia coli* O157 infection due to lettuce, September – October 2007

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Between 14 September and 20 October 2007, an outbreak of Shiga toxin-producing *Escherichia coli* (STEC) O157 simultaneously occurred in the Netherlands and Iceland. A total of 50 laboratory-confirmed cases were reported with a STEC O157 infection caused by the same clone. The strain was of type O157:H-, PT8, positive for $stx_1$, $stx_2$, *eae* and *e-hly*, and sorbitol negative. The most probable cause of this international outbreak was contaminated lettuce, shredded and pre-packed in a Dutch food processing plant. Samples of the environment, raw produce and end products, taken at several vegetable growers and processing plants all tested negative for STEC O157. However, the only epidemiological link between the cases in the Netherlands and in Iceland was the implicated Dutch processing plant. In Europe, food products are often widely distributed posing the risk of potential spread of food borne pathogens simultaneously to several countries. This international outbreak emphasises the importance of common alert and surveillance systems in earlier detection of international outbreaks and better assessment of their spread.

## Introduction

Infections with Shiga toxin-producing *Escherichia coli* (STEC) typically present as diarrhoea which can range from mild and watery to bloody (haemorrhagic colitis). The infection can progress to haemolytic uremic syndrome (HUS), a serious condition that can result in death [1,2]. Children under 10 years of age with a verified STEC infection have approximately 15% risk of developing HUS [3]. The serotype most often associated with severe disease is STEC O157:H7, but many other serotypes are also known to cause symptoms [4-6].

A great majority of STEC O157 outbreaks can be traced back to ruminants, especially cattle [7]. Numerous studies have been done on faecal excretion of STEC O157 from cattle to estimate the carriage rate of STEC [7]. All agree that faecal excretion exists, although the rate found varies. Inevitably, contact with farm animals has been reported as a source for STEC outbreaks [8,9]. Spreading of cattle manure over land or in water can contaminate water and produce, and meat can be contaminated in the slaughterhouse or later in the production process. Water [10,11] and food products [12], such as meat [13-15], dairy products [16-18], and fresh produce [19,20] are therefore often reported as sources of outbreaks caused by STEC O157.

In September-October 2007, national outbreaks of STEC O157 infection occurred simultaneously in the Netherlands and Iceland, of which preliminary reports were published in November 2007 [21,22]. As the isolates of STEC O157 from the patients of both outbreaks had an identical and unique pulsed-field gel electrophoresis (PFGE) pattern, a common source was suspected. In the present report, we have combined the results of the outbreak investigations done in both countries into one description of the international outbreak, with lettuce as the most probable cause.

## Methods

### The Netherlands

Since 1999, an enhanced laboratory-based surveillance of STEC infections has been implemented in the Netherlands. This means that all Dutch medical microbiological laboratories are required to send STEC isolates to the National Institute for Public Health and the Environment (RIVM) for O- and H-typing. The isolates are also tested for genes encoding Shiga toxin type 1 and type 2 ($stx_1$ and $stx_2$), the *E. coli* attaching-and-effacing gene (*eae*) and the haemolysin encoding EHEC-*hly* gene (*e-hly*). DNA fingerprints are made by PFGE, using *Xba*I as the restriction enzyme. The fingerprints are processed with BioNumerics® (Applied Maths, Kortrijk, Belgium; Dendrogram type=UPGMA, Similarity coefficient=Dice).

Additionally, as part of the surveillance, municipal health services contact every laboratory-confirmed STEC patient in the Netherlands to collect information about clinical symptoms and exposures to known risk factors in the week before illness onset using a standardised questionnaire [23,24]. When a marked increase in the numbers of reported STEC cases was observed in the end of September 2007, in addition to the standard questionnaire a special outbreak questionnaire was designed, providing more detailed information on consumption of meat, dairy and raw

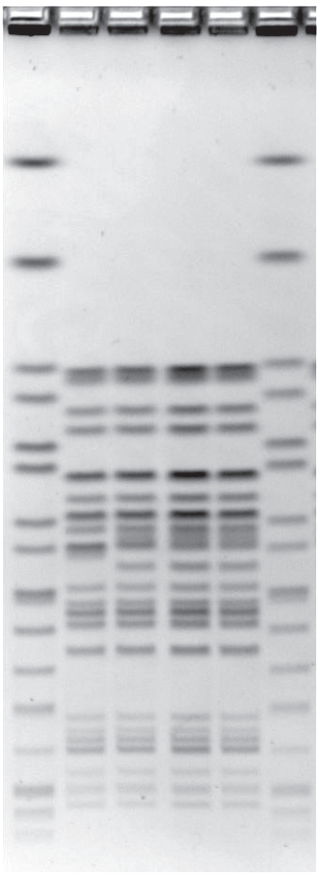
vegetables and contact with farm animals and manure. All cases with onset of symptoms after 1 September 2007 were asked to complete both questionnaires.

An outbreak-related case was defined as having an isolate matching the outbreak fingerprint for at least 95% and the date of onset of symptoms later than 1 September 2007. A case-case comparison was made between non-outbreak cases of the enhanced surveillance (1999-2007) and the outbreak-related cases using the standardised questionnaire.

When lettuce was suggested as a possible source, the Dutch Food and Consumer Product Safety Authority (VWA) started investigating the distribution channels of packed fresh vegetables and the individual ingredients, and visited several vegetable producing and processing companies. During these visits, samples of the environment, raw produce and end products were collected and tested for STEC O157. During the visit of VWA at one of the processing plants, it was noted that during the outbreak period a high number of workers had been absent due to illness. The municipal health service visited the plant shortly afterwards to gather additional information on the symptoms and to collect stool and blood samples from those who had been ill. Blood samples were tested for the presence for antibodies against LPS O157 using ELISA and immunoblotting [25].

**Figure 1**

**Pulsed-field gel electrophoresis (PFGE) pattern of the outbreak strain (middle four lanes) and the reference H9812 *S. Braenderup* (both side-lanes), international outbreak of Shiga toxin-producing *Escherichia coli* (STEC) O157 in Iceland and the Netherlands, September-October 2007**



**Iceland**

In Iceland, STEC infections are subject to mandatory notification which requires laboratories and treating physicians to report cases without delay. When a clear rise in the number of domestically acquired STEC O157 infections was observed in early October 2007, an outbreak investigation was initiated.

The case definition used for the outbreak investigation included all domestically acquired STEC O157 infections with onset of symptoms after 1 September 2007, pending PFGE and testing for $stx_1$ and $stx_2$ genes. A trawling questionnaire on food consumption, mass gathering and travel as well as purchase records from two weeks prior to onset of infection were collected from cases.

In Iceland, detection of STEC O157 is carried out only by the reference laboratory which performs DNA fingerprinting with PFGE, using *Xba*I as the restriction enzyme. Testing for $stx_1$ and $stx_2$, however, is not done in Iceland therefore isolates were sent to the Laboratory of Enteric Pathogens at the Health Protection Agency (HPA) in the United Kingdom for detection of these genes.

The food division of the Environment and Food Agency in Iceland is responsible for surveillance in food and when results from the trawling questionnaire and purchase records were available, surveillance of lettuce was intensified with increased sampling.

## Results

On 11 October 2007, Iceland notified other European countries about the ongoing outbreak of STEC O157 through the urgent inquiries system of the European Food and Waterborne Diseases Network administered by the European Centre for Disease Prevention and Control (ECDC). When the Netherlands responded by reporting a similar outbreak, contact between these two countries was established and information exchange was facilitated. PFGE patterns of the first set of STEC isolates from cases in both countries were available for comparison on 22 October revealing identical fingerprints and a definite link between the two countries. The PFGE-pattern is shown in Figure 1.

In total, isolates from 48 cases from both countries had identical PFGE patterns. This pattern had not been observed previously in either of the two countries or the rest of Europe. Isolates from two other individuals generated a PFGE pattern that matched the outbreak pattern in 95-97%. Both patients were included as cases, resulting in a total number of 50 cases. The distribution of these cases by date of symptom onset is shown in Figure 2. Forty-seven cases (94%) reported diarrhoea, including 41(87%) with bloody diarrhoea. No cases of HUS were reported.

Twelve cases, seven males and five females, were regarded as secondary cases, as they most likely had contracted the infection from another case. Six of them were children aged 0-8 years, and six were adults aged 34-82 years.

The 38 primary cases included 21 females and 17 males. Their median age was 24.5 years (range 1-74 years), and about half of

the cases were aged between 10 and 30 years. The detailed age and sex distribution of the primary cases is shown in Figure 3.

**The Netherlands**

In the Netherlands, the annual number of STEC cases reported between 1999 and 2006 ranged from 32 to 57. In the end of September 2007, a marked increase in the number of reported cases was noted. An outbreak of STEC O157 was identified including 41 cases with dates of symptom onset between 14 September and 20 October 2007, of whom 31 were primary cases, and ten were secondary cases. Thirteen patients were admitted to hospital; in two cases information on hospitalisation was missing. All 41 isolates were of serotype O157:H-, contained $stx_1$, $stx_2$, *eae* and *e-hly* genes, and were sorbitol negative.

Answers to at least one of the two questionnaires were available for 29 of the 31 primary cases. Descriptive epidemiology suggested a link between STEC infection and consumption of lettuce as 25 cases (86%) reported eating lettuce in the week before illness. Comparison of the standard questionnaire results for the outbreak-cases with those of the sporadic cases of the surveillance showed highest odds ratios for pre-packed lettuces: 4.41 (95% confidence interval 1.91-10.19) compared to the sporadic cases of 1999-2007, and 7.33 (95% CI 2.19-24.50) compared to the sporadic cases of 2007.

A total of 99 environmental and food samples taken at the vegetable producing and processing plants were tested and found negative for STEC O157. In one company, which exported pre-packed lettuce to Iceland, a total of 32 employees had been on sick leaves because of gastroenteritis during the outbreak period. However, faeces and blood samples of these workers tested negative for STEC O157 and interviews with them suggested that the clinical presentation was more compatible with a norovirus outbreak than with STEC O157 infection.

**Iceland**

In Iceland, only up to two cases of STEC infection had been reported annually in the 10-year period preceding 2007 (with the exception of four cases notified in 2004), and no outbreak had ever been detected. In the outbreak in 2007, nine cases were identified with onset of symptoms between 23 September and 18 October. Seven cases were considered primary cases and two were secondary cases. Seven patients were hospitalised.

The isolates from the first three cases were sent to the Laboratory of Enteric Pathogens at the HPA in the United Kingdom and were identified as STEC O157, phage type 8, carrying the $stx_1$ and $stx_2$ genes with a PFGE pattern identical to the pattern for the Dutch strains. PFGE done on all nine isolates at the Department of Microbiology at Landspitali University hospital revealed a pattern identical to the pattern from HPA.

The seven primary cases lived in different parts of the country: three cases resided in the capital area, two in the northern part of the country, one in the eastern part and one on the Westman Islands. It was clear that the product that had caused the infection had been widely distributed.

Eight cases (seven primary) answered the trawling questionnaire and two primary cases provided purchase records. Results from the outbreak questionnaire showed that six of the seven primary cases had consumed either fish or sliced precooked ham. But since these products originated from different producers or local fishermen, they were considered to be an unlikely source of the outbreak. The purchase records and the outbreak questionnaire also revealed that five of the seven primary cases had consumed ready-to-eat lettuce mixtures of one brand pre-packed in and imported from the Netherlands. However, of the 80 samples of lettuce collected between 22 October and 5 November none tested positive for *E. coli* O157.

## Discussion

Between mid-September and mid-October 2007, in Iceland and the Netherlands a total of 50 patients were diagnosed with a STEC O157 infection caused by the same strain. The actual number of cases may have been considerably higher seeing that infections with STEC O157 may pass uncomplicated or even symptom-free, especially in adults [26,27], and those affected do not seek medical help and are not tested for STEC O157. No HUS-cases have been reported in the outbreak. The age of the cases is probably a relevant indication of the cause, as only three of the 38 primary cases and five of the 12 secondary cases were younger than five years.

**Figure 2**

**Epidemic curve of the international outbreak of Shiga toxin-producing *Escherichia coli* (STEC) O157 in Iceland and the Netherlands, September-October 2007, by date of onset of symptoms (n=50)**

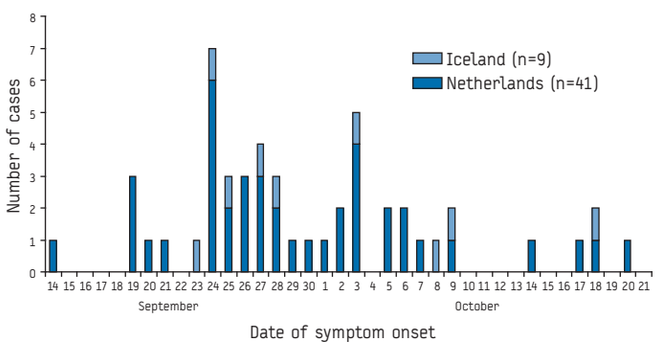
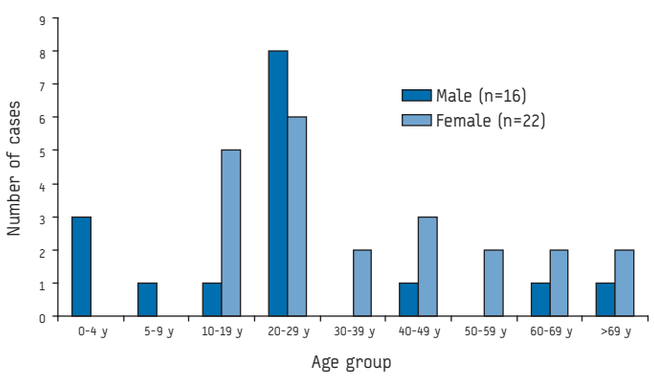



**Figure 3**

**Age and sex distribution of all primary cases of Shiga toxin-producing *Escherichia coli* (STEC) O157 related to the international outbreak in Iceland and the Netherlands, September-October 2007 (n=38)**



The most probable cause of this international outbreak was contaminated lettuce, shredded and pre-packed in a Dutch food processing plant. Packages with several combinations of different types of lettuce but belonging to the same brand imported from the Netherlands were reported by the cases. Contamination of lettuce can occur either during growth by the application of water, soil or manure contaminated with animal faeces or as a result of cross-contamination during processing, for example through contaminated transport containers, human transmission or in the shredding process. However, microbiological evidence pointing to the source of this outbreak was not found. Furthermore, none of the workers of the implicated food processing plant tested positive for STEC O157 infection. It is likely that the contamination had already faded out at the time samples were taken at the food producing and processing plants and that contaminated products had not been present in the supermarkets anymore. The sampling started in mid-October, which is around the date the last cases had onset of symptoms.

The outbreak highlights the importance of fresh produce as a vehicle in STEC infections. Although it has been shown that lettuce can become infiltrated by *E. coli* O157 making it impossible to wash off [28], in most cases the bacteria stay on the surface of the leafs. However, the fact that salad vegetables are usually eaten raw is compounded by the increase in popularity of pre-packed salad products that are unlikely to be washed by the consumer. In one outbreak caused by lettuce, the wash water used by the grower was the most likely source of contamination, as it contained *E. coli* O157:H7 [29]. Contaminated water was also suspected as the source in an STEC O157 outbreak related to iceberg lettuce in Sweden, although no microbiological evidence was found [30]. The trace-back investigation in another lettuce outbreak in the United States implicated two possible sources: one at a local farm and another in six farms shipping under the same label [31]. Microbiological evidence could not be established, so the transmission route remained unclear.

Food products are widely distributed within the European Union (EU) and from and to countries outside the EU thus creating the potential for the spread of food borne pathogens simultaneously to several countries. This international outbreak emphasises the importance of common alert and surveillance systems in the EU for earlier detection of international outbreaks and better assessment of the size and the spread of such outbreaks. The e-mail urgent inquiries system of the European Food and Waterborne Diseases Network administered by the ECDC has proven its value to detect similar outbreaks occurring simultaneously in more than one country. In this outbreak, the link to Dutch lettuce products was suspected two weeks after the first e-mail informing about the cases in Iceland. As both countries promptly joined forces, direct action by the Dutch food authorities could then be taken, which shows the added value in joint outbreak investigation within the EU. Analysing compiled data when possible and collecting supporting findings from more than one country, at the same time increases the possibility to detect potential sources at an earlier stage and strengthens the epidemiological evidence. Thus, cooperation allows for earlier implementation of actions aimed at identifying and eliminating the source of infections and therefore contributes to the decrease of both morbidity and mortality due to communicable diseases within the EU.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# Tuberculosis outbreak associated with a mosque: challenges of large scale contact tracing

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In this report, we describe the investigation and management of an outbreak of TB associated with a mosque in Scotland, and consider the implications of large-scale TB contact tracing. In 2005, an Algerian man living in the north-east of Scotland was found to be sputum smear-positive for TB. Initial investigation identified three (18.8%) close contacts with active disease. Due to the high rate of transmission, contact tracing was extended to casual contacts of the index case at a mosque. No sub-group at highest risk of exposure could be defined at the mosque. Screening of mosque attendees identified two cases (0.53%), with a further two identified by review of existing cases and enhanced surveillance. Two additional cases were linked to the outbreak by genetic profile. Response to the screening exercise was initially poor, but after modification of the communication strategy, 438 people were offered screening with 86% attending. The investigation and management of a TB outbreak is challenging and requires a complex message about risk to be communicated. In a mosque setting, there were additional complexities that, to the best of our knowledge, have not been reported previously. It was crucial, in designing the communication strategy, to identify key individuals within the community to assist with tailoring the message to address risk perception and to help to deliver the message. Despite this, approximately 50% of those considered to have the highest exposure (adult males regularly attending Friday lunchtime prayer meetings) did not come forward for screening. The screening of casual contacts in this setting was complex and time-consuming with a low detection rate.

## Introduction

In 2005, an Algerian man, living in the north-east of Scotland was found to be sputum smear-positive for tuberculosis (TB). He had lived in three houses in Aberdeen and was a member of the Aberdeen mosque. Initial investigation identified three (18.8%) close contacts to have active TB disease. Therefore, NHS Grampian undertook a large contact tracing exercise in early 2006, focusing on the mosque community. In this report, we describe the outbreak investigation and management, and consider the implications of large-scale TB contact tracing. As far as we are aware, this is the first reported exercise of its kind associated with a mosque.

### Epidemiology of TB in Scotland

Since the mid 1980s, the incidence of TB has been increasing in many parts of the developed world [1]. In low-incidence European countries, it has long been assumed that reactivation of latent infections was responsible for causing the majority of disease [2]. However, molecular epidemiology studies have shown that the contribution of recent transmission to the overall burden of disease is greater than previously thought [3-6]. The rising incidence of TB in England, Wales and Northern Ireland has been attributed to an increase in cases among those who have recently arrived from high-prevalence countries, with 72% of cases in 2005 occurring in people not born in the United Kingdom (UK) [7]. In contrast, case numbers in Scotland have been relatively stable, with approximately 400 new TB cases annually over the past decade and non-UK born cases representing only 30% of the total number of cases [8].

The outbreak we report here was unusual in Scotland, representing recent transmission of infection rather than reactivation of latent disease. Occurrence within a community where a high proportion of members were not born in the UK is an epidemiological pattern more akin to England and Wales than to Scotland.

### Guidelines for the management of TB

At the time of this outbreak, the National Institute for Health and Clinical Excellence (NICE) guidelines were in draft form and the British Thoracic Society (BTS) guidelines (2000) formed the basis for the actions taken [9,10].

## Investigation of index case

### Setting

Grampian, in the north-east of Scotland, has a population of approximately 524,000 people spread over around 7,700 km2. Approximately half the population live in Aberdeen. In Aberdeen, there is one mosque, although Muslim meetings also occur at other premises. In the 2001 census, 0.82% of people living in Aberdeen City reported that their current religion was Muslim, a figure similar to the Scottish national average of 0.84% [11].

### Index case

The index case was identified in October 2005 after a prolonged illness lasting several months. It was estimated that his symptoms, including a productive cough, had begun around March 2005. A sputum sample was smear-positive for numerous acid- and alcohol-fast bacilli (AAFB) and was confirmed to be fully sensitive *Mycobacterium tuberculosis* by the Scottish Mycobacteriaum Reference Laboratory (Box 1).

### Screening of close contacts

The definition of a close contact (Box 2) was complicated by the index case having been resident at three separate addresses during his illness. The time period of concern also included the start of

**Box 1**

**Details of cases of tuberculosis linked with the outbreak in a mosque, Aberdeen, 2005**

**Index Case**
- adult male
- 7-8 month history of symptoms including productive cough, weight loss and night sweats
- Chest X-ray showed extensive bilateral changes
- Sputum smear-positive 8 October 2005
- Cultured as fully sensitive *Mycobacterium tuberculosis*
- High transmission risk period defined as July to October 2005
- Completed treatment May 2006; no adherence issues

**Close Contacts – Linked Cases**

Linked Case 1
- Child (intermittent household contact over risk period)
- Grade 3 Heaf test, no previous BCG vaccination
- Chest X-ray changes, weight loss, night sweats, slight unproductive cough
- Considered clinically to be non-infectious
- No samples for culture obtained
- Completed treatment May 2006; directly observed treatment

Linked Case 2
- Child (occasional contact at mosque)
- Grade 4 Heaf test, no previous BCG vaccination
- Chest X-ray changes, weight loss, night sweats, slight unproductive cough
- Considered clinically to be non-infectious
- No samples for culture obtained
- Completed treatment May 2006 no adherence issues

Linked Case 3
- Adult male (intermittent household contact over risk period)
- Chest X-ray changes, weight loss, night sweats, no cough
- Considered clinically to be non-infectious
- Bronchoalveolar lavage – smear-negative, culture-positive, fully sensitive *M. tuberculosis*
- Culture specimen genetically identical to index case
- Completed treatment August 2006; no adherence issues

**Review of Grampian Cases and enhanced surveillance**

Linked Case 4
- Adult male (mosque attendee with no known direct contact)
- Grade 1 Heaf test
- Chest X-ray changes, abnormal computer tomography chest scan
- Considered clinically to be non-infectious
- Diagnosed on bronchoalveolar lavage – smear-negative culture positive for fully sensitive *M. tuberculosis*
- Culture specimen genetically identical to index case
- Completed treatment June 2006; no adherence issues

Linked case 5
- Adult male (mosque attendee with no known direct contact)
- New arrival in United Kingdom (UK), September 2005 – no BCG vaccination
- Mantoux 18 mm, minor chest X-ray changes
- Considered clinically to be non-infectious
- No samples for culture obtained
- Incomplete treatment; lost to follow up (left UK) July 2006

**Screening detected cases**

Linked Case 6
- Child (mosque attendee with no known direct contact)
- Mantoux 12 mm, no previous BCG vaccination
- No changes on chest X-ray or symptoms suggestive of tuberculosis
- Completed three-month course of chemoprophylaxsis

Linked Case 7
- Adult male (mosque attendee with no known direct contact)
- Mantoux >15 mm and blistered, minor chest X-ray changes
- Weight loss, night sweats, cervical lymph nodes swollen
- Sputum sent for culture and found to contain fully sensitive *M. tuberculosis*
- Lymph node aspirated and sent for culture; found to be fully sensitive *M. tuberculosis*
- Culture specimens genetically identical to index case
- Completed treatment October 2006; no adherence issues

**Late cases**

Linked Case 8
- Adult female from England, no known links to mosque or Aberdeen
- Culture specimens genetically identical to index case
- No additional information available

Linked Case 9 (Grampian)
- Adult male household contact intermittently over at risk period
- Screened as a close contact but no chest X-ray changes
- Presented in 2007 with productive cough, no changes on chest X-ray
- Sputum sent for culture and found to contain fully sensitive *M. tuberculosis*
- Culture specimens genetically identical to index case
- Completed treatment September 2007; no adherence issues

BCG = Bacillus Calmette Guérin

Ramadan and, as a result, the index case had spent substantial periods of time in close contact of people beyond his residence. Sixteen people were classed as close contacts, although their degree of contact was variable.

From the screening of the 16 close contacts, three cases of TB were identified (18.8%); one adult and two children (see Box 1: linked cases 1, 2 and 3).Screening of the contacts of the three linked cases resulted in a further three people being tested (two children and one adult); all were negative for TB disease.

### Conclusion of initial investigation

To find such a high rate of spread among close contacts (18.8%) was unusual. BTS 2000 guidelines advised that casual contact tracing should be considered if the index case was highly infectious, indicated by transmission to more than 10% of close contacts [10]. Therefore an outbreak was declared and an outbreak control team assembled to consider further investigation and control measures.

The outbreak control team identified two settings where significant casual contact with the case could have occurred:

1) The index case's work place, a small food outlet (however, all contacts through work had already been screened as part of the close contacts);

2) The mosque where the index case had attended Friday lunchtime and Friday evening prayer meetings throughout his illness.

## Outbreak investigation and management

### Review of recent TB cases and enhanced surveillance

The outbreak control team decided that a review of recently diagnosed cases, along with enhanced surveillance, of any new cases was appropriate to identify potential association with the index case. Association was considered if there was evidence that the case could have been attending the mosque at the same time as the index case. Through this process, two cases of TB were linked to this outbreak: two adult males (Box 1: linked cases 4 and 5), diagnosed with TB in December 2005 and January 2006.

### Initial microbiological investigation

Where bacteriological specimens were available, genotype testing was requested to establish potential linkage to the index case. The Scottish Mycobacterial Reference Laboratory undertook molecular typing and comparison of genetic profiles using Mycobacterial Interspersed Repetitive Unit-Variable Number Tandem Repeat (MIRU-VNTR), a method introduced in Scotland in August 2005 [12].

Of the three cases detected as part of the close contact investigation only one (linked case 3) had culture-positive specimens

**Box 2**

**Definition of a close contact (British Thoracic Society guidelines 2000 [10])**

**Close Contacts:**
Someone from the same household (sharing a kitchen), very close associates, or frequent household visitors.

available for genotyping. This specimen had a genetically identical profile to the index case.

Of the two cases detected as potentially linked to the outbreak from the review of all recent Grampian TB cases, bacteriological specimens were available for genotyping for one case, and this specimen was also found to be genetically identical to the index case. A third possibly linked case, a 33 year-old male with a lymph node biopsy positive for TB, was found to have a different genotype from the index case.

**Environmental investigation**

Members of the health protection team (a TB specialist nurse and a colleague) visited the Aberdeen mosque accompanied by the Imam outwith prayer meeting times. For Friday lunchtime prayer meetings, attended by adult males only, the series of rooms that made up the mosque were reported to be full and found to be poorly ventilated. The Friday evening meetings, though substantially less well attended, also included women and children. Children were considered to be more susceptible to infection due to their potentially immature immune systems [13].

Attempts were made to identify a subgroup considered to have had greatest exposure or to be particularly susceptible to infection. However, there was no list of contact details for the mosque attendees nor was there a regular pattern as to where within the mosque the attendees prayed. It was, therefore, impossible to identify any "high-risk" sub-group within the mosque using the traditional "ripples from a stone in the pond" approach recommended in the BTS guidelines [10].

**Risk assessment of potential to spread to casual contacts and definition of casual contacts**

The outbreak control team identified a risk of spread to casual contacts through attendance at the mosque at the same time as the index case. The key factors in reaching that decision were:

- Evidence of high rate of infection among the close contacts (with genotype as evidence of link),
- Long period of symptomatic disease in the index case prior to diagnosis as demonstrated by numerous AAFBs in the sputum samples,
- Evidence linking other Grampian cases with the index case for whom the only opportunity for common exposure appeared to be through attendance at the Friday lunchtime mosque meetings (with genotype as evidence of link),
- Relatively overcrowded conditions and poor ventilation at the mosque,
- Presence of children, with their less mature immune systems, among the contacts.

The outbreak control team therefore established a definition of casual contacts (Box 3) and made the decision that a larger scale contact tracing exercise should be undertaken.

**Large scale contact tracing exercise of casual contacts attending the mosque**

***Organisation and methods***

Communication with the mosque community was via the Imam. Religious beliefs dictated that only male Muslims could attend the Friday lunchtime prayer meetings. As a result, no one from the (all female) health protection team could attend. It was necessary to rely on the Imam communicating our complex message to the mosque attendees requesting casual contacts to come forward for screening. Figure 1 summarises the communication process.

On the advice of the Imam that the mosque community would provide translation as required, standard information letters and leaflets in English were made available for collection. The information provided advice about TB and the risk of transmission, along with advice that anyone who met the definition of a casual contact should call a dedicated National Health Service (NHS) helpline to provide their personal details, so that invitations for screening appointments could be sent out.

The initial response was low, with less than 40 names received. A further meeting was held to discuss approaches to improve the response. The NHS Grampian Equity and Diversity Manager, through a network of contacts within the NHS, identified the chair of the mosque committee and a number of well-respected mosque attendees, including a local general practitioner (GP), to assist with communication. With the help of these individuals, a number of potential barriers were identified:

- Risk perception – the level of anxiety was thought to be low, with many considering TB to be easily treated and not a major cause for concern;
- Misunderstanding of risk – some people believed that if they did not currently have clinical symptoms of TB they could not

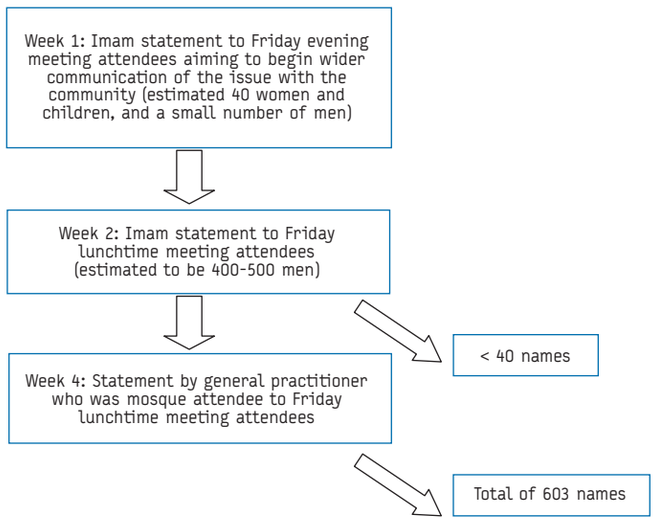
**Box 3**

**Definition of casual contacts of the index case in the tuberculosis outbreak in a mosque, Aberdeen, 2005**

**Casual Contact:**
"Anyone who regularly attended mosque prayer meetings on Friday lunchtimes or Friday evenings between the beginning of July 2005 and the end of October 2005." ('regularly' defined as attending at least three times during the defined time period)

**Figure 1**

**Summary of the communication process with the mosque, Aberdeen, 2005**



have the disease, especially since some months had elapsed since the time of exposure;
- Protection by Bacillus Calmette Guérin (BCG) vaccine – there was a misconception that previous BCG vaccination would guarantee life-long protection;
- Language barriers – complex messages, provided in English, may not have been translated or passed on effectively; where English was not the first language, having to contact a helpline number was believed to be daunting;
- Confidentiality – some people expressed anxiety about sharing of personal information.

To address these issues, the outbreak control team decided to re-iterate among all regular attendees the message about the importance of contact tracing and the need for them to come forward. The message was modified to address the potential barriers and was delivered by the male GP. In view of the potential difficulties calling the help-line, forms were also made available for attendees to complete and return to the health protection team in a freepost envelope.

Communication with the two universities in Aberdeen, which many international students attend, and local media attention raised general awareness. As the first people began to attend for screening, they were asked to encourage others to come forward.

These actions led to a greatly increased response.

**Screening**

All adults (aged 16 years and over) were offered a chest X-ray. Clinics were staffed by X-ray department staff as well as the TB specialist nurse or another member of the health protection team. People who came for a chest X-ray, were also asked to take part in a questionnaire survey of potential symptoms and risk factors.

One respiratory physician reviewed all chest X-rays, together with the relevant questionnaire survey findings. The radiology departments provided a second review of the chest X-ray films. If an abnormality was identified, appropriate follow-up was arranged. All children aged under 16 years were offered a Mantoux test and BCG vaccination as appropriate.

Screened participants, and their GPs, were informed of their test results.

## Results of large scale casual contact screening

Although no formal register of mosque attendees was kept, estimates from various sources suggested that between 400 and 500 adult males (over 15 or 16 years of age) regularly attended Friday lunchtime meetings; a further 40 women and children were thought to regularly attend Friday evening sessions.

A total of 603 mosque attendees contacted the health protection team but, after discussion with a member of the team, only 438 who had had sufficient contact with the index case to qualify as casual contacts were screened; 336 (76.7%) of these 438 were male and 102 (23.3%) were female (Figure 2).

258 adult males (aged 16 years or over) were identified for screening (59% of the total identified for screening). This represented approximately half the number of attendees estimated of the Friday lunchtime prayer meetings.

180 women and children under the age of 16 years were offered appointments for screening; this number was 4.5 times that of the estimated number of attendees to the Friday evening prayer meeting.

Of the 438 individuals identified as casual contacts and eligible for screening, 378 attended the screening (86%). Of the 60 who were offered appointments but did not attend, 57% were adult males.

**Screening findings**

Two individuals with TB (one latent, one active) were identified among the 378 who attended screening (a rate of 0.53%) (see Box 1: linked cases 6 and 7).

The screening identified a further three adults with chest X-ray abnormalities, and one with possible clinical symptoms. All required additional follow-up before TB was excluded.

**Additional molecular genotyping information**

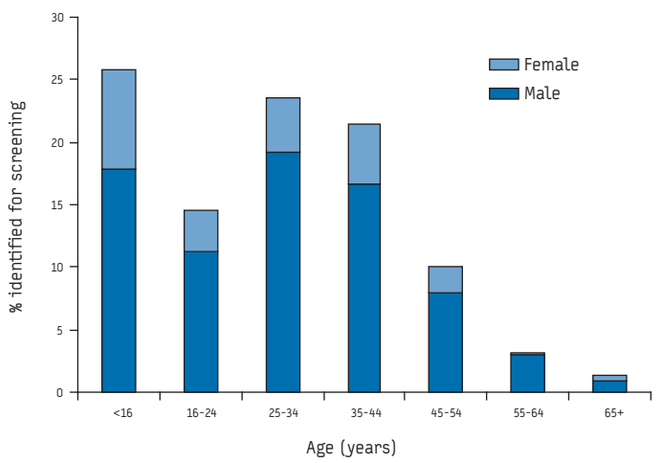
A UK-wide database of MIRU-VNTR profiles, developed by the UK TB Diagnostic and Molecular Epidemiology (DAME) Group, was searched to identify whether the 15 digit profile associated with this outbreak had been detected previously in the UK. It was the first time this particular fully sensitive MIRU-VNTR profile had been observed in the UK. There is no known link of this strain with Algeria nor is there any known pre-deposition for acquiring it. Since the outbreak, this MIRU-VNTR profile has been detected in a UK-born person in England in May 2006, and a further case in a previous household contact in February 2007 (see Box 1: linked cases 8 and 9; personal communication: Dr Ian Laurenson, Oct 2008).

## Discussion

We have described the experience of undertaking a large-scale contact tracing exercise for an outbreak of TB associated with a mosque. Literature searches failed to identify any other such exercises associated with a mosque community, although similar

FIGURE 2

**Age and sex distribution of people identified as casual contacts for screening, tuberculosis outbreak, Aberdeen mosque, 2005**



experiences had been reported in other community settings [14-18].

In total, our screening identified five cases of active disease (1.3% of those screened) with a further four cases identified by other means. The detection rate from screening of casual contacts was low, at 0.53%. A review of outbreaks of TB in the UK involving screening of more than 100 contacts reported an estimated mean detection rate of 0.37% [19], raising questions about the effectiveness of large scale screening of contacts [20].

The national Collaborating Centre for Chronic Conditions report, funded by NICE for the development of guidelines on the control and prevention of TB in the UK, suggested the following definition of a significant casual contact:

*"Contacts with a cumulative total exposure to a smear-positive case of TB exceeding eight hours within a restricted area equivalent to a domestic room are equivalent to domestic contacts" [9].*

Faced with a high incidence of active disease found in close contacts, there was an imperative to identify any potentially significant casual contacts. Reviewing the type of contact between the index case and attendees at the mosque, it was considered that at least some of the attendees would have met the NICE criteria. In this instance, no definable subgroup of contacts from the mosque who definitely met the criteria could be identified to enable the traditional "stone in the pond" approach. In addition, the degree of contact with the index case, reported by the linked cases, was variable and did not indicate a minimum level of exposure that could be used to focus screening. To further restrict our definition of a casual contact would have substantially increased the complexity of the message delivered. We were therefore faced with the difficult decision of whether to screen all attendees or no attendees.

We experienced some specific challenges in managing the outbreak that related to the mosque setting. Delivering our message was difficult because, for religious reasons, it was not possible for a member of the health protection team to directly address mosque attendees at a mosque meeting. Identifying all appropriate communication avenues as well as key individuals, who would be seen as respected and influential by their community, to deliver the message, was crucial.

Substantial work was generated for the health protection team because of a lack of clarity in communicating the definition of a casual contact. 603 individuals gave their names and details as casual contacts but closer interview identified that 165 of them had had no, or only minimal, contact with the index case at the mosque. Often the details of entire households were given when only one or two of the male members of the house regularly attended the mosque. Had we been clearer, we might have been able to reduce the number of individuals that were worried or answered the invitation for screening unnecessarily.

Attempts to operate by standard local radiology procedures in the X-ray department identified some language and cultural challenges. For Muslim women, changing into gowns before the X-ray examination was problematic. Cubicles for changing were located in mixed sex waiting areas, so women changed into gowns and then put their outer garments back on over the gowns while waiting for their X-ray. This increased the required appointment time substantially but was unavoidable as many of the women preferred to have their husbands present during interview and X-ray.

The contact tracing exercise was based on the then current BTS guidelines (2000) for the management and control of TB [10]. This guidance recommended that those under 16 years of age should have a tuberculin skin test, irrespective of BCG status, with a follow up chest X-ray for those who had a positive result. This meant that both latent and active TB cases could be identified. Those over 16 years, with a previous BCG vaccination, were recommended to have a chest X-ray, which would only detect those with active TB disease. Linked case 9 was not identified at initial screening because latent TB was not detected. Even when presenting with symptoms approximately 15 months after exposure to the index case, chest X-ray was normal.

However, had the latest NICE guidance (2006) been followed, all those under 35 years would have been offered a tuberculin skin test and, where positive, followed up by interferon gamma blood test and chest X-ray as necessary [9]. The case finding rate, especially for latent TB, might then have been higher. That said, it would still have been impossible to ascertain and report on who had developed latent TB due to recent infection at the mosque. Many of those who were screened were born in countries with a high prevalence of TB (>40 per 100,000) and, therefore, prior latent TB infection could not have been ruled out.

By using the BTS guidelines (2000) for screening adults with a single chest X-ray, we required attendance at only one appointment. We experienced a failure to attend rate of 14%, and a number of attendees required multiple appointments before they finally did attend. Anecdotal evidence suggested that offering screening that required more than one attendance may have led to a higher default rate. Freudenstein *et al.* reported their experience of a large casual contact tracing exercise in a UK village community where screening offered Heaf testing and reading, followed by chest X-ray as required. 20% of the casual contacts failed to complete the screening process [21].

While extensive casual contact tracing was undertaken in this outbreak, we know that a substantial number of individuals who were exposed did not come forward for screening. Standard communication about TB is aimed at reducing anxiety. The message focuses on emphasising the low risk of transmission and treatable nature of TB. It seems that some individuals in the mosque community may have interpreted our initial message as meaning there was no need to come forward for screening. A more direct message, delivered by medical members of the mosque community, and a change in the written information provided, instigated a more active response. However, we continued to have difficulty convincing adult males, who potentially had the highest exposure, to come forward for screening. And yet more children and women attended for screening than were estimated to be at risk. Risk perception contributed to this discrepancy, as it appeared that this community perceived the highest risk to be to women and children, but less concern was given to the risks faced by adult men. Ethnicity and religious beliefs have been reported to influence risk perception of other health issues [22-25].

In summary, enhanced surveillance and, where possible, the use of molecular genetic techniques to link TB cases to the outbreak was, in our experience, useful in defining the extent of the outbreak

[26]. The screening of a large number of casual contacts was a complex and time consuming exercise with a low detection rate.

The following insights were gained in the course of this investigation:

- Screening casual, multi-ethnic contacts in a mosque posed particular challenges;
- Communication of the risk of need for screening must be tailored to meet the specific needs of the community;
- Those at highest risk of TB infection in this setting, adult males, were least likely to attend for screening;
- The detection rate among screened casual contacts was low;
- We describe the first UK case with this particular pan-sensitive TB strain.

**Policy implications**

Where the identity of those individuals who form a group of causal contacts cannot be established and the group are asked to self-assess against given criteria and then volunteer for screening, the uptake of screening and the case yield are low.


## Acknowledgements

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# Cohort study of an outbreak of viral gastroenteritis in a nursing home for elderly, Majorca, Spain, February 2008

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An outbreak of acute gastroenteritis occurred in a nursing home for elderly in Majorca between 4 and 23 February 2008. To know its aetiology and mechanism of transmission a retrospective cohort study was conducted with a fixed cohort including 146 people (96 residents and 50 employees). The data were collected from clinical histories and through a survey by questionnaire. In total 71 cases were identified (53 residents, 18 employees), corresponding to an overall attack rate (AR) of 48.6%. The consumption of tap water, adjusted by age, sex and consumption of meals provided at the nursing home, presented a relative risk (RR) of 4.03 (95%CI, 1.4-11.4). The microbiological analyses confirmed the presence of norovirus and/or rotavirus in five of the seven stool samples submitted. The slow appearance of cases at the beginning of the outbreak is characteristic of a person to person transmission, while the sudden peak in the middle of the month suggests a common source such as the tap water. We therefore concluded that the outbreak likely originated from two sources: an infected employee of the nursing home and the tap water. The high number of dependent residents most probably facilitated the spread of the outbreak.

## Introduction

The progressive aging of the Spanish population increases the demand for residential services. The resulting increase of the numbers of nursing homes and their residents has favoured the emergence of acute gastroenteritis outbreaks in these institutions over the past years [1]. Given the risk characteristics of this particular population, these outbreaks are characterised by high morbidity with high attack rates and long duration [2].

Enteropathogenic viruses, including caliciviruses, are the most common causal agents in these outbreaks [3-5]. Rotaviruses are also responsible for severe diarrhoea, but mainly in children [6,7]. Nevertheless, outbreaks of acute gastroenteritis in nursing homes for elderly caused by rotavirus have been described in the literature [8-10].

In Spain, little information is available on morbidity and mortality associated with norovirus infection, its distribution among the population, and many of its epidemiological characteristics. This is primarily due to the fact that sample collection and laboratory screening for noroviruses is not done routinely [11]. Compared to other EU countries, not many studies of gastroenteritis outbreaks caused by norovirus are described in general and in nursing homes in Spain in particular [4, 12-15].

It is estimated that norovirus is the most common cause of acute gastroenteritis in some European Union countries, with 6% and 11% of all intestinal infectious diseases attributed to norovirus in the United Kingdom and the Netherlands, respectively [16,17].

Noroviruses are transmitted primarily through the faecal-oral route, either by direct person-to-person spread or by faecally contaminated food or water. Secondary and tertiary cases appear quickly through a person-to-person transmission. Noroviruses can also spread via a droplet route from vomits [18,19].

In healthcare facilities, transmission can additionally occur through hand transfer of the virus to the oral mucosa via contact with materials, fomites, and environmental surfaces that have been contaminated with either faeces or vomits. These circumstances make it extremely difficult to control outbreaks in institutional settings [20,21].

Between 4 and 23 February an outbreak of acute gastroenteritis occurred in an elderly nursing home in Majorca, Spain. The outbreak was characterised by a slow start followed by an explosive increase in the number of cases which may be linked to a common source. To contain the outbreak, between 9 and 11 February, the nursing home authorities implemented the following control measures: enteric isolation, cleaning of areas contaminated by vomit, restriction of visitors, suspension of the consumption of tap water, distribution of bottled water, cleaning and chlorination of the water cistern, and stool sampling. The notification of a suspected gastroenteritis outbreak was sent to the health authorities of the Balearic Islands on 13 February. In view of the microbiological confirmation of a mixed viral aetiology (norovirus and rotavirus) and the high attack rate, an epidemiological investigation to determine the causes and transmission routes of the outbreak was launched on 5 March.

## Methods

### Study design

A retrospective cohort study was conducted including all residents and employees (health workers, cleaning, laundry and maintenance service and administration) who were present in the nursing home in February. The observation period covered 29 days, from 1 to 29 February 2008. Persons who were admitted to or began employment in the nursing home after 29 February or those who were not present for the entire period of 29 days were excluded from the study.

A case of gastroenteritis was defined as any person working or residing in the nursing home during the month of February 2008 who had an episode of acute diarrhoea (defined as three or more liquid stools in 24 hours) or vomiting, or two or more of the following signs: fever, abdominal pain, malaise and nausea.

### Data source and epidemiological survey

Two data sources were used. The first one was a computerised database with the medical history of all residents of the nursing home. Information on the employees was obtained through an epidemiological self-administered survey. The questionnaire collected data on the employment position, working shifts and location within the nursing home (ground- or first floor, module A, B or C), as well as consumption of meals and drinking of tap water at the workplace during the month of February and on days 8, 9 and 10 of the same month (these dates were chosen taking into consideration the peak in case numbers on 13 February and the 72-hour incubation period). Finally, questions concerning symptoms experienced during the month of February, history of the disease and information on family members affected.

### Microbiological analysis

Stool samples were collected by the medical doctor of the nursing home and sent for routine bacteriological testing to the reference laboratory in the Balearic Islands. Subsequently, as viral origin was suspected in this outbreak, the health authorities of the Balearic Islands sent the available samples to the laboratory of the National Centre of Microbiology in Majadahonda near Madrid where polymerase chain reaction (PCR) was used for identifying norovirus and Elisa test for the identification of rotavirus.

Samples of drinking water could not be taken by the outbreak investigation team because on 26 February cleaning and chlorination of the water cistern of the residence was carried out. Food samples from different meals were collected during the week between 11 and 17 February, i.e. before the arrival of the outbreak investigation team, and were tested for bacteria only.

### Statistical analysis:

Common statistical methods were used for describing the variables related to personal data and place of work or residence within the nursing home. A univariate descriptive analysis was done to study the risk factors of employees and residents. The attack rate, the incidence densities, incidence density ratios (IDR) and the aetiological fractions due to exposure were calculated with their respective 95% confidence interval. The incidence densities were expressed in person-days. The differences of rates were analysed through the Fisher's Exact Test [22]. Finally, multivariate analyses using Cox regression with explanatory purpose were done to test the foodborne and the waterborne hypotheses, adjusted for age and sex. The overall significance of the model was verified through a maximum likelihood ratio test and the individual significance using p value of $\chi 2$ (chi square) by Wald's test. The resulting model relative risks (RR) were expressed with their respective 95% CI. The verification of the hypothesis of proportionality of risks was carried out using the graphic method of logarithmic survival curves: $Ln(-Ln\hat{S}(t))$. The study of outliers and influential values was done through the analysis of the residuals. EPIDAT v.3.1 was used for the data collection and Stata v.10 for the data analysis.

## Results

The study population consisted of 168 persons, 96 of them were residents and 72 employees of the nursing home. Information was obtained from 146 people; 100% (n= 96) of the residents and 69% (n=50) of the employees. Among the 50 employees included in the study, 38 (76%) were health workers. Among the 22 employees who did not respond to the questionnaire, nine worked in administration, management or services (laundry, cleaning and cooking) and 13 were health workers.

### Descriptive analysis of the residents

The sex ratio (males to females) among residents was 0.2 and the median age was 82 years, with an interquartile range (IQR) of 12. Over 60% of the residents needed help to perform activities of daily living. Dementia was present in 54% and 41% were incontinent. Among the residents, 53 (55%) fulfilled the case definition. The most common symptom was diarrhoea, present in 98% of the cases. All residents ate the meals provided by the nursing home and drank the tap water of the nursing home until distribution and consumption of bottled water was ordered by the director on 11 February.

### Descriptive analysis of the employees

Among the employees, the sex ratio (males to females) was 0.06. The median age was 37.7 years (IQR: 17). The median time at workplace was six months. Five (10%) employees drank tap water

**Figure 1**

**Epidemic curve of cases, by date of onset of symptoms, outbreak of gastroenteritis in a nursing home for elderly, Majorca, February 2008 (n=71)**

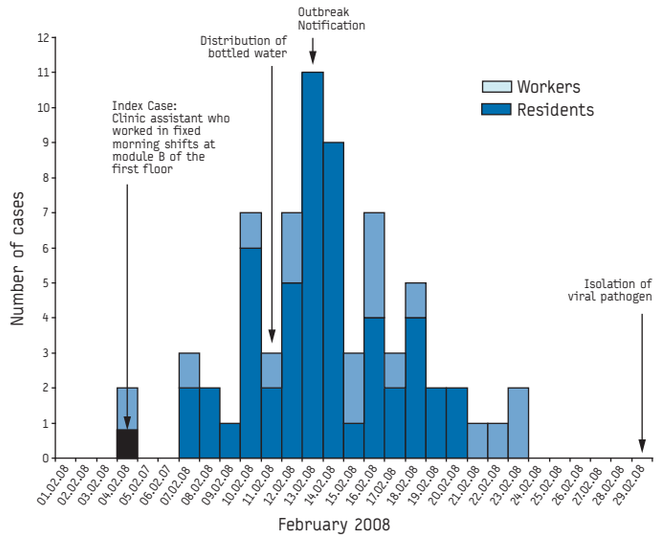

**Table 1**

**Attack rates and incidence densities in the cohort, by residents, employees, consumption of meals and drinking of tap water; outbreak of gastroenteritis in a nursing home for elderly, Majorca, February 2008 (n=146)**

| Variables | Cohort | Cases | Attack rate (%) (95% CI) | Incidence density* (95% CI) | P value** |
|---|---|---|---|---|---|
| | 146 | 71 | 48.6 (38.5-61.3) | 2.3 (1.8-2.8) | |
| **Cohort** | | | | | 0.02 |
| Residents | 96 | 53 | 55.2 (42.1-72.2) | 2.7 (2.1-3.6) | |
| Employees | 50 | 18 | 36.0 (22.6-57.1) | 1.5 (0.9-2.4) | |
| **Employees** | | | | | 0.13 |
| Health workers | 37 | 16 | 42.1 (25.7-68.7) | 1.8 (1.1-2.9) | |
| Others*** | 13 | 2 | 16.6 (4.2-66.6) | 0.6 (0.1-2.5) | |
| **Sex** | | | | | 0.39 |
| Male | 23 | 13 | 48.7 (37.5-63.2) | 2.2 (1.7-2.9) | |
| Female | 123 | 58 | 56.5 (32.3-97.3) | 2.8 (1.6-4.8) | |
| **Drinking of the nursing home tap water** | | | | | < 0.05 |
| Yes | 102 | 58 | 56.8 (43.9-73.5) | 2.8 (2.2-3.7) | |
| No | 44 | 13 | 29.5 (17.1-50.8) | 1.1 (0.6-1.9) | |
| **Diet** | | | | | 0.09 |
| Standard | 59 | 32 | 54.2 (38.3-76.7) | 2.6 (1.9-3.7) | |
| Diabetic | 19 | 12 | 63.1 (35.8-111.2) | 3.3 (1.8-5.7) | |
| Soft | 13 | 8 | 61.5 (30.7-123.0) | 3.5 (1.7-6.9) | |
| Pureed | 14 | 4 | 28.5 (10.7-76.1) | 1.2 (0.4-3.1) | |
| Do not eat meals at the nursing home | 41 | 15 | 36.5 (22.0-60.6) | 1.5 (0.9-2.5) | |

* Incidence density per 100 people and day
** P value of $\chi^2$ of Fisher's exact test
*** Cleaning, laundry and maintenance service and administration

**Table 2**

**Univariate analyses of gastroenteritis cases in residents of the nursing home, outbreak in Majorca, February 2008 (n=96)**

| Variables | Cohort of residents | Cases | Attack rate (%) (95%CI) | Incidence density* (95%CI) | Incidence density ratio (95%CI) | Attributable fraction (exposed) (95%CI) |
|---|---|---|---|---|---|---|
| **Sex** | | | | | | ** |
| Female | 76 | 41 | 53.9 (39.7-73.2) | 2.6 (1.9-3.5) | 1 | |
| Male | 20 | 12 | 60.0 (34.0-105.6) | 3.1 (1.7-5.4) | 1.2 (0.5-2.2) | 15.2 ([-7.7]-5.6) |
| **Age in years** | | | | | | ** |
| ≤ 80 years | 43 | 25 | 58.1 (39.2-86.0) | 2.9 (2.0-4.4) | 1.2 (0.6-2.1) | 15.6 ([-5.1]-52.6) |
| ≥ 81 years | 53 | 28 | 52.8 (36.4-76.5) | 2.5 (1.7-3.6) | 1 | |
| **Independent in the activities of daily living** | | | | | | ** |
| Yes | 38 | 21 | 55.1 (39.2-78.0) | 2.6 (1.7-4.0) | 1 | |
| No | 58 | 32 | 55.2 (36.0-84.7) | 2.8 (1.9-3.9) | 1.1 (0.6-1.9) | 5.7 ([-6.8]-48.3) |
| **Physical disability*** ** | | | | | | ** |
| Yes | 36 | 18 | 50.0 (31.5-79.3) | 2.4 (1.5-3.8) | 1 | |
| No | 59 | 35 | 59.3 (42.5-82.6) | 2.9 (2.1-4.1) | 1.22 (0.7-2.3) | 18.2 ([-48.2]-56.4) |
| **Dementia** | | | | | | ** |
| Yes | 52 | 26 | 50.0 (34.0-73.4) | 2.4 (1.6-3.6) | 1 | |
| No | 44 | 27 | 61.3 (47.1-89.4) | 3.0 (2.1-4.5) | 1.2 (0.7-2.2) | 21.0 ([-40.5]-55.7) |
| **Control sphincters** | | | | | | ** |
| Yes | 56 | 28 | 50.0 (34.5-72.4) | 2.4 (1.7-3.6) | 1 | |
| No | 40 | 25 | 62.5 (42.2-92.4) | 3.1 (2.1-4.6) | 1.3 (0.7-2.2) | 20.0 ([-43.0]-55.0) |
| **Diet** | | | | | | ** |
| Standard | 50 | 29 | 58.0 (40.3-83.4) | 28.8 (20.0-41.5) | 2.4 (0.8-9.5) | 59.1 ([-16.3]-89.5) |
| Diabetic | 19 | 12 | 63.1 (35.8-1112) | 32.6 (18.5-57.4) | 2.7 (0.8-11.7) | 63.8 ([-19.3]-91.4) |
| Soft | 13 | 8 | 61.5 (30.7-123.0) | 34.6 (17.3-69.2) | 2.9 (0.8-13.3) | 65.9 ([-27.1]-92.5) |
| Pureed | 14 | 4 | 28.5 (10.7-76.1) | 11.8 (4.4-31.4) | 1 | |
| **Type of room** | | | | | | ** |
| Simple | 46 | 23 | 50.0 (36.1-63.8) | 2.3 (1.5-3.5) | 1 | |
| Double | 50 | 30 | 60.0 (46.1-72.4) | 3.1(2.1-4.4) | 1.3 (0.7-2.7) | 24.1 ([-30.6]-55.9) |
| **Floor** | | | | | | ** |
| Ground floor | 24 | 11 | 45.8 (25.3-82.7) | 1.9 (1.0-3.5) | 1 | |
| Second floor | 72 | 42 | 58.3 (43.1-48.0) | 3.0 (2.2-4.1) | 1.5 (0.8-3.3) | 35.8 ([-26.7]-70.2) |

* Incidence density per 100 people and day
** P value > 0.05 of $\chi^2$ of Fisher's exact test
*** Information on physical disability was available for 95 of the 96 residents in the cohort (one missing)

**Figure 2**

**Survival function of the tap water adjusted by age, sex and consumption of meals at the nursing home, outbreak of gastroenteritis in Majorca, February 2008 (n=146, 48.6% cases)**

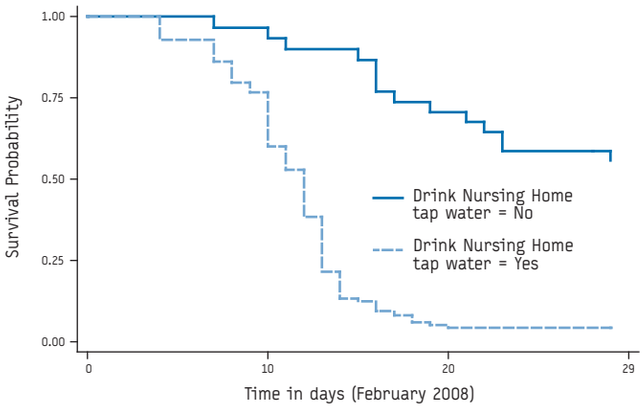


**Figure 3**

**Verification of the hypothesis of proportional risks assumption, logarithmic survival curves, Ln(-LnŜ (t)), outbreak of gastroenteritis in a nursing home for elderly, Majorca, February 2008**

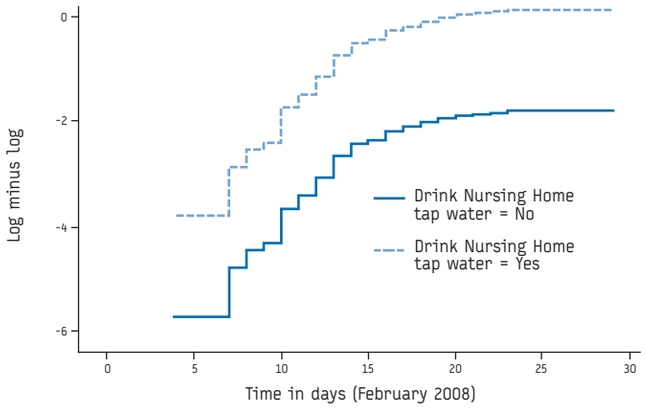


**Table 3**

**Univariate analyses of gastroenteritis cases in employees of the nursing home, outbreak in Majorca, February 2008 (n=50)**

| Variables | Cohort of employees | Cases | Attack rate (%) (95% CI) | Incidence density* (95% CI) | Incidence density ratio (95% CI) | Attributable fraction (exposed) (95% CI) |
|---|---|---|---|---|---|---|
| **Sex** | | | | | | ** |
| Female | 47 | 17 | 36.1 (22.4-58.1) | 1.5 (0.9-2.3) | 1.1 (0.2-48.5) | 13.9 ([-4.5]-97.9) |
| Male | 3 | 1 | 33.3 (4.7-23.6) | 1.2 (0.2-9.2) | 1 | |
| **Age in years*** ** | | | | | | |
| ≤ 24 | 8 | 3 | 37.5 (12.116.2) | 1.5 (0.5-4.8) | 1.5 (0.2-1.7) | 36.3 ([-37.5]-91.4) |
| 25-34 | 12 | 3 | 25.0 (8.0-77.5) | 0.9 (0.3-3.0) | 1 | |
| 35-44 | 12 | 6 | 50.0 (22.4-111.2) | 2.1 (0.9-4.8) | 2.1 (0.4-13.5) | 54.2 ([-11.4]-92.6) |
| ≥ 45 | 15 | 6 | 40.0 (17.9-89.0) | 1.7 (0.7-3.8) | 1.7 (0.4-6.9) | 43.2 ([-12.2]-85.5) |
| **Job position** | | | | | | |
| Health workers | 38 | 16 | 42.1 (25.7-68.7) | 1.8 (1.1-2.9) | 2.9 (0.7-2.6) | 65.7 ([-45.6]-96.1) |
| Others**** | 12 | 2 | 16.6 (4.1-66.6) | 0.6 (0.1-2.4) | 1 | |
| **Working hours** | | | | | | |
| Day shift | 16 | 8 | 50.0 (25.0-99.9) | 2.2 (1.1-4.4) | 2.3 (0.5-13.4) | 56.5 ([-81.2]-92.5) |
| Afternoon shift | 8 | 3 | 37.5 (12.0-116.2) | 1.6 (0.5-5.0) | 1.6 (0.2-12.5) | 40.5 ([-34.3]-92.0) |
| All shifts | 3 | 1 | 33.3 (4.6-23.6) | 1.4 (0.2-10.1) | 1.5 (0.1-18.6) | 33.0 ([-342.2]-94.6) |
| Day/night shift | 11 | 3 | 27.2 (8.7-84.5) | 1.1 (0.3-3.4) | 1.1 (0.1-8.6) | 13.1 ([-54.8]-88.3) |
| Day/Afternoon shift | 12 | 3 | 25.0 (8.0-77.5) | 0.9 (0.3-2.9) | 1 | |
| **Length of employment in months** | | | | | | ** |
| 11-14 months | 10 | 5 | 50.0 (20.8-120.1) | 2.3 (9.9-57.4) | 2.6 (0.5-13.2) | 61.2 ([-76.9]-92.4) |
| 7-10 months | 11 | 5 | 45.4 (18.9-109.2) | 2.0 (8.5-49.4) | 2.2 (0.5-11.3) | 55.7 ([-105.7]-91.2) |
| 4-6 months | 12 | 4 | 33.3 (12.5-88.8) | 1.2 (4.8-34.0) | 1.4 (0.3-7.35) | 28.7 ([-282.7]-86.7) |
| 0-3 months | 17 | 4 | 23.5 (8.8-62.6) | 0.9 (3.4-24.2) | 1 | |
| **Location at the workplace (floor and module) in February** | | | | | | ** |
| Ground floor | 10 | 3 | 30.0 (9.6-93.0) | 1.1 (0.3-3.5) | 1.4 (0.2-8.2) | 28.0 ([-391.7]-87.8) |
| Second floor, module A | 7 | 2 | 28.5 (7.1-114.2) | 1.1 (0.2-4.3) | 1.3 (0.1-9.1) | 23.7 ([-743.3]-89.0) |
| Second floor, module B | 6 | 4 | 66.6 (25.0-117.6) | 4.5 (1.7-12.1) | 5.5 (1.0-29.6) | 81.8 (2.5-96.6) |
| Second floor, module C | 8 | 5 | 62.5 (26.0-150.1) | 2.7 (1.1-6.5) | 3.3 (0.7-16.6) | 69.6 ([-41.0]-93.9) |
| Both floors | 19 | 4 | 21.0 (7.9-56.0) | 0.8 (0.3-2.1) | 1 | |
| **Consumption of the nursing home meals in February** | | | | | | ** |
| Yes | 9 | 3 | 33.3 (10.7-103.3) | 1.4 (0.4-4.3) | 1 | |
| No | 41 | 15 | 36.5 (22.0-60.6) | 1.5 (0.9-2.5) | 1.1 (0.3-5.8) | 7.4 ([-227.0]-82.8) |
| **Drinking of the nursing home tap water in February** | | | | | | **0.01** |
| Yes | 5 | 4 | 80.0 (30.0-213.1) | 6.5 (2.4-17.4) | **5.3 (1.2-17.0)** | **81.3 (0.2-94.1)** |
| No | 45 | 14 | 31.1 (18.4-52.5) | 1.2 (7.2-20.7) | 1 | |

*Incidence density per 100 people and day
** P value > 0.05 of $\chi^2$ of Fisher's exact test
*** Information on age was available for 47 of the 50 employees in the cohort (three missing)
**** Cleaning, laundry and maintenance service and administration; (working in administration was not reported by any case)

from the cistern of the nursing home during the month of February, and nine (18%) ate the standard menu during the same time. 18 cases (36%) were identified among the employees. The most common symptom reported by employees was diarrhoea, followed by abdominal discomfort.

### Descriptive temporal analysis

The outbreak began on 4 February and lasted until 23 February. The first two cases with onset of symptoms on 4 February were employees of the centre. Both were included in the study but only one provided detailed answers to all questions in the questionnaire. This index case was a nursing assistant who during the month of February worked in fixed morning shifts in the module B on the first floor. This person was diagnosed with acute gastroenteritis by a physician. The relatives of the index case were also affected and began to show symptoms on 6 February (Figure 1).

The outbreak peaked on 13 and 14 February (11 and 9 cases, respectively). The latest reported date of onset of symptoms was 23 February (two cases).

### Attack rates, incidence densities

The overall attack rate (AR) was 48.6% (95% confidence interval, CI, 38.5-61.3). The AR among the employees (n=50) was 36% (95%CI, 22.6-57.1). The AR among the residents (n= 96) was 55.2% (95%CI, 42.1-72.2).

There were no significant differences between attack rates and the incidence densities according to sex and consumption of the menu. However, the risk of illness following consumption of tap water from the nursing home was significantly higher among those who drank it compared to those who did not (Table 1).

### Univariate analysis

Among residents, women of any age and people of both sexes below 80 years of age were most affected. Being resident of the first floor in a double room, incontinent and dependent on the staff to handle the basic activities of daily living, posed a greater risk of infection. The risk of residents of double rooms was 30% higher (IDR: 1.3 CI95% [0.7-2.2]) than those of single rooms. There were no significant differences between risks related to different diets (i.e. standard, diabetic, pureed, etc.) within the meals consumed in the nursing home (chi square of Fisher's exact test for unequal rates, p= 0.098) (Table 2).

Among employees, health workers between 34 to 44 years of age, with fixed morning shifts attached to the module B of the first floor and more than 10 months at the workplace had a higher risk of acute gastroenteritis. The consumption of tap water during the month of February is the highest risk factor associated with the acute gastroenteritis (Table 3).

### Multivariate analysis

The consumption of tap water during the month of February is a clear risk factor for gastroenteritis within employees and residents. The unadjusted risk ratio for drinking tap water was 2.5 95% CI (1.3-4.5). Regardless of age, sex and consumption of the menu, individuals from the cohort, who drank water, were four times more at risk of acute gastroenteritis than those that did not consume (Table 4, Figures 2 and 3).

### Laboratory results

On 29 February the results of laboratory analysis of samples taken during the outbreak confirmed the isolation of viral enteropathogenic agents in five of the seven samples submitted: norovirus in three of them, rotavirus in one and both norovirus and rotavirus in the fifth one. The food samples tested during the outbreak were negative for bacteria.

## Discussion

The description and the epidemiological analysis of the outbreak allow us to reconstruct the possible source and subsequent transmission of infection in the nursing home. The index case of 4 February was a clinic assistant who worked in fixed morning shifts at module B of the first floor. In this module, where the outbreak began among residents, it is likely that the index case introduced the virus into the residence. This hypothesis is also supported by the fact that the washing and changing clothes of residents is done during the morning shift, the workload is bigger than in the other shifts and the contact between employees and residents is closer.

Regarding the transmission of the outbreak, the epidemic curve with mild start and slow spread until 9 February would support the hypothesis of introduction of rotavirus from outside through the index case. However, on 13 and 14 February an explosive peak, lasting two-days, occurred affecting only residents. Knowing the pathogenesis of norovirus, its epidemiological characteristics and the fact that calicivirus outbreaks have been associated with a common water source [23-27], it is likely that this peak was due to consumption of tap water from the nursing home. All residents and five (10%) of employees in the centre drank tap water until 11 February, when distribution of bottled water was imposed due to the suspicion of an acute gastroenteritis outbreak. Within 72 hours after the closure of the cistern the highest case load per day were reported. In addition, all these cases had drunk tap water before. If we take into consideration the incubation period of these viral agents, the epidemic peak of day 14 and 15 corresponds well to the prohibition to consume tap water and the provision of bottled drinking water. This assumption is further supported by the results of the statistical analysis. The risk of gastroenteritis was four times higher in those individuals of the cohort who had consumed tap water regardless of age, sex and consumption of the nursing home meals. From the qualitative information obtained from staff interviews, we understood that days before the start of the outbreak there were complaints from residents of a bad taste of the tap water.

**Table 4**

**Multivariate analyses by Cox regression model of gastroenteritis cases categorised by age, sex and consumption of meals and tap water at the nursing home, outbreak in Majorca, February 2008 (n=146)**

| Variables | Beta coefficient | Standard error | Relative risk (95%CI) | P value* |
|---|---|---|---|---|
| Age (in years) | -0.01 | 0.01 | 0.99 (0.97-1.01) | 0.17 |
| Sex (female vs male) | -0.03 | 0.31 | 1.03 (0.53-1.79) | 0.93 |
| Drinking of tap water (yes vs no) | 1.39 | 0.53 | **4.03 (1.42-11.38)** | **0.01** |
| Consumption of meals (yes vs no) | -0.05 | 0.25 | 0.96 (0.58-1.56) | 0.85 |

* P value of $\chi^2$ Wald's test

There were no differences between the risks related to different diets, so the alimentary hypothesis was rejected. The risk of acute gastroenteritis was similar for those who usually ate at the residence as for those who did not, and multivariate analysis confirmed the absence of association between the outbreak and having meals at the nursing home.

Therefore, disregarding the hypothesis of food source, we consider as very likely the coexistence of two routes the outbreak was introduced into the nursing home. The first was infection imported from outside, most likely by the index case we identified, which progressed by a person to person transmission. The second was a common source, most likely the tap water.

The outbreak took place in a closed setting which usually results in high attack rates. However, in this outbreak, the double source could also explain the high virulence and high transmissibility of infection, that affected half of the cohort and a density incidence of 2.3 (95%CI: 1.8-2.8) cases per 100 person-days. The unique dynamics in the transmission of this outbreak makes it markedly different from other outbreaks in nursing homes studied in Spain [4, 10-13].

After the peak on 13 and 14 February, the outbreak adopted a person to person transmission pattern affecting employees and residents. This hypothesis is supported by the high attack rates in both incontinent and dependent residents and health workers in fixed morning shifts of module B of the first floor. In addition, the risk of becoming ill among health workers was greater for those with fixed morning shifts, when as previously commented workers usually have more contact with residents. This phenomenon of spreading the disease by person to person is recurrent in different outbreaks described in nursing homes in Spain and in health care settings in other European countries [4,12-15, 28].

The greatest risk of becoming ill in the group of dependent residents and those with incontinent sphincter may be related to the special care they required. In this group of residents, the health worker per resident ratio is one per 12. As 60.4% of the residents needed assistance in performing activities of daily living, and 41% were incontinent, this might be a factor to take into account when trying to understand the difficulty of controlling the mechanism of person to person transmission in an outbreak in such setting.

Considering the limitations of this study, we must be prudent in interpreting the results where statistically significant associations were not found, since there is a possibility of false negative results in the statistical analyses. We should not overlook the possibility of a classification bias due to the memory at the time of completing the epidemiological questionnaire. Another limitation includes the selection bias introduced with the loss of selective information in the subgroup of employees in our cohort, linked to the non-response of the epidemiological questionnaire. This represents a 21.6% rate of non-response among employees. Therefore, apart from being cautious in extrapolating the results to the subgroup of employees, we should bear in mind that when we report the relative risks in the bivariate analysis of this group, the statistical power of our results is 22%. And finally, the impossibility to confirm by laboratory the presence of viruses in the drinking water of the cistern of the residence can subtract the attribution force of water as the causal hypothesis.

We can conclude that the studied outbreak showed a high attack rate and affected both residents and employees. The aetiology of the outbreak was mixed, with the involvement of norovirus and rotavirus. It is likely, that the high level of dependence of the residents had been a facilitating factor of the spread of the outbreak.

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# Methicillin-resistant Staphylococcus aureus nasal carriage among healthy employees of the Hellenic Air Force

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The prevalence of methicillin-resistant Staphylococcus aureus nasal carriage among 959 healthy employees of the Hellenic Air Force was investigated from November 2004 to October 2005. Nine participants were found to be colonised by methicillin-resistant Staphylococcus aureus (MRSA) (SCCmec type IV). Eight of the MRSA isolates were PVL-negative and belonged to ST30 by MLST, while the remaining one isolate was PVL-positive and classified as ST-80.

## Introduction

The incidence of infections caused by methicillin-resistant Staphylococcus aureus (MRSA) apparently acquired in the community (CA-MRSA) is increasing. CA-MRSA isolates are commonly non-multi-drug resistant and belong to lineages distinct from those of MRSA strains prevailing in hospitals [1]. Recent reports from Greece indicated community emergence of MRSA mainly implicated in skin and soft tissue infections in children [2,3]. Yet, the extent of the spread of CA-MRSA in the community has not been studied. We attempted to evaluate the prevalence as well as the microbiological and epidemiological characteristics of MRSA strains in a population of healthy adults in Greece.

## Methodology

The study population consisted of employees of the Hellenic Air Force (HAF), residing in different geographical areas of Greece, visiting the Air Force General Hospital in Athens from November 2004 to October 2005, for a scheduled biannual medical examination. Before joining the HAF, all participants had been in good health. For operational reasons, they trained and maintained good physical fitness. Additionally, they underwent an obligatory medical examination at least once every two years. Therefore, this study population was considered as approximating "healthy adults". Demographic data and medical history over the preceding year, including hospitalisation, surgery, use of antibiotics or other medication and underlying diseases, were obtained for each participant during a short interview by a medical doctor.

Swabs obtained from both anterior nares of each individual were immediately streaked onto mannitol salt agar containing 2 µg/ml oxacillin (Oxacillin Resistance Screening Agar Base, Oxoid Ltd.). Plates were incubated at 35ºC for 48 h. Colonies demonstrating an intense blue colour were subcultured onto blood agar and incubated overnight at 35ºC. Species identification was performed by standard methods. Susceptibility profile to a wide variety of antimicrobial agents was determined by the disk diffusion method according to the current CLSI guidelines. Isolates were also tested by an oxacillin disk (1 µg) and a cefoxitin disk (30 µg) to confirm methicillin resistance. MRSA isolates were defined as community-associated according to established criteria [4].

MRSA isolates were characterised by multi-locus sequence typing (MLST) and pulsed-field gel electrophoresis (PFGE) of chromosomal DNA SmaI digests. Macrorestriction patterns were compared to previously identified clones [5]. Multi-locus sequence typing (MLST) was performed to all PFGE/SCCmec types. MRSA were additionally characterised by spa typing. Sequences of amplified parts of the spa gene were analysed using the Ridom StaphType software (Ridom GmbH, Würzburg, Germany). Detection of mecA as well as SCCmec typing was carried out by PCR. Genes lukS-PV and lukF-PV encoding Panton-Valentine leukocidin (PVL) were also identified.

Data were processed and analysed by using the SPSS statistics software, version 12 for Windows. Bivariable comparisons were carried out by the $\chi 2$ or Fisher's exact test for categorical variables and the t-test for continuous variables.

## Results

A total of 959 individuals (874 males) aged 18 to 60 years (mean age 33) were enrolled in the study. Nine of the 959 participants (0.94%, 95% confidence interval [CI] 0.33% to 1.55%) were colonised with MRSA. All MRSA carriers were males. Two of the colonised individuals were smokers. One of the MRSA carriers reported systematic use of inhaled corticosteroids during the two months preceding enrolment. Another carrier had been treated with antibiotics two months prior to sampling. Three of the colonised individuals had been admitted to different hospitals at least once in the year before enrolment in the study. Two of them had been hospitalised in medical wards while the third one had been admitted to a surgical ward. In two MRSA carriers none of the investigated risk factors was identified. Among the demographic and clinical variables, prior hospitalisation and use of inhaled corticosteroids appeared to be correlated with an increased risk for MRSA colonisation ($P<0.01$) (Table 1).

Characteristics of the MRSA isolates are presented in Table 2. All nine isolates were susceptible to imipenem, gentamycin, erythromycin, clindamycin, ciprofloxacin, trimethoprim-

sulfamethoxazole, rifampicin, linezolid, teicoplanin and vancomycin. One isolate (Sa-344) was resistant to tetracycline (Tet) and two isolates (Sa-344, Sa-784) exhibited intermediate susceptibility to fusidic acid (Fus).

Eight isolates exhibited similar PFGE patterns (type A) not differing by more than three bands, correlated to ST30 by MLST. The chromosomal fingerprint of isolate Sa-344 was distinct (type C) belonging to ST80 by MLST. A total of five spa types were identified. Five of the eight ST30 isolates were classified as t012 (three strains) and t018 (two strains) that are common among strains of this ST. ST80 strain was classified as t044, a spa type strongly associated with this particular lineage. SCCmec typing revealed that all isolates possessed the SCCmec type IV. Genes lukF-PV and lukS-PV encoding PVL were detected only in the ST80 isolate.

## Discussion

This study confirms the circulation of PVL-positive t044/ST80-IV which is common among CA-MRSA in Europe [6] as well as several spa variants of a PVL-negative ST30-IV MRSA frequently encountered in Greek hospitals [5]. While only one of the nine isolates belonged to ST80, this type seems to predominate among community-acquired infections requiring hospitalisation [2,3] most likely reflecting a higher virulence. In addition, since the PVL-positive strain was one of the two fusidic acid-resistant MRSA

**Table 1**

**Risk factors tested for MRSA colonisation, study of Hellenic Air Force employees, Greece, 2004-2005 (n=959)**

| Characteristics | Number (%) of MRSA-colonised subjects | Total number of subjects | Statistically significant difference |
|---|---|---|---|
| Sex | | | P>0.05 |
| Male | 9 (1.03) | 874 | |
| Female | 0 (0) | 85 | |
| Smoking | | | P>0.05 |
| No | 7 (1.39) | 501 | |
| Yes | 2 (0.44) | 458 | |
| Antibiotic use (within the past two months) | | | P>0.05 |
| No | 8 (0.89) | 902 | |
| Yes | 1 (1.75) | 57 | |
| Corticosteroid use (within the past two months) | | | |
| No | 8 (0.85) | 943 | |
| Yes (inhaled) | 1 (10) | 10 | P<0.01 |
| Yes (*per os*) | 0 (0) | 6 | |
| Hospitalisation (during the past year) | | | |
| No | 6 (0.71) | 844 | |
| Yes (medical ward patients) | 2 (6.25) | 32 | P<0.01 |
| Yes (surgical ward patients) | 1 (1.2) | 83 | |

**Table 2**

**Characteristics of nine CA-MRSA isolates from healthy carriers, study of Hellenic Air Force employees, Greece, 2004-2005**

| Isolate | Resistance to non-β-lactams | PFGE type (MLST) | mecA type | spa type | PVL | Factors potentially associated with MRSA colonisation |
|---|---|---|---|---|---|---|
| 43 | - | A (ST30) | IV | t1051 | - | Smoking |
| 196 | - | A (ST30) | IV | t046 | - | Antibiotics |
| 344 | Tet, Fus | C (ST80) | IV | t044 | + | Hospitalisation (medical ward)* |
| 408 | - | A (ST30) | IV | t046 | - | Inhaled corticosteroids* |
| 714 | - | A (ST30) | IV | t018 | - | - |
| 778 | - | A (ST30) | IV | t018 | - | - |
| 784 | Fus | A (ST30) | IV | t012 | - | Hospitalisation (surgical ward) |
| 901 | - | A (ST30) | IV | t012 | - | Smoking |
| 933 | - | A (ST30) | IV | t012 | - | Hospitalisation (medical ward)* |

* Denotes factors that appeared as significantly associated with MRSA colonisation

isolates, the emergence of MRSA with fusidic acid resistance could be a convenient means for the timely detection of any increase in the incidence of PVL-positive MRSA in the community [7].

Differences in MRSA colonisation rates of apparently healthy community-dwelling persons have been observed in various settings. In western European countries colonisation rates are comparable to the rate observed here in Greece [6]. In other countries such as Taiwan, however, the respective rate is as high as 3.5% and has been partly attributed to the excessive community use of antibiotics [8]. Although consumption of antibiotics in Greece ranks among the highest in Europe, the MRSA isolation rate in this study was relatively low. This could be partly due to the fact that the study population was composed of individuals healthier than average adults and with limited exposure to antibiotics and healthcare.

Eight of the isolates were indistinguishable from the ST30 strain that has been established in Greek hospitals [5,9]. Notably, three of the eight respective carriers had been admitted to a hospital at least once in the year preceding enrolment in the study. Hence, a hospital origin of the ST30 strains circulating in this community cannot be excluded.

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# Point prevalence study of antibiotic use in a paediatric hospital in Italy

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A survey aimed to describe the prevalence of antibiotic use in hospitalised children was conducted in June 2007, in Bambino Gesù Children's Hospital in Rome which has the highest annual number of inpatients among paediatric hospitals in Italy. Data were collected by reviewing medical charts of all patients hospitalised for >48 hours. A total of 412 hospitalised children were evaluated; their median age was 42.3 months, and 55.6% were males. Antibiotics were prescribed to 181 of the 412 patients (43.9%). The prevalence was lowest (37.7%) in medical wards, higher (51.1%) in intensive care units and highest (52.2%) in surgical wards. Of the patients treated with antibiotics in surgical wards, 71% received the treatment as prophylaxis. The most frequently prescribed antibiotics were ceftazidime and the combination of amoxicillin and clavulanic acid. The observed prevalence of antibiotic use was within the range recently reported from other paediatric hospitals in Europe; however, it is advisable to collect data from all over the country in order to identify priority areas and design interventions. These results also highlight the need to implement guidelines for surgical prophylaxis in children, and to further investigate reasons for prescription of parenteral antibiotic therapy in paediatric hospitals.

## Introduction

Antibiotics are among the drugs most commonly prescribed for children. In Italy it has been estimated that 40-50% of children below 15 years of age receive at least one outpatient antibiotic prescription per year [1,2].

Although the vast majority of antibiotics are consumed in primary care [3], the pressure to select antimicrobial drugs in hospitals appears to be even higher than in outpatient care [4]. An estimated proportion of 36-49% of hospitalised infants and children receive antibiotics [5-9]. The frequent use of antibiotics is considered to be one of the main reasons for the high prevalence of antimicrobial resistance observed in hospitals [10]. Adverse drug events and excessive costs of treatment are also reasons for concern [8,11], particularly considering that 15-45% of antibiotic treatment regimens for paediatric patients may be inappropriate [6,12,13].

Surveillance of antimicrobial use in hospitals is therefore important to identify prescribing trends, to link results with antimicrobial resistance data, and to identify areas for improvement.

In this study, we present the results of a survey conducted in 2007 to describe the prevalence of antibiotic use in hospitalised children in Italy. Data have been collected in Bambino Gesù Children's Hospital in Rome, which is the paediatric hospital with the highest annual number of inpatients in Italy.

## Materials and methods

### Description of the hospital

Bambino Gesù Children's Hospital is one of the nine children's hospitals in Italy. It is a research hospital within the National Healthcare System and includes two different sites, one located in Rome and the other in Palidoro on the sea coast north of Rome. It is organised in 13 departments and has a total of 607 inpatient bed capacity (444 in Rome and 163 in Palidoro).

In 2007, there were 33,050 hospital inpatient admissions, with a mean length of stay of 5.3 days. The mean number of monthly admissions was 2,738, ranging from 2,016 in August to 3,049 in March. In June, there were 2,893 inpatient admissions.

### Population under study

The point prevalence study was conducted in all hospital departments from 4 to 16 June 2007. Data on antibiotic use were collected by reviewing medical charts of all patients hospitalised for >48 hours. For each hospitalised child, information was collected on age, sex, main diagnosis at admission and the type and number of antibiotics administered. Data was also recorded on whether the antimicrobial drugs were prescribed on the basis of clinical signs suggestive of infection, but without microbiological confirmation (i.e. on an empirical basis), or administered for infections that were laboratory confirmed (i.e. based on microbiological findings), or related to prophylaxis.

The antibiotic prescription rates were calculated for the entire hospital and by type of unit, i.e. intensive care units (ICUs), surgical wards and medical wards, including all non-surgical wards apart from ICUs.

### Statistics

Statistical analyses were conducted using STATA 8.2 (Stata Corporation, College Station, Texas, USA).

Differences in rates between groups were compared using the chi-square test or Fisher's exact test; t-test or Mann-Whitney non-parametric test were used to compare continuous variables.

## Results

A total of 412 hospitalised children were evaluated; their median age was 42.3 months (range 0-806 months), and 229 were males (55.6%). Antibiotics were prescribed for 181 of the 412 patients (43.9%). The prevalence of antibiotic use was higher in older children, ranging from 33.7% in 0-6-month-old infants (32/95) to 42.4% in children aged from seven months to five years (61/144) and 49.1% in children older than five years (85/173) (chi-square for trend: p=0.049). No statistically significant differences by sex were noted.

Out of the total 412 children, 236 were hospitalised in medical wards, 129 in surgical wards and 47 in ICUs. The median age of patients differed significantly, being lowest in ICUs and highest in surgical wards (Table 1). The prevalence of antibiotic use was 37.7% in medical wards, 51.1% in ICUs and 52.2% in surgical wards (Table 1). Prevalence by diagnosis at admission is shown in Table 2.

Of the 181 children who were treated with antibiotics, 78 (43.8%) received more than one drug. The prevalence of combination therapy was thus 18.9%.

The total number of antibiotic courses was 255, i.e. a mean of 1.4 drugs per treated child.

As shown in Figure 1, the top five ranking antibiotics were amoxicillin in combination with clavulanic acid, ceftazidime, ceftriaxone and amikacin.

**Figure 1**

**Number of prescriptions by antibiotic drug, Bambino Gesù Children's Hospital, Rome, Italy, June 2007**

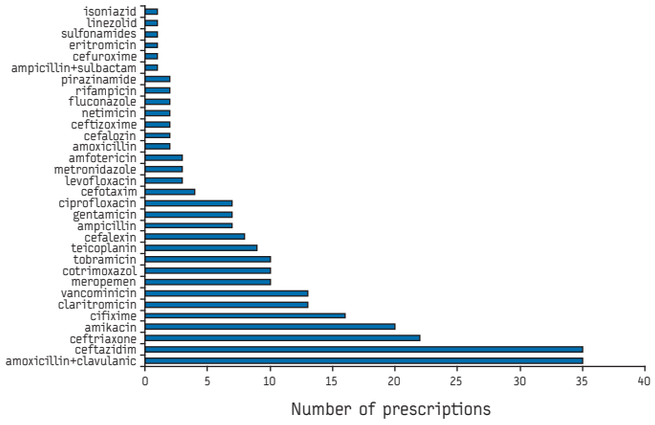


**Table 2**

**Prevalence of antibiotic use, by diagnosis at admission, Bambino Gesù Children's Hospital, Rome, Italy, June 2007**

| Diagnosis at admission | Number of patients | Number of patients receiving antibiotics (%) |
|---|---|---|
| Symptoms, signs and ill-defined conditions | 72 | 33 (45.8) |
| Congenital malformations | 48 | 22 (45.8) |
| Diseases of the cardiovascular system | 43 | 11 (25.6) |
| Diseases of the respiratory system | 42 | 25 (59.5) |
| Diseases of the digestive system | 30 | 12 (40.0) |
| Diseases of the musculoskeletal system and connective tissue | 22 | 10 (45.5) |
| Conditions originating in the perinatal period | 17 | 4 (23.5) |
| Diseases of the genitourinary system | 16 | 10 (62.5) |
| Disorders of the nervous system | 13 | 2 (15.4) |
| Neoplasms | 11 | 8 (72.7) |
| Injury and poisoning | 11 | 5 (45.5) |
| Infectious and parasitic diseases | 9 | 7 (77.8) |
| Diseases of the sense organs | 8 | 6 (75) |
| Mental disorders | 6 | 0 (0) |
| Diseases of the blood and blood-forming organs | 4 | 2 (50.0) |
| Endocrine, nutritional and metabolic diseases, and immune system disorders | 4 | 0 (0) |

**Table 3**

**Number of prescriptions by antibiotic class and reasons for prescription (microbiological data, clinical data, prophylaxis), Bambino Gesù Children's Hospital, Rome, Italy, June 2007**

| Antibiotic class | Microbiological data (%) | Clinical data (%) | Prophylaxis (%) | Total |
|---|---|---|---|---|
| cephalosporins | 2 (2.2) | 40 (44.4) | 48 (53.4) | 90 |
| penicillins | 5 (11.4) | 23 (52.3) | 16 (36.3) | 44 |
| aminoglycosides | 5 (12.8) | 18 (46.2) | 16 (41.0) | 39 |
| macrolides | 0 (0) | 13 (93.0) | 1 (7.0) | 14 |
| vancomycin | 1 (7.0) | 12 (86.0) | 1 (7.0) | 14 |
| carbapenems | 1 (10.0) | 7 (70.0) | 2 (20.0) | 10 |
| Others | 7 (16.0) | 17 (39.0) | 20 (45.0) | 44 |
| Total | 21 (8.2) | 130 (51.0) | 104 (40.8) | 255 |

**Table 1**

**Prevalence of antibiotic use, by basis for prescription (microbiological data, clinical data or prophylaxis), and by type of ward, Bambino Gesù Children's Hospital, Rome, Italy, June 2007**

| | Medical ward | Surgical ward | Intensive care units (ICUs) | p-value |
|---|---|---|---|---|
| Number of patients | 236 | 129 | 47 | - |
| Patients' median age in months (range) | 36.8 (0-512) | 68.7 (0-807) | 2.6 (0-222) | < 0.001 |
| Number of patients receiving antibiotics based on microbiological data (%) | 5 (2.1) | 1 (0.07) | 6 (12.7) | < 0.001 |
| Number of patients receiving antibiotics based on clinical data (%) | 65 (27.5) | 19 (14.7) | 9 (19.1) | n.s. |
| Number of patients receiving antibiotics for prophylaxis (%) | 19 (8.0) | 48 (37.2) | 9 (19.1) | < 0.001 |
| Total number of patients receiving antibiotics (%) | 89 (37.7) | 68 (52.7) | 24 (51.1) | 0.013 |

Antibiotics were prescribed empirically in 51.0% of cases; in 40.8% of cases the drugs were used for prophylaxis, and in 8.2% of cases the treatment was based on microbiological data (Table 3).

The use of cephalosporins was almost evenly distributed between empirical therapy and prophylaxis, while penicillins were most frequently used for empirical therapy.

Penicillins and aminoglycosides were the two categories of drugs that were most commonly prescribed on the basis of microbiological data.

The highest proportion of children receiving antibiotics prescribed on the basis of microbiological data was found in ICUs (25.0% vs. 5.7% and 1.5% in medical and surgical wards, respectively; $p \leq 0.01$), while medical wards ranked first in proportion of empirical treatments (73.0% vs. 37.5% in ICUs and 27.9% in surgical wards; $p<0.01$), and surgical wards in prophylactic use (70.6% vs. 37.5% in ICUs and 21.3% in medical wards; $p<0.01$).

## Discussion

In 2005, Italy ranked third among European countries with the highest consumption of antibiotics in outpatient care [14], and a recent literature review of studies published in USA, Canada, north-central Europe and Italy found that Italy also has one of the highest paediatric outpatient antibiotic prescription rates [15]. Although a strong positive correlation between the extent of antibiotic consumption in outpatient and inpatient care has been shown [4], no national data on hospital consumption have been collected in Italy up to now, and no national policies on the prudent use of antibiotic have been implemented.

In western Europe, studies on hospital use of antibiotics in children are few [5,6,9]. In comparison with these findings, our results show higher prevalence of antibiotic use than those observed in the Netherlands and Switzerland in the late 1990s and early 2000s where prevalence rates were 36% [5,6], yet lower than those reported from UK in 2006 (49%) [9]. The proportion of prescriptions that had been based on microbiological data was also similar to that reported by these European surveys.

Our study has some limitations. Firstly, it was conducted in one hospital only, and its results cannot be considered representative of the whole country. Secondly, it was conducted in June, when the number of children admitted with respiratory infections could have been lower than observed in other periods of the year. Since respiratory tract infections are one of the leading causes of antimicrobial use in children [2], we could have underestimated the prevalence. Thirdly, information on the start of antibiotic therapy was not collected, so we cannot exclude the possibility that some children had already been on therapy at admission. Lastly, we did not evaluate the appropriateness of antibiotic prescriptions and we did not investigate if prescriptions were due to nosocomial infections.

In our study, the most frequently used antibiotic was the combination of amoxicillin plus clavulanic acid, as observed in primary care [1,14]. This finding confirms that hospital antimicrobial use tends to display a similar distribution pattern to that observed in the ambulatory use [4].

A number of interventions including persuasive and restrictive methods have been shown to be effective in reducing antimicrobial use in hospitals [16]. The commonly prescription pattern observed in hospitalised and outpatient children underscore the need to implement actions targeting both primary care and hospital paediatricians. However, it is well known that health indicators, such as infant mortality rate, vaccination coverage and hospitalisation rates, vary widely across Italy [17]. Variability in outpatient antibiotic prescribing profiles by geographical area has also been shown [18], and it is likely that antibiotic use in children would also differ by hospital. It is therefore advisable to collect data at both hospital and national level, in order to identify priority areas and design interventions tailored to specific circumstances.

Since early 2000s, Bambino Gesù Children's Hospital has implemented a series of measures, including collection of data on antimicrobial resistance, introduction of guidelines for diagnosis and treatment of infectious diseases such as bronchiolitis and acute gastroenteritis, which could have affected the prescribing habits.

An important issue identified in our results is the high proportion of children who received surgical prophylaxis. In fact, 71% of patients treated with antibiotics in surgical wards received their prescription for prophylaxis, compared to 13-42% reported by other authors [6,7].

The fact that ceftadizime, a parenteral third-generation cephalosporin, ranked first (together with amoxicillin + clavulanic acid) in prescription frequency is also a reason for concern.

Though we did not evaluate the appropriateness of antibiotic use, these results highlight the need to introduce guidelines for surgical prophylaxis in children, and to further investigate the reasons for prescribing parenteral antibiotic therapy in paediatric hospitals.

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# Mapping the future dynamics of disease transmission: risk analysis in the United Kingdom Foresight Programme on the detection and identification of infectious diseases

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This paper reflects on the qualitative risk analysis framework developed for a Foresight study on the Detection and Identification of Infectious Diseases, which was coordinated in 2005 by the United Kingdom (UK) under what is now the Government Office for Science, Department for Innovation, Universities and Skills. The risk assessment covered human, plant and animal diseases in the UK and Africa in the years 2015 and 2030. Through engaging a diverse pool of experts, we developed a model conceptualising disease spread as the outcome of interactions among sources, pathways and drivers. We then used this model to conduct a Delphi survey of experts. The factors perceived most likely to contribute to infectious disease spread in 2015 and 2030 included geographic extension of existing pathogens (partially due to climate change), over-use of antibiotics/antivirals/pesticides leading to drug resistance, and zoonoses. Our methodology provides a framework for those who need to integrate a wide range of perspectives and factors into their planning and analyses.

**Table 1**

**Main categories of drivers associated with emergence and reemergence of human pathogens (reproduced from Woolhouse *et al.* (2005) [5])**

| Rank* | Driver |
|---|---|
| 1 | Changes in land use or agricultural practices |
| 2 | Changes in human demographics and society |
| 3 | Poor population health (e.g., HIV, malnutrition) |
| 4 | Hospitals and medical procedures |
| 5 | Pathogen evolution (e.g., antimicrobial drug resistance, increased virulence) |
| 6 | Contamination of food sources or water supplies |
| 7 | International travel |
| 8 | Failure of public health programs |
| 9 | International trade |
| 10 | Climate change |

* Ranked by the number of pathogen species associated with them (most to least).

## Introduction

It is by now well documented that a wide range of factors, including changes in land use and agricultural practices, changes in human demography, pathogen evolution, international travel and trade, climate change, and poor public health infrastructures can all trigger or exacerbate the spread of infectious diseases, determining how and where they will emerge in the future and the circumstances under which they could progress to epidemic or even pandemic proportions (Table 1) [1-5].

Less widely documented are methods for analysing these factors in ways that enable a better understanding of how they are interlinked and how to prioritise their importance. One of the key challenges is that relevant information, when available, is not consolidated in a few hands but spread across numerous institutions and disciplines. Anticipating the emergence or altered transmission of any disease is likely to require expertise in biology, epidemiology, animal and human medicine, demographics, economics, and even sociology and anthropology. Although the importance of cross-sectoral collaboration in disease control is increasingly recognised [6-8], there remains the need to develop new ways of ensuring that diverse and sometimes divergent perspectives are accounted for. Doing so is essential for developing multi-sectoral understanding and commitment – increasingly required for the pursuit of pubic health action in a rapidly changing world.

With a long-term vision in mind, the United Kingdom (UK), under what is now the Government Office for Science, Department for Innovation, Universities and Skills, conducted a Foresight project on Detection and Identification of Infectious Diseases (DIID) with the objective of supporting strategic investment in disease detection, identification and monitoring technologies and systems [9-12]. This paper reflects on the risk analysis component of the DIID project, describing a methodology that could be adapted to subsequent analyses.

## Methodology

We analysed expert opinion on infectious disease risks in plants, animals and humans, in sub-Saharan Africa and the UK in 2015

and 2030 (comprehensive details on the methodology, workshop and survey results are available at the Foresight website [12]). Potential changes in sources, pathways and drivers of disease risks were identified and assessed according to how the magnitude and nature of risks are evolving, as well as the range of plausible future risk patterns. Research questions focused on:

- Factors driving changes in infectious disease risks ('risk drivers') and how they might evolve;
- Future risks for infectious diseases and their importance;
- Uncertainty attached to future risks;
- Comparisons among plant, animal and human disease risks.

To answer these questions a preliminary scoping phase, which included an expert workshop, developed an understanding of important issues and their interactions and formulated the overall approach to the research. A Delphi survey was then carried out in order to assess a broad range of expert opinions on future risks in the UK and Africa.

### Scoping phase

The scoping workshop brought together 22 UK infectious disease experts (recommended by the UK Foresight Scientific Advisory Group) to advise on the challenges presented by new and emerging infectious diseases. A disease systems model was developed (Figure 1), as well as an initial list of key factors ("drivers") likely to give rise to changes in disease patterns and emergence of new diseases, such as biological changes and socio-economic factors acting on disease sources and pathways of disease spread. The initial long list of drivers derived at the workshop was refined and clustered under the six main headings listed in Table 2.

### Identification and selection of participants for the survey

The experts who took part in the Delphi survey were scientists selected to cover a broad range of expertise in plant, animal and human diseases, from epidemiological modelling, disease identification and disease pathology to disease control, regulation and policy making. They were selected upon the advice of approximately 30 senior advisers who took part in the DIID Foresight project, including members of the UK Foresight Scientific Advisory Group, the UK Foresight High Level Stakeholder Group and UK Health Protection Agency staff, to represent the best available informed judgement across our six areas of interest – the future development of plant, animal and human diseases in the UK and in sub-Saharan Africa.

African respondents from 20 countries in sub-Saharan Africa were invited on the basis of the best available expertise, rather than ensuring geographical equity. Francophone countries were under-represented as we did not have sufficient time within the project to translate questionnaires. This omission may have influenced the findings. There was, however, no evidence of any specific bias among the 55% respondents who completed the questionnaires, with relatively equal representation across the six survey areas (Table 3), and also across relevant areas of expertise (20 areas of expertise were mentioned in the questionnaire responses).

### Questionnaire development

A two-stage questionnaire-based survey was sent to 145 experts in infectious diseases from the UK and sub-Saharan Africa. In the second stage of this Delphi-type process [13], respondents were given the results from the first phase and asked to re-assess their own responses. Where their opinions diverged from those of others they were asked to explain their reasons rather than being encouraged to reach a consensus.

The questionnaire was based on the disease systems model (Figure, Table 2), but slightly different versions were sent out depending on whether the participants were being asked about human, plant, or animal diseases. Nonetheless, the questionnaires were designed so as to be as comparable as possible. For example, question 3.2.4 in Table 2 was worded as "lack of availability of new vaccines or engineered resistance", broadening the scope of the question from vaccines (mainly relevant for humans and animals) to also include engineered resistance (mainly relevant for plants and animals). As another example, question 2.9 in Table 2 shows a question that was worded differentially depending on whether it was considering animal or human diseases; however, this question was not included in the plant diseases survey.

Each questionnaire asked about future changes in disease sources, pathways and drivers, leading to future disease outcomes. These terms were defined as follows:

- *Sources:* phenomena or biological events that give rise to potential new diseases, enable existing diseases to become

**Figure**

**The disease systems model as a tool for assessing future infectious disease risks**

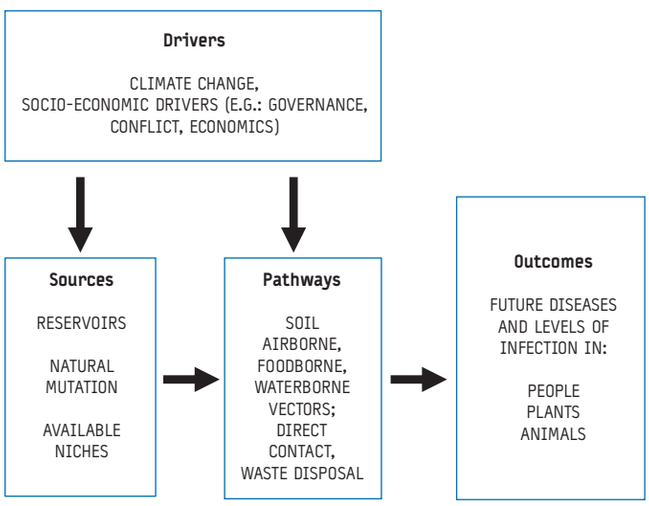


**Table 3**

**Sample size, UK Foresight questionnaire, 2005**

| Questionnaire type | No. distributed | No. of responses (Round 1) | No. of responses (Round 2) |
|---|---|---|---|
| UK animals | 20 | 10 | 6 |
| UK humans | 20 | 12 | 5 |
| UK plants | 24 | 13 | 5 |
| Africa animals | 29 | 18 | 11 |
| Africa humans | 27 | 13 | 9 |
| Africa plants | 25 | 14 | 6 |
| Total | 145 | 80 | 42 |

**Table 2**

**Classification of factors influencing the spread of infectious disease, Foresight questionnaire, 2005**

| | |
|---|---|
| | **Sources** |
| 1.1 | New pathogens or new strains of existing pathogens arising through natural genetic change |
| 1.2 | Geographical expansion of pathogens |
| 1.3 | Emergence of new disease vectors |
| 1.4 | Failure of engineered resistance (e.g. vaccines, genetically manipulated animals/crops) |
| 1.5 | Increased number of accidental introductions of pathogens |
| 1.6 | Increased pathogen resistance (e.g. to microbicides, antivirals, pesticides) |
| 1.7 | Decreased immuno-competence of target populations |
| 1.8 | Emergence of new diseases from other species reservoirs, including wild species reservoirs |
| | **Pathways** |
| 2.1 | Increased role of soil-borne route for disease spread |
| 2.2 | Increased role of air-borne route for disease spread |
| 2.3 | Increased role of water-borne route for disease spread |
| 2.4 | Increased populations of disease vectors |
| 2.5 | Increased host-to-host transmission due to increased density of host populations |
| 2.6 | Increased role of food-borne (or feed-borne) route for disease spread (plant diseases excluded) |
| 2.7 | Increased role of food-borne (or feed-borne) route for disease spread (plant diseases excluded) |
| 2.8 | Increased spread of disease in veterinary hospitals and/or herding of animal for veterinary interventions (animal diseases) OR Increased spread of disease in hospitals (human diseases) (plant diseases excluded) |
| 2.9 | Increased spread of disease through mass veterinary interventions (e.g. campaign vaccinations with shared needles) (animal diseases) OR Increased spread of disease through blood/tissue (e.g. needle sharing, blood transfusions, transplantation) (human diseases) (plant diseases excluded) |
| 2.10 | Increased spread of disease due to sexual contact (human diseases only) |
| | **Drivers** |
| 3.1 | Legislation and government systems |
| 3.1.1 | Lack of adequate systems for disease control |
| 3.1.2 | Lack of adequate surveillance systems to detect and monitor diseases |
| 3.1.3 | Poor implementation of national legislation on disease surveillance and control |
| 3.1.4 | Poor implementation of international legislation on disease surveillance and control |
| 3.1.5 | Lack of or ineffective biosecurity legislation regarding disease surveillance and control |
| 3.1.6 | Low degree of inter-institutional cooperation |
| 3.1.7 | Failure of government bodies to accurately or honestly report disease incidences |
| 3.2 | Technology and innovation |
| 3.2.1 | Lack of innovation in relevant and rapid technologies for detection and identification of existing diseases |
| 3.2.2 | Lack of innovation in technologies for detection and identification of new diseases |
| 3.2.3 | Lack of innovation in information technology for disease surveillance and communication |
| 3.2.4 | Lack of availability of new vaccines or engineered resistance |
| 3.2.5 | Development of potential new pathogens for bioterrorism |
| 3.2.6 | Drug use leading to the emergence of drug-resistant disease organisms |
| 3.2.7 | Lack of new food preservation and decontamination technologies |
| 3.2.8 | Lack of new drugs (or pesticides for plants) to control disease |
| 3.3 | Conflict and war |
| 3.3.1 | Loss of effective detection and identification systems |
| 3.3.2 | Increased movement of people (e.g. refugees, armies) spreading disease |
| 3.3.3 | Damage to infrastructure (e.g. water, sewage, power supplies) |
| 3.3.4 | Increased bioterrorism, exploiting existing diseases |
| 3.3.5 | Increased use of wild species as alternative human food source (plant diseases excluded) |
| 3.4 | Economic factors |
| 3.4.1 | Decreased economic prosperity |
| 3.4.2 | Increased disparity between rich and poor |
| 3.4.3 | Increase in trade and transport of animals and crops |
| 3.4.4 | Decreased average education levels |
| 3.4.5 | Reduced quality of sanitation and water supplies |
| 3.4.6 | Increased movement of migrant workers, spreading disease |
| 3.4.7 | Increased number of disease-susceptible individuals in the population |
| 3.5 | Human activity and social pressures |
| 3.5.1 | Decrease in public willingness to change behaviour in order to help contain or prevent disease |
| 3.5.2 | Decrease in individuals' readiness to report disease incidences |
| 3.5.3 | Increase in illegal practices leading to spread of disease |
| 3.5.4 | Malnutrition/poor husbandry of animals/crops affecting resistance to disease |
| 3.5.5 | Increased travel related to tourism and international business, spreading disease |
| 3.6 | Climate change |
| 3.6.1 | Increase in mean temperature in the range of 0.5-2.0 °Celsius |
| 3.6.2 | Increase in frequency of heavy rainfall events and/or flooding |
| 3.6.3 | Increase in frequency of drought in arid and semi-arid areas |

more harmful, enable existing diseases to infect new hosts, or enable existing diseases to spread to new areas;
- *Pathways*: mechanisms or routes by which a disease-causing organism can be transferred from one host to another, within or between species;
- *Drivers:* social, economic, biological or environmental factors that affect disease outcomes, by changing the behaviour of disease sources or pathways;
- *Outcomes:* plants and animals at the individual, community and ecosystem, or farming system level, and humans at individual and societal levels, that are affected by infectious diseases.

'Drivers' operate in the infectious disease system through 'sources' of disease emergence and/or 'pathways' of disease transmission to determine the 'outcome' in terms of the emergence of future diseases and the levels of infection.

'Risk' was defined as the product of 'the future extent of a hazard' and 'the probability of occurrence of that hazard'. For each factor listed in Table 2, the respondents were asked to rate the extent and probability of different outcomes in the years 2015 and 2030, on a three-point scale. The survey thus provided a systematic method for gathering informed opinions on rankings of the impact of drivers on sources and pathways, as well as on the importance of changes in sources and pathways themselves.

The questionnaires also asked respondents for additional observations, including the phenomena or processes they thought were likely to decrease risk and what they expected to be future risks (for example, which classes of diseases or organisms were likely to represent the greatest risk).

### Data analysis

Questionnaires generated qualitative scores for both the perceived extent of the hazard and the perceived probability of its occurrence (1, 2 or 3; low, medium or high). The risk associated with a particular factor for each source, pathway and driver was then calculated as the product of these two scores, giving a range of potential values: 1, 2, 3, 4, 6 or 9. , Thus we compared the perceived importance of sources, pathways and drivers in contributing to future disease outcomes for the six risk questionnaire categories (permutations of host and location: Africa-human (AH), UK-human (UKH), Africa-animal (AA), UK-animal (UKA), Africa-plant (AP), UK-plant (UKP)). We focused on factors that were consistently predicted to be of higher risk through a data filtering process - risk assessments were categorised as low, moderate or high as follows:
- Low risk: an overall score in the range 1-3, i.e. either hazard or probability were scored as low (1);
- Moderate risk: an overall score of 4, i.e. both hazard and probability were scored as moderate (2);
- High risk: an overall score of 6 or 9, i.e. either hazard or probability were scored as high (3) and the other was scored as moderate or high (2 or 3).

The first filter selected the cases for which more than 50% of the responses were in the moderate or high category (scores 4, 6 or 9). The second filter selected cases for which more than 50% of responses were in the high category (scores 6 or 9).

## Survey results

### Participants

The response rate in the first round of the survey was 55%, and 53% of the first round respondents contributed to the second round (Table 3). The respondents' self-reported areas of expertise were primarily: epidemiology (12%), virology (9%), pest and disease management (8%) and animal health and veterinary science (7%). This participation rate was more than sufficient to conduct the analysis, as breadth of expertise was deemed to have priority over absolute number of respondents. The declining number of respondents from the first and second round partially reflects those participants that did not feel that they needed to alter their responses.

### Risk assessments

The complete survey results are available on the UK Foresight website [10]. Table 4 compares the factors which, for 2015 and 2030, passed the first and second filters of 50% or more of respondents.

The highest perceived risks (for 2030) related to:
- new pathogens or new strains of existing pathogens arising through natural genetic change;
- and geographical expansion of pathogens from within or outside the UK and Africa.

In five of the six categories there was a perceived high risk of:
- new diseases from other species reservoirs, including wild species reservoirs;
- drug use leading to the emergence of drug-resistant disease organisms;
- an increase in disease due to a mean temperature increase in the range 0.5-2 °C.

Changes in *sources* were seen as important in all six categories (plants, animals and humans; UK and Africa), and there was little difference between UK and Africa in perceived overall risks generated by changes in sources.

Changes in *pathways* were seen as less important generators of disease risks across all categories than were changes in sources, although there were marked differences between UK and Africa. Increased host-to-host transmission due to increased density of host populations was seen as important for animals, plants and humans in Africa, but not at all in the UK. Increased disease vector populations were seen as important for plants and animals in the UK and for plants in Africa.

Many more disease *drivers* were considered important in Africa than in UK. For Africa, intriguingly, many respondents predicted lower risks arising from 'Legislation and Systems of Government' and 'Conflict and War' in 2030 compared to 2015, which reflects optimism about the future.

Finally, the three elements of climate change that were examined (increased temperature, rainfall and drought) were all seen as important drivers for human disease risks in Africa; yet only drought was highlighted for animals, and only temperature and rainfall was highlighted for plants.

In the UK, drivers seen as generating high levels of risk for human diseases were: drug use leading to the emergence of drug-resistant disease organisms and climate change, specifically rising temperatures. For UK plant diseases, the emergence of pesticide-resistant disease strains and the lack of new pesticides, increased trade and transport of crops and higher ambient temperatures, were seen as important risk drivers. For UK animal diseases, lack of adequate systems for disease control, poor implementation of international systems of disease surveillance and control, increased ability to engineer new diseases or to exploit existing diseases for

**Table 4**

**Responses for the years 2015 and 2030 that passed the first filter (moderate and high > 50%) and the second filter (high >50%), Foresight questionnaire 2005**

| | Africa animals | | UK animals | | Africa plants | | UK plants | | Africa humans | | UK humans | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Year | 2015 | 2030 | 2015 | 2030 | 2015 | 2030 | 2015 | 2030 | 2015 | 2030 | 2015 | 2030 |
| Source | | | | | | | | | | | | |
| 1.1 | | | | | | | | | | | | |
| 1.2 | | | | | | | | | | | | |
| 1.3 | | | | | | | | | | | | |
| 1.4 | | | | | | | | | | | | |
| 1.5 | | | | | | | | | | | | |
| 1.6 | | | | | | | | | | | | |
| 1.7 | | | | | | | | | | | | |
| 1.8 | | | | | | | | | | | | |
| Pathway | | | | | | | | | | | | |
| 2.1 | | | | | | | | | | | | |
| 2.2 | | | | | | | | | | | | |
| 2.3 | | | | | | | | | | | | |
| 2.4 | | | | | | | | | | | | |
| 2.5 | | | | | | | | | | | | |
| 2.6 | | | | | | | | | | | | |
| 2.7 | | | | | n/a | n/a | n/a | n/a | | | | |
| 2.8 | | | | | n/a | n/a | n/a | n/a | | | | |
| 2.9 | | | | | n/a | n/a | n/a | n/a | | | | |
| 2.10 | | | | | n/a | n/a | n/a | n/a | | | | |
| 2.11 | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | | | | |
| Driver: Legislation & government | | | | | | | | | | | | |
| 3.1.1 | | | | | | | | | | | | |
| 3.1.2 | | | | | | | | | | | | |
| 3.1.3 | | | | | | | | | | | | |
| 3.1.4 | | | | | | | | | | | | |
| 3.1.5 | | | | | | | | | | | | |
| 3.1.6 | | | | | | | | | | | | |
| 3.1.7 | | | | | | | | | | | | |
| Driver: Technology & innovation | | | | | | | | | | | | |
| 3.2.1 | | | | | | | | | | | | |
| 3.2.2 | | | | | | | | | | | | |
| 3.2.3 | | | | | | | | | | | | |
| 3.2.4 | | | | | | | | | | | | |
| 3.2.5 | | | | | | | | | | | | |
| 3.2.6 | | | | | | | | | | | | |
| 3.2.7 | | | | | | | | | | | | |
| 3.2.8 | | | | | | | | | | | | |
| Driver: Conflict & war | | | | | | | | | | | | |
| 3.3.1 | | | | | | | | | | | | |
| 3.3.2 | | | | | | | | | | | | |
| 3.3.3 | | | | | | | | | | | | |
| 3.3.4 | | | | | | | | | | | | |
| 3.3.5 | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | | | | |
| Driver: Economic factors | | | | | | | | | | | | |
| 3.4.1 | | | | | | | | | | | | |
| 3.4.2 | | | | | | | | | | | | |
| 3.4.3 | | | | | | | | | | | | |
| 3.4.4 | | | | | | | | | | | | |
| 3.4.5 | | | | | | | | | | | | |
| 3.4.6 | | | | | | | | | | | | |
| 3.4.7 | | | | | | | | | | | | |
| Driver: Human activity & social factors | | | | | | | | | | | | |
| 3.5.1 | | | | | | | | | | | | |
| 3.5.2 | | | | | | | | | | | | |
| 3.5.3 | | | | | | | | | | | | |
| 3.5.4 | | | | | | | | | | | | |
| 3.5.5 | | | | | | | | | | | | |
| 3.5.6 | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | | | | |
| Driver: Climate change | | | | | | | | | | | | |
| 3.6.1 | | | | | | | | | | | | |
| 3.6.2 | | | | | | | | | | | | |
| 3.6.3 | | | | | | | | | | | | |

The numbers in the first column correspond to the variables listed in Table 2.
Black cells represent 'high risks' (factors passed the first and second filter); grey cells represent 'moderate risks' (factors that passed the first filter but not the second); empty cells represent 'low risks' (factors that passed neither filter).

bio-terrorism, emergence of drug resistance and the lack of new drugs, increased trade in animals, increase in illegal practices leading to the spread of diseases and climate change, specifically increased temperatures, were highlighted as important.

**High hazard, low probability responses**

We also examined high hazard, low probability risks, which would be scored as a 3 (1 for probability multiplied by 3 for hazard) and therefore would not have passed through the data-filtering analysis. However, only 17 out of the total 636 possible responses were of this nature, and in each case only between two and four respondents had categorised the risk in this way.

## Discussion: Employing Foresight to understand future disease outcomes

If it is clear that a wide range of factors influence the spread of infectious disease [1-3,14], then there is a need to better understand and prioritise them:

*"The rate and scale of global change in agriculture, trade, demographics, species translocations and invasions, microbial adaptation, and other complex factors, have evidently outstripped our ability to understand and respond to EIDs [emerging infectious diseases], and exposed serious limitations of approaches that fail to engage with the wider contexts from which infectious diseases emerge."* [15]

For each factor, it is important to: identify and quantify the relevant sources, pathways and drivers, model their relationships and interactions, and identify potential intervention points where synergistic interactions promoting disease emergence can be arrested. Quantitative analyses are ideally suited for this, yet in many instances crucial knowledge gaps exist, creating the need for complementary analyses to help guide decision-making and priority-setting until more hard evidence becomes available. Although some analysts have called for interaction across a very broad range of expertise [15-17], there has been little discussion about how this could be practically done.

Foresight projects, such as the UK DIID project, aim to develop scientific and technological priorities, integrate multi-disciplinary perspectives, co-ordinate research opportunities with economic and social needs, and stimulate communication and partnerships between researchers, research users and research funders [18,19]. Meanwhile, survey methodologies such as Delphi enable a systematic approach to eliciting, aggregating and synthesising expert opinions [20-22]. The approach we describe here begins to develop a framework for identifying, assessing and prioritising infectious disease spread by incorporating a wide range of perspectives and insights into the analysis. Through engaging a wide range of expertise, we identified and developed a preliminary prioritisation of the myriad factors relevant to plant, animal and human disease.

There are, of course, limitations to this approach. One is that in order to cover the broad geographic and disease range mandated by this project, it was inevitable that the disease systems model on which the research was based would be rather general; the predictions should be interpreted with this in mind.

One other limitation of our study, and perhaps of Foresight in general, is that the answers are not 'evidence-based' in the scientific sense of the word. In our study, the respondents' predictions are based on their experience and knowledge, and represent the respondents' expectations of future courses of events. Where little data exist (necessarily the case when mapping the future), or where these data are not easily comparable, we would suggest that demonstrating general agreement – or the lack thereof – on common themes across a broad range of disciplines and institutions can be an important starting point for framing and pursuing multi-agency action.

Finally, we are also aware that our disease systems approach has been unrealistically linear. For any specific disease, dynamic interactions and feedback loops among drivers, sources and pathways will amplify or diminish overall disease risks. However, it was not possible to include this level of sophistication in a general, meta-level model applicable to all the disease categories in this study. Future studies would be well advised to focus on specific classes of disease, or even on specific drivers, pathways or sources of disease.

Ultimately, the challenge is to identify the processes that influence the spread of new and emerging diseases before they become significant problems for national public health systems or public health emergencies of international concern. The approach described here, appropriately applied, could help facilitate this.


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# The burden of genital warts in Slovenia: results from a national probability sample survey

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The objective of this study was to estimate the lifetime age-specific cumulative incidence of self-reported genital warts diagnosis in Slovenia and to explore the association with demographic characteristics and self-reported sexual behaviour. Data were collected in the period from November 1999 to February 2001 from a national probability sample of the general population aged 18-49 years through a combination of face-to-face interviews at the respondents' homes and anonymous self-completed questionnaires. In total, 849 men and 903 women were interviewed (response: 63.3% men, 70.9% women). Among sexually experienced respondents with available information (752 men and 842 women), previous diagnosis of genital warts was reported by 0.3% of men (95% confidence interval (CI): 0.0%-1.3%) and 0.4% of women (95% CI: 0.1%-1.1%), and in the age group of 40-49 year-olds by 0.5% of men (95% CI:0.0-3.2) and 0.7% of women (95% CI: 0.2%-2.9%). In comparison to women with fewer than 10 lifetime male partners, those who reported to have had at least 10 male partners were more likely to have a previous diagnosis of genital warts (adjusted odds ratio: 7.2 (95% CI: 1.1%-47.8%). The lifetime cumulative incidence of self-reported genital warts diagnosis among Slovenians was relatively low in comparison to other published estimates from probability sample surveys in the general population in European countries. Our findings will inform the Slovenian vaccination policy against human papillomaviruses (HPV) and contribute to a better understanding of the differences between European countries regarding the burden of genital warts.

## Introduction

Anogenital infections with human papillomavirus (HPV) types 6 and 11 are responsible for almost all genital warts, in Slovenia as well as in other countries [1-3]. Prophylactic quadrivalent HPV vaccine has been shown to be highly effective in preventing anogenital disease, including genital warts, associated with HPV types 6, 11, 16, and 18 in young women [4]. Since this vaccine has recently become available, and many Member States of the European Union (EU) consider introducing HPV vaccination into their national immunisation schedules [5], understanding the burden of genital warts in the general population is important in order to make informed vaccination policy decisions. Few studies about the overall and age -specific lifetime cumulative incidence of self-reported genital warts diagnosis have been conducted in probability samples of the general population of European countries [6,7].

Surveillance of sexually transmitted infections in Slovenia, including genital warts, is based on mandatory notification of all diagnosed cases by clinicians. The annual reported incidence of newly diagnosed genital warts in the period from 2001 to 2007 was relatively low. The lowest rate, 3.5 per 100,000 general population, was reported in 2001, and the highest rate in 2002, with 6.7 per 100,000. The incidence in 2007 was 4.7 per 100,000 general population [8]. Although the sensitivity of our surveillance system has not been formally assessed, these reported rates are assumed to underestimate the true incidence [8].

We used data from the Slovenian national Sexual Lifestyles, Attitudes and Health Survey to estimate the overall and age-specific lifetime cumulative incidence of self-reported genital warts diagnosis in Slovenia and to explore the association with selected demographic characteristics and self-reported sexual behaviours.

## Methods

Details on the employed methods have been published previously [9]. In brief, data were collected over the period between November 1999 and February 2001 from a national probability sample of the general population aged 18-49 years by a combination of face-to-face interviews conducted at respondents' homes and anonymous self-administered pencil and paper questionnaires. The data collection methods were an adaptation from the British National Survey of Sexual Attitudes and Lifestyles conducted in 1990 and have been thoroughly piloted in Slovenia [10,11]. They were very similar to the methods used in the second British survey conducted in 2000 [6,12]. Ethical approval was obtained from the Medical Ethics Committee of the Republic of Slovenia. Informed consent was obtained from each study participant.

We used stratified two-stage probability sampling. Individuals aged between 18 and 24 years were sampled with twice the probability of older individuals. The sampling frame was designed using the list of enumeration areas provided by the Central Population Registry. Within each of the 12 statistical regions of Slovenia, communities were implicitly stratified according to their type and size as follows: rural communities with less than 2,000 inhabitants, non-rural communities with less than 2,000, communities with 2,000-9,999 inhabitants, those with 10,000-100,000, and two cities with more than 100,000 inhabitants. The entire sampling frame included 9,850 primary sampling units of approximately 120 inhabitants at 18-49 years of age. 270 primary sampling units were sampled

independently from the 12 regions with the probability proportional to the size of the eligible population, which was defined as the sum of the individuals at 25-49 years of age and twice as many individuals at 18-24 years of age. On average, 10 individuals at the age of 18-49 years were randomly selected from each unit.

Questions about demographic characteristics and first heterosexual intercourse were asked in face-to-face interviews. Only those who reported any sexual experience were asked to anonymously complete self-administered questionnaires that included questions on the details of sexual lifestyles, risk behaviours and previous diagnosis of sexually transmitted infections. The question designed to estimate age-specific lifetime cumulative incidence of self-reported genital warts diagnosis was: "Have you ever been told by a doctor that you have genital warts?"

Weights were computed to adjust for over-sampling of the age group of 18-24 year-olds and the differences in survey response between different regions, and different types and sizes of communities. A multidimensional calibration procedure was applied to adjust for any remaining differences between the achieved sample and available Slovenian population estimates according to statistical regions, types of communities, and gender and age groups, based on Central Population Registry data for the year 2000.

Analyses were conducted using STATA version 7.0 statistical methods for complex survey data (svy commands) to account for stratification, clustered sampling, over-sampling of 18-24 year-olds. Response rates were calculated from unweighted data. Weighted estimates of cumulative proportions of respondents who reported genital warts diagnosis, overall and according to different demographic and sexual behaviour characteristics, were obtained together with 95% confidence intervals (CI). Tests for independence for complex survey data (the Pearson chi-squared statistics corrected for the survey design) were computed. For women only, multivariate analyses of the association between self-reported genital warts diagnosis and marital status as well as having at least 10 heterosexual partners in one's lifetime (two variables associated with self-reported genital warts diagnosis in the univariate analyses, $p<0.05$) were performed by logistic regression accounting for complex survey design (svylogit command) to obtain pseudo-maximum likelihood estimates of adjusted odds ratio (AOR) together with 95% CI, and adjusted Wald tests of significance.

## Results

A total of 849 men (survey response: 63.3% of those selected) and 903 women (survey response: 70.9%) were interviewed. The 807 men and 874 women who reported sexual experience were asked to anonymously complete self-administered questionnaires.

The question for previous diagnosis of genital warts was answered by 752 sexually experienced men and 842 sexually experienced women (item response: 93.2% among men; 96.3% among women). Overall, two men and three women (unweighted counts) reported previous diagnosis of genital warts. Table 1 shows the proportions of those who reported previous diagnosis of genital warts, overall and by selected demographic characteristics and sexual behaviours with the results of univariate analyses of association (p values). Previous diagnosis of genital warts was reported more often by

**Table 1**

**Proportion (cumulative incidence) of sexually experienced* men and women aged 18-49 years who reported previous diagnosis of genital warts, Slovenia, 1999-2001**

| | Men | | | | Women | | | |
|---|---|---|---|---|---|---|---|---|
| | % (95% CI) | Base WT | Base UWT | p value† | % (95% CI) | Base WT | Base UWT | p value† |
| All | 0.3 (0.0 - 1.3) | 801 | 752 | | 0.4 ( 0.1–1.1) | 823 | 842 | |
| Age | | | | 0.58 | | | | 0.37 |
| 18-29 years | 0 | 280 | 363 | | 0 | 283 | 381 | |
| 30-39 years | 0.5 (0.0 - 3.7) | 254 | 174 | | 0.4 ( 0.0–2.7 ) | 265 | 235 | |
| 40-49 years | 0.5 (0.0 - 3.2) | 268 | 215 | | 0.7 ( 0.2–2.9 ) | 276 | 226 | |
| Marital status | | | | 0.55 | | | | 0.04 |
| Married/cohabiting | 0.5 (0.1 - 2.1) | 497 | 391 | | 0.3 (0.0 – 1.3) | 605 | 553 | |
| Widowed/separated/divorced | 0 | 17 | 12 | | 2.9 (0.4 -18.4) | 34 | 27 | |
| Single | 0 | 286 | 349 | | 0 | 184 | 262 | |
| Heterosexual partners in lifetime | | | | 0.58 | | | | 0.04 |
| Less than 10 | 0.3 (0.0 – 1.8) | 547 | 528 | | 0.3 (0.0 – 1.1) | 762 | 778 | |
| 10 or more | 0.5 (0.1 – 3.7) | 227 | 203 | | 2.1 (0.3 -13.8) | 47 | 49 | |
| Concurrent heterosexual partners | | | | 0.07 | | | | 0.41 |
| Never | 0 | 454 | 439 | | 0.3 (0.0 – 1.2) | 658 | 668 | |
| At least once | 1.0 (0.2 – 3.7) | 271 | 245 | | 0.8 (0.1 – 5.6) | 123 | 133 | |

* Sexually experienced respondents are defined as those who reported to have had sexual intercourse (oral, vaginal or anal).
† Pearson's chi-squared statistics corrected for the survey design were computed (univariate analyses of association between self-reported genital warts diagnosis and selected demographic and sexual behaviour characteristics).
CI: confidence interval; WT: weighted count of individuals; UWT: unweighted count of individuals.
Numbers of individuals (bases) vary according to the number of missing values for individual variables.
The data were weighted to be representative of the Slovenian population based on the Central Population Registry data for the year 2000 and analysed using STATA version 7.0 to account for complex survey design (stratification, clustered sampling, over-sampling of 18-24 year-olds).

older than by younger respondents: by 0.5% of the 40-49 year-old men (95% CI: 0.0-3.2) and by 0.7% of the 40-49 year-old women (95% CI: 0.2-2.9).

We found no evidence of association of previous genital warts diagnosis with the level of education, first heterosexual intercourse before the age of 16, having ever paid for sex, or condom use.

In multivariate analysis, women with at least 10 lifetime partners had higher odds of previous genital warts diagnosis (AOR (adjusted for marital status): 7.2 (95% CI: 1.1-47.8)) in comparison to those with fewer than 10. In comparison to married/cohabiting and single women, women who had been married previously were also more likely to have a previous genital warts diagnosis (AOR (adjusted for 10+ lifetime partners): 5.8 (95% CI: 0.9-38.8)); however, the statistical significance was borderline (p=0.07).

## Discussion and conclusion

Our findings indicate a relatively low overall and age-specific lifetime cumulative incidence of self-reported genital warts diagnosis in the general population of Slovenia.

The lifetime cumulative incidence of self-reported genital warts seems to vary substantially between European countries. In the general population probability sample of 16-44 year-old British men and women interviewed in 2000, 3.6% (95% CI: 3.1-4.2) of sexually experienced men and 4.1% (95% CI: 3.6-4.7) of sexually experienced women reported ever being diagnosed with genital warts [6]. In the general population probability sample of 18-45 year-old women interviewed in the period 2004-2005 in four Nordic countries, clinically diagnosed genital warts were reported by 10.1% (95% CI: 9.7-10.5) in Denmark, 12.0% (95% CI: 11.5-12.6) in Iceland, 9.5% (95% CI: 9.0-9.9) in Norway, and 11.3% (95% CI: 10.8-11.8) in Sweden [7]. These differences in the estimated lifetime cumulative incidence of self-reported genital warts between the studies in Slovenia, the United Kingdom (UK) and the Nordic countries are consistent with a recent review on the epidemiology of sexually transmitted infections in the European Union which concluded that the prevalence of herpes simplex virus type 2 (HSV-2) in Scandinavia was higher than in other countries [13].

Differences in sexual behaviours may contribute to the differences in the lifetime cumulative incidence of self-reported genital warts diagnosis between these European countries. The occurrence of genital warts has been linked to higher-risk sexual behaviours, most often with higher numbers of sexual partners [7,14,15]. Both of the above-mentioned European studies conducted in general population probability samples, reported higher mean numbers of lifetime sexual partners than our study (see Table 2).

Our results provide some evidence that Slovenian women with at least 10 lifetime male partners were more likely to have a previous genital warts diagnosis than those with fewer partners. Since the numbers were small and we used logistic regression accounting for the complex survey design (svylogit command) to obtain pseudo-maximum likelihood estimates of adjusted odds ratio (AOR) together with 95% CI and adjusted Wald tests of significance, it is possible that the statistically significant association we calculated may have been an association of only borderline significance. The lack of evidence in our data for an association of previous genital warts diagnosis with the level of education, first heterosexual intercourse before the age of 16, having ever had concurrent heterosexual partnership, having ever paid for sex, and condom use may be due to the relatively low prevalence of self-reported genital warts diagnosis as well as the relatively small sample size.

We may have underestimated the true overall and age-specific lifetime cumulative incidence of genital warts among Slovenian men and women due to the survey limitations that include validity constraints of self-reported information and to possible participation biases inherent to all behavioural surveys. Another possible factor is under-diagnosis caused by people that do not consult a doctor for genital warts or by barriers with respect to referral to sexually transmitted infections (STI) outpatient clinics. The ability to self-diagnose genital warts, a precondition to seeking health care, has been questioned [14]. However, we have no reason to believe that differences of such magnitude exist between general populations of Slovenia, Britain and Nordic countries with regards to the ability to self-diagnose genital warts, health-care seeking behaviour or access to STI outpatient clinics. Nor do we think that the ability to recall a previously diagnosed episode of genital warts or the number of lifetime sexual partners is responsible for the differences between these European studies [6,7,12].

It is noteworthy that our estimates, for both men and women, of the self-reported lifetime cumulative incidence of any STI (rather than of genital warts only), although still lower, were closer to the estimates obtained in the British survey (Slovenian men: 5.5%; Slovenian women: 5.1%; British men: 10.8%; British women: 12.6%) [6,17]. Further, the measured prevalence of sexually transmitted Chlamydia trachomatis infection among sexually experienced Slovenians aged 18-24 years in our survey was substantial, at 4.7% (CI 2.5%-8.5%) in both sexes, while the corresponding estimates for the UK were appreciably lower, with 2.7% (CI 1.2%-5.8%) among men and 3.0% (CI 1.7%-5.0%) among women, although the differences between the two countries were not statistically significant [6,18].

In conclusion, we found a relatively low lifetime cumulative incidence of self-reported genital warts diagnosis among Slovenian men and women in comparison to other published estimates from general population probability sample surveys in European countries. Differences in high-risk sexual behaviours may have contributed to these differences. Our findings will inform the Slovenian HPV vaccination policy as well as broader sexual and reproductive health

**Table 2**

**Estimates of mean numbers of lifetime sexual partners from surveys conducted in representative samples of general populations in selected European countries**

| Country | Mean numbers of lifetime sexual partners | | Year of study | Reference |
|---|---|---|---|---|
| | Men | Women | | |
| United Kingdom | 12.7 | 6.5 | 2000 | [12] |
| Denmark | n.i. | 8.4 | 2004-2005 | [7] |
| Iceland | n.i. | 8.8 | | |
| Norway | n.i. | 7.4 | | |
| Sweden | n.i. | 8.6 | | |
| Slovenia | 8.3 | 3.2 | 1999-2001 | Unpublished results |

n.i.: not included in the study.

policies. Our results also contribute to a better understanding of the differences in the burden of genital warts between European countries and may inform mathematical models aimed at projecting the long-term benefits and costs of vaccination with prophylactic quadrivalent HPV vaccine.


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# HIV and risk behaviour among men who have sex with men in Denmark – the 2006 Sex Life Survey

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Behavioural surveys among men who have sex with men (MSM) are important for HIV surveillance. The Danish 2006 Sex Life Survey was carried out as a self administered questionnaire, which was distributed at gay venues and bars and posted on the internet. The questionnaire was completed by 3,141 MSM. We describe the methods, the respondent group and the results of the 2006 Sex Life Survey, and discuss it implications. The main finding of this survey is that 33% of the respondents have practised unsafe sex, defined as unprotected anal intercourse with one or more partners of different or unknown HIV status. In the three previous Sex Life Surveys of 2000, 2001 and 2002, this figure was between 26% and 28%.

## Introduction

Following a period of decreasing incidence of newly diagnosed cases of human immunodeficiency virus (HIV) infection from the early 1990s to 2000, the rate of newly detected HIV infections began to rise again in Denmark, as it did in many EU countries [1,2]. The main mode of HIV transmission in Denmark is unprotected sex between men who have sex with men (MSM). An important approach to understand the dynamics of this rising trend and contribute to evidence-based HIV prevention, is to conduct second generation surveillance – that is surveillance which combines monitoring of new HIV cases and indicators of sexual behaviour among persons in the groups at highest risk for infection [3] .

Since 2000, four Sex Life Surveys monitoring sexual behaviour and responses to HIV issues among MSM in Denmark have been carried out in cooperation between STOP AIDS – Gay Men's HIV Organization and Statens Serum Institut, with financial support from the National Board of Health. All four surveys were quantitative analyses with data collection on sexual behaviour and self-reported HIV prevalence among MSM in Denmark [4]. This paper describes the results of the most recent, fourth survey performed in 2006.

## Methods

The 2006 survey was carried out between mid-August and mid-October 2006 by handing out questionnaires during the annual Copenhagen Gay Pride event and placing questionnaires in gay bars, clubs and other venues in Copenhagen and in the second largest city in Denmark, Aarhus. Questionnaires were also distributed as inserts in magazines both gay and HIV-related journals. In addition, the questionnaire was posted on several sites on the internet, both gay and HIV-related websites. This sampling method was the same as in the other Sex Life Surveys.

The questionnaire was constructed so that it would be possible to compare the results with those from earlier Danish surveys and with the outcomes of other European surveys among MSM (e.g. Gay Men's Sex Survey by Sigma Research in the United Kingdom and Barometre Gay by INVS in France). Most questions and the recall period of 12 months were identical in all four Sex Life Surveys. The questionnaire was limited to 28 questions in order to be contained within a single paper sheet.

The questions were arranged in four categories: a) demographic data/background data (age, education, residence, homo/bisexual behaviour and HIV status); b) sexual behaviour (frequency of sex, number of partners, unprotected anal sex, etc.); c) knowledge about and attitudes towards HIV and sex-related matters; d) response to various safe sex campaigns.

The internet version of the questionnaire contained exactly the same questions as the paper version, but had a number of additional pop-up double-check questions in case of answers that were inconsistent (e.g. the date of the last positive test being earlier than the year of HIV detection). Both versions were tested in a pilot study of 30 MSM contacted in gay bars in Copenhagen.

Data analysis was performed using Stata version 8. Chi-square test was used for bivariate comparisons, and multivariate logistic regression was applied to assess odds ratios (OR) and significance of independent variables for main sexual behaviour outcomes. A non-parametric test (Kruskal-Wallis) was used to compare number of partners in different groups.

When analysing the data the following definitions were used:

- Unprotected anal sex = penetrative anal sex without a condom, no distinction between insertive and receptive anal sex.
- Unsafe sex = unprotected anal sex when serostatus of the respondent is unknown, or with a partner with unknown HIV serostatus, or with a partner whose HIV serostatus is different from the perceived or known serostatus of the respondent.

In the data analyses different denominators are used, i.e. not the total number of respondents but the number of respondents who provided particular information.

## Results

### Demographic and background data

A total of 3,141 responses from survey participants were analysed. Of these, 2,026 (64%) responses were obtained from

questionnaires posted on the internet, 468 (15%) from those handed out at the gay pride, 411 (13%) from those disseminated in gay bars or saunas, and 236 (8%) from those distributed via magazines.

The mean age of the respondents was 33 years (range: 15-85 years). Fifty-six percent lived in the Copenhagen Area, 27% in Aarhus, Odense or Aalborg, and 27% outside the large cities.

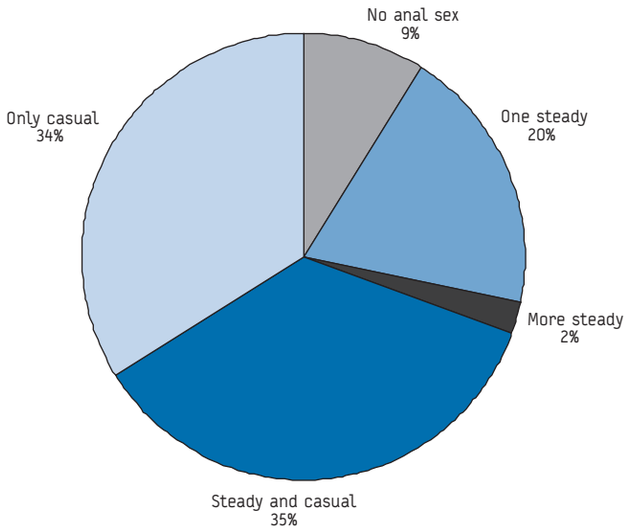
Fifty-five percent had either completed, or were attending post-secondary vocational or post-secondary academic education. This level of education was high compared to the general population, but it did not differ from other surveys among MSM.

During the 12-month study period, 2,755 (88%) of the respondents had sex with men exclusively, whereas 386 (12%) had sex with both men and women (hereafter referred to as homo or bisexual behaviour, respectively). Bisexual behaviour was stated by 343 (17%) of the 2,026 internet respondents, but only by 43 (4%) of those 1,115 who responded to paper questionnaires. This difference can be associated with a geographical pattern, as a higher proportion of paper respondents were Copenhagen residents, and a higher proportion of internet respondents were non-Copenhagen residents. Accordingly, 88 (6%) of respondents in Copenhagen reported bisexual behaviour, compared to 197 (18%) of respondents outside Copenhagen (p<0.001).

Two hundred thirty-one (8%) of the 2,918 respondents who answered this question reported to be HIV-positive, 2,188 (75%) to be HIV-negative, and 499 (17%) stated that they did not know their HIV status. There was a significant difference in geographic distribution, as 10% of the respondents living in the Copenhagen area were HIV-positive whereas among those living in the rest of the country this proportion was only 4% (p<0.001). Among Copenhageners, 17% did not know their HIV-status, and among non-Copenhageners this figure was 19% (non significant).

**FIGURE**

**Type of anal sex partners reported in 2006 Sex Life Survey, Denmark (n=2,932)**




**Sexual behaviour**

A total of 2,836 (92%) of the 3,095 respondents who answered this question had practised anal sex within the past 12 months. In comparison, 86% of the respondents in the Sex Life Surveys of 2000 and 2002 had practised anal sex, and the percentage was 84% in 2001. A logistic regression analysis controlling for age, residence, HIV status and homo or bisexual behaviour showed the 2006 increase to be significant (OR= 1.9, 95%CI=1.6-2.3, p<0.001), when comparing participants of the present survey to participants of any of the three former surveys.

The average number of anal sex partners reported by respondents in the 2006 survey was 9.4. In the previous three Sex Life Surveys this number was 8. Likewise, the median has increased from two in previous years to three in 2006 (p<0.001).

The proportion of men who had practised anal sex was the same for HIV-positive and HIV-negative men, but HIV-positive respondents had more anal sex partners (mean 17, median 6) than HIV-negative respondents (mean 8, median 3) (p<0.001). Among HIV-negatives, 180 (10%) of the 1,867 respondents who stated the number of anal sex partners had more than 20 anal sex partners, while this was the case for 47 (25%) of 186 HIV-positive respondents who stated number of anal sex partners (p<0.001).

The respondents were asked to state the number of both steady and casual partners during the last 12 months. The terms "steady" and "casual" were not defined in the questionnaire, and it is unknown to what extent partners may have overlapped or succeeded each other.

Thirty-four percent had only casual partners – one or more - during the last 12 months. A similar proportion, 35%, had both steady and casual partners, whereas only 20% had one steady partner. A small group (2%) reported more than one steady partner but no casual partners (Figure). In comparison, in the 2000 Sex Life Survey (the only one of the former surveys that distinguished between steady and casual partners), 26% of the respondents had only one steady partner, 28% had only casual partners and 29% had both steady and casual partners. A similarly small proportion (2%) had more than one steady partner but no casual ones (p<0.001).

The survey did not distinguish between insertive and receptive anal sex. When asked about unprotected anal sex, that is anal sex without condom, it was declared by 58% of the respondents.

Among HIV-positive respondents, 66% had practised unprotected anal sex, while this was the case for 55% of the HIV-negatives and for 60% of respondents who did not know their HIV status (p=0.001).

Among respondents with one steady partner, 73% had practised unprotected anal sex. This proportion was 71% among respondents with both steady and casual partners, 50% among respondents with only casual partners, and 51% among respondents with more than one steady, but no casual partners (p<0.001). Compared to the 2000 Sex Life Survey, the respondents who had only casual partners had a significantly higher proportion of unprotected anal sex in 2006 (p<0.001). For the respondents who had both steady and casual partners, the increase in the proportion of those who had unprotected anal sex was only marginally significant (p=0.06). Among respondents who had only one steady partner, there was no

difference in the proportion of those who had unprotected anal sex between the two Sex Life Surveys.

Among homosexual MSM, 59% had practised unprotected anal sex, compared with 48% of bisexual men (p<0.001).

Among respondents under 30 years of age, 62% had practised unprotected anal sex, compared with 55% of respondents aged 30 years or older (p<0.001). When stratified by number and type of anal sex partners, there was no difference between the age groups, if respondents had only steady partners. Among respondents who had both steady and casual partners, 301 of 404 (75%) respondents less than 30 years old had unprotected anal sex, compared to 341 out of 499 (68%) respondents aged 30 years or more (p=0.04). Among respondents who had only casual partners, 195 of 360 (54%) respondents less than 30 years old had unprotected anal sex compared to 242 of 556 (46%) respondents aged 30 years or more (p=0.02).

Education level or place of residence was not of significant importance with regard to having practiced unprotected anal sex.

Among men who had only had one anal sex partner during the last 12 months (casual or steady), 66% had unprotected anal sex. Among men who had two or more partners, the fraction of those who had unprotected anal sex ranged from 55% (2-5 partners) to 71% (>20 partners).

**Unsafe sex**

In this survey, unsafe sex is defined as unprotected anal sex when serostatus of the respondent is unknown, or with a partner with unknown HIV serostatus, or with a partner whose HIV serostatus is different from the perceived or known serostatus of the respondent. Of the respondents, 33% stated that they had practised unsafe sex at least once during the last 12 months.

To assess possible predictors of unsafe sex, a multivariate logistic regression analysis was carried out. Six factors turned out to be independently associated with unsafe sex: Number of anal sex partners, HIV status, risk perception, age, education level and frequency of having sex (Table 1). There was no association between unsafe sex and residence, homo- or bisexual behaviour, or whether the questionnaire was submitted online or on paper, neither in bivariate nor multivariate analyses. Having had a new anal sex partner within the last 12 months was a significant predictor for unsafe sex in the bivariate analyses, but not in the multivariate analysis.

The number of partners was the strongest predictor of unsafe sex; the probability of having had unsafe sex ranged from 17% in men with one partner to 58% in men with more than 20 partners (p< 0.001).

HIV status was also a strong predictor. In a bivariate analysis, 49% of HIV-positive men had practised unsafe sex compared to 25% of HIV-negative men (p<0.001). Men who did not know their HIV status were the group among whom unsafe sex was practised by the biggest proportion (60%). This is due to the fact that all unprotected anal sex in this group was considered unsafe sex.

In the three earlier Sex Life Surveys, the proportions of respondents reporting unsafe sex ranged from 26% to 28%. As the populations in the four surveys differed in demographic composition, it is not possible to make a direct comparison. However, a multiple regression analysis shows that the proportion of respondents who practised unsafe sex had increased by 20-30% since the 2000, 2001 and 2002 surveys, when controlling for age, HIV status, education, number of partners, and frequency of sex. Unsafe sex was further stratified by discordant/unknown status. Table 2 presents the different strata of safe/unsafe sexual behaviour.

**Risk perception and assessment of the risk of HIV transmission**

Unprotected anal sex is known to be the most risky sexual practice for HIV transmission. Respondents were asked to state their perception of the risk of HIV transmission when practising anal sex with and without condom use, and with and without ejaculation, respectively. The majority (88%) stated that the risk of HIV transmission during anal sex without a condom and with ejaculation inside the partner was "risky" or "very risky". As noted above, perceiving the risk as "low" or "not risky" was a predictor of having practised unsafe sex. Whether the respondents perceived the practices to be risky or not, we examined the individual answers according to the level of risk assigned to the different anal sex practices (with and without a condom and with and without ejaculation inside the partner). The way the respondents ranked the

**Table 1**

**Frequency and odds ratios (OR) for independent variables which were significant predictors of unsafe sex in a logistic regression analysis; 2006 Sex Life Survey, Denmark**

| | N (%)* | Multivariate OR |
|---|---|---|
| Anal sex partners < 3, >0<br>Anal sex partners ≥ 3 | 1,046 (36%)<br>1,627 (55%) | 1<br>4.4 (3.50-5.62) |
| HIV-negative**<br>HIV-positive | 2,188 (75%)<br>231 (8%) | 1<br>3.1 (2.20-4.37) |
| High risk perception***<br>Low risk perception | 2,569 (87%)<br>377 (13%) | 1<br>2.67 (1.98-3.61) |
| Age ≥ 30 years<br>Age < 30 years | 1,673 (60%)<br>1,124 (40%) | 1<br>1.7 (1.32-2.06) |
| Post-secondary vocational or academic education<br>Primary and secondary education | 1,526 (55%)<br>1,266 (45%) | 1<br>1.3 (1.04-1.61) |
| Frequency of sex: once a month or less often<br>Frequency of sex: several times a month or more often | 1,041 (33%)<br>2,100 (67%) | 1<br>1.3 (1.01-1.61) |

OR: odds ratio
* Number and proportion (%) of respondents who answered the question concerned
** Respondents with unknown HIV status were excluded from the analysis
*** High risk perception: attributing great or very great risk of unprotected anal sex; low risk perception: attributing low or no risk of unprotected anal sex

**Table 2**

**Overview of respondents' sexual behaviour within the last 12 months; 2006 Sex Life Survey, Denmark**

| Behaviour | Number of respondents (%) |
|---|---|
| No anal sex | 231 (8%) |
| Only protected anal sex | 974 (35%) |
| Unprotected anal sex with concordant partners | 663 (24%) |
| Unprotected anal sex without knowing own and/or partners' HIV status | 737 (28%) |
| Unprotected anal sex with discordant partners | 187 (5%) |
| **Total (who stated HIV status and sexual behaviour)** | **2,792 (100%)** |

risk levels was used as a marker for knowledge of HIV transmission risk, so that anal sex without a condom with ejaculation inside the partner had to be ranked as more risky than without ejaculation, which in turn had to be ranked more risky than anal sex with a condom. Ninety-seven percent ranked the levels of risk satisfactorily.

**HIV testing**

Seventy seven percent of the respondents had undergone HIV testing one or more times in their lifetime.

Among respondents who stated the year of the last test, 36% had been tested in 2006. The questionnaire was distributed in the period August-October, so the answers could not reflect test activity in a full year. When including respondents whose last test had taken place in 2006 or 2005 (i.e. max 22 months ago), the figure was 59%. The corresponding figure was 51% in 2001 and 50% in 2002 survey (data were not available in 2000 survey) ($p<0.001$).

There was no difference in whether an individual had been practising unsafe sex during the last 12 months or not, in relation to whether he had ever been tested. However, among men who had practised unsafe sex during the last 12 months and were not HIV-positive, 48% had been tested in 2006 or 2005, while this was the case for only 43% of those who had not practised unsafe sex and were not HIV-positive. Two thirds of the respondents who had unsafe sex also stated how often they had it. Among those, testing frequency did not reflect risk taking; respondents who had unsafe sex once or twice during the last 12 months were more often tested recently than respondents who had unsafe sex 3-10 times, who, in turn, were tested recently more often than respondents who had unsafe sex more than 10 times during the last 12 months. This trend was, however, only marginally significant ($p=0.06$)

**Disclosure and condom use with a new partner**

In the course of the last 12 months, 66% of respondents had practised anal sex with a new partner with whom they had not previously had anal sex. Of these, 22% did not use a condom during the most recent occasion they had anal sex with a new partner, i.e. they had practiced unprotected anal sex.

Overall, 31% of those who had anal sex with a partner with whom they had not previously had anal sex informed their partner of their HIV status (disclosure) prior to having sex (only 1% disclosed it after sex). The same number of men were informed about their partner's status (received disclosure). There was an almost total overlap in these two groups, indicating that people either practised mutual disclosure or that neither of them disclosed.

**Table 3**

**Disclosure of HIV status and condom use; 2006 Sex Life Survey, Denmark**

| Disclosure/condom | Number of respondents | Proportion of total (%) |
|---|---|---|
| No disclosure, no condom | 196 | 11 |
| Condom but no disclosure | 975 | 56 |
| Disclosure but no condom | 171 | 10 |
| Both disclosure and condom | 394 | 23 |
| Total (who provided this information in the questionnaire) | 1,736 | 100 |

Forty-nine percent of the respondents who had not been using a condom last time they practised anal sex with a new partner disclosed their HIV status, compared with 30% of those who did use a condom. As shown in Table 3, 11% did not use condoms and did not disclose their HIV status the last time they practiced sex with a new partner, matching the study definition of unsafe sex.

Nearly half (48%) of the respondents had met their new partner on the internet. This figure was higher among internet respondents (57%) and lower (33%) among those who submitted paper questionnaires ($p<0.001$). The internet, bars/discotheques and saunas/sex clubs constituted a total of 79% of the answers to the question on where the respondents had met their latest new partners, regardless of the questionnaire source.

**Gay magazines, venues and websites**

Sixty percent read gay magazines, 82% used websites for homosexuals and 74% frequented gay venues. Fifty nine percent of the respondents used both gay venues and websites, 15% used venues exclusively, 12% only websites, and 14% used none of these.

## Discussion

This survey included 3,141 MSM representing 6.4% of the estimated 50,000 MSM in Denmark who in turn constitute 2.5% of the adult male population (aged 15-80 years) [5].

It is not possible to calculate a response rate, nor can it be known if the MSM who were not reached with the questionnaire or who chose not to answer, differ from the respondents in demographical or behavioural parameters. Even though the large number of internet respondents facilitated the inclusion of MSM outside the big cities, it is quite possible that MSM who answered the questionnaire represent a more outgoing and sexually active fragment of the Danish MSM population.

In this survey, only 20% of the respondents appeared to be practising a monogamous sex life with one steady partner, whereas the majority had both steady and casual partners or only casual partners. The extensive change of partners facilitates the spread of sexually transmitted infections, including HIV infection.

The main finding of this survey is that 33% of the respondents have practised unsafe sex, defined as unprotected anal intercourse with one or more partners of different or unknown HIV status. In the three previous Sex Life Surveys of 2000, 2001 and 2002, this figure was between 26% and 28%, indicating an increase of 20-30%, when controlled for population differences. There is no perfect way of dealing with differences when trying to compare different convenience samples, but controlling for factors that were shown to influence the risk of unsafe sex in bivariate analyses of both the present and the former Sex Life Surveys goes some way to overcome this issue. Furthermore, the same logistic regression analysis showed no difference in unsafe sex between the years 2000, 2001 and 2002, when controlling for the same factors. The fact that more respondents were recruited via the internet did not have an independent impact, when different rates of unsafe sex were analysed.

Several studies in other European countries [6-8] have reported increased frequency of unsafe sex among MSM in the early 2000s. However, during the recent years, unsafe sex levels seem to have

stabilised among MSM in some countries [9]. The increase in proportion of MSM practising unsafe sex between the 2002 and the 2006 Sex Life Surveys could have taken place at any time during this four-year period, and only repeated surveys will show if the trend in Denmark is still increasing.

The fact that MSM who are aware of their positive HIV status reported the highest levels of unsafe sex is problematic, but it mirrors recent findings elsewhere [6,10,11].

Among HIV-positive respondents, 66% had unprotected anal sex, but the proportion of those HIV-positive respondents who had unprotected anal sex with partners they did not know to be seroconcordant (of same HIV status) was 49%, suggesting that some amount of serosorting (the practice of having unprotected anal intercourse with a partner believed to be of the same HIV status [12,13]) among HIV-positive MSM takes place. The difference among HIV-negative respondents who have unprotected anal sex (55%) and HIV-negative respondents who have unsafe sex (25%) is even bigger. Whether this is due to HIV-negative MSM practicing active serosorting, or it is merely due to the more easy access that HIV-negative MSM have to seroconcordant partners, is not known. Serosorting among men who perceive themselves to be HIV-negative is only of value if both partners have had no risk of becoming infected since last negative test, and several studies have demonstrated that relying on negative serosorting with casual partners often leads to HIV transmission [10,14,15].

An even stronger predictor of unsafe sex than HIV positivity was the number of anal sex partners. This issue is recurrent in all the previous

Sex Life Surveys as well as in surveys in other countries [16]. In this context it is noteworthy that the average number of anal sex partners has increased since the 2002 Sex Life Survey.

The present survey does not offer an explanation as to why the numbers of partners, unprotected anal intercourse and unsafe sex are increasing. Among the reasons suggested by researchers in the field are treatment optimism, "safe sex fatigue", and the absence of the deterring effect of friends and lovers who are ill [17]. Especially the younger generations have begun their sex life in this day and age when HIV is no longer considered a threat of early death. This may partly explain why in our study younger MSM had unsafe sex more often than the older MSM.

It may be that the findings reflect a general tendency towards a more liberal and uninhibited sex life following a couple of decades of caution. Men's sex life is influenced by other factors than those that have to do with risk and HIV. An additional reason for increase of unprotected anal sex could be a switch from risk avoidance towards risk management strategies, e.g. serosorting.

Although 33% of people practising unsafe sex is a high percentage, there are still many MSM who exclusively had safe sex. The respondents were not asked about the number of partners with whom they had practised safe sex, or how many times.

The survey included assessment of the risk of HIV transmission in the case of unprotected anal intercourse with ejaculation in the partner. However, the participants were not asked to assert whether they practiced insertive or receptive anal sex or both, so some respondents could have interpreted the question in light of their own practices, and not, as intended, as the general possibility of transmitting HIV by ejaculating into the partner, i.e. transmission from the insertive partner to the receptive one.

Men who had a low estimation of the risk were more likely to have practised unsafe sex than men who estimated that the risk was high. However, on the basis of this survey results, it cannot be determined whether individuals choose to practise unsafe sex because they estimate the risk to be low, or they may be rationalising – after having practised unsafe sex – that the risk might not be that high after all.

MSM with an education level corresponding to post-secondary vocational or academic education had a lower risk of having unsafe sex than the less well educated MSM in this survey. The level of knowledge regarding safe sex practice was very high regardless of educational level, so this finding is surprising. Also, education level has not been a significant predictor in the former Sex Life Surveys.

Finally, the frequency of sex was an independent predictor of unsafe sex, but not as strong as the number of anal sex partners.

The overall HIV prevalence in the study was 8%, with a higher HIV prevalence among residents of the capital (10%) than among respondents from the rest of the country (4%). The wide use of the internet questionnaire in the 2006 Sex Life Survey has contributed to a larger proportion of responses from internet respondents living outside of Copenhagen in the 2006 survey than in the previous three Sex Life Surveys. Consequently, the overall HIV prevalence was lower than in the past surveys when it ranged between 10 and 11%. The prevalence estimate obtained by using data from the national surveillance system and the Danish HIV Cohort [18] is 5%. The result of our study is in line with this, taking into account that the survey still contained a disproportionately big fraction of Copenhageners with higher HIV prevalence than in the rest of the country. However, this can not be quantified, since the population distribution of MSM in Denmark is not known. Furthermore, the very high prevalence among the 61 respondents who had received the questionnaire as an insert in a HIV related magazine contributed to increase the overall prevalence.

Practically all respondents ranked different anal sex practice risks in the right order. This indicates a very high level of awareness concerning risky sex behaviours. Also in 2000, respondents demonstrated a good knowledge of risky sex behaviour. The awareness level is thus still high, a fact that may be ascribed to earlier information campaigns.

Future prevention initiatives must not only aim at maintaining this high level but also address the fact that unsafe sex is taking place despite the widespread and thorough knowledge of risks.

More than three-fourths of the respondents had undergone HIV testing one or more times in their lifetime. In other European countries, this figure varies between 50% and 80% [19]. Half of the respondents who had practised unsafe sex (and who had not previously been tested positive) had been tested in 2006 or 2005, implying that half of those respondents who could in principle have been infected within the last 12 months had not been tested within this period [12]. This was the case for a somewhat smaller number of respondents who – according to their questionnaire replies – had run no risk of HIV infection. From a prevention perspective, the

point is not to make as many people as possible take the test, but to make the relevant people take the test – those who have run a risk of being infected.

Two thirds of the respondents had anal sex with a new partner during the last 12 months, confirming the impression of a high partner turnover. Eleven percent did not know the HIV status of their new partner, but they still did not use a condom at the latest intercourse with the new partner. This method of assessing unsafe sex (at the last anal intercourse) adds an additional level to the measure of unsafe sex during the last 12 months.

Nearly half of the respondents had met their new partner via internet. From a prevention perspective, it is relevant that the internet is such a popular contact place. Gay venues such as bars/discos and sauna/sex clubs were used by two thirds of the respondents, and more than half of the respondents used both gay venues and gay websites. The proportion of respondents who used only venues or websites, or neither of these were much smaller (15%, 12% and 14%, respectively. In the light of both internet and gay venues playing a considerable role in the social and sexual life of MSM, preventive efforts focused on both these information media should make it possible to reach a large number of this population.

HIV among MSM is still a serious problem in Denmark and in the rest of Europe, and will continue to be so as a considerable proportion of MSM practise unsafe sex. The present survey demonstrates a high level of knowledge in this target group. However, knowledge is not enough to ensure safe sex practices, and the frequency of unsafe sex among MSM seems to be increasing. This finding has been used in safer sex campaigns conducted by STOP AIDS – Gay Men's HIV Organization, who tailor campaigns to influence attitudes and actions and not just knowledge about HIV transmission [20]. Monitoring developments and trends in the sexual behaviour among MSM is thus important, not only on a national level, but also in a European and a global context. Hopefully, the Danish Sex Life Survey will be continued regularly in the future, and behavioural surveys among MSM on a European scale will be undertaken.

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# The Russian influenza in Sweden in 1889-90: an example of Geographic Information System analysis

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Using data from a study of the 1889-90 Russian flu in Sweden, this article describes how the application of Geographic Information System (GIS) may improve analyses and presentation of surveillance data. In 1890, immediately after the outbreak, all Swedish doctors were asked to provide information about the start and the peak of the epidemic, and the total number of cases in their region and to fill in a questionnaire on the number, sex and age of infected persons in the households they visited. General answers on the epidemic were received from 398 physicians and data on individual patients were available for more than 32,600 persons. These historic data were reanalysed with the use of GIS, in map documents and in animated video sequences, to depict the onset, the intensity and the spread of the disease over time. A stack diagram with the observations grouped into one week intervals was produced to depict the spread in one figure only. To better understand how the influenza was disseminated, Thiessen polygons were created around 70 places reported on by the doctors. Having prepared GIS layers of the population (divided into parishes), estimations could be made for all the Swedish parishes on the number of infected persons for each of the 15 weeks studied. The described models may be useful in current epidemiological investigations, as well.

## Introduction

The aim of this paper is to demonstrate how Geographic Information System (GIS) improves prospective surveillance and our knowledge on the diffusion of influenza epidemics. As an example we describe the application of this method to analyse historic data on the Russian influenza epidemic of 1889.

The cause of influenza was disputed in the 19th century. One old theory dating from the days of Hippocrates (460-377 BC), saying that diseases are disseminated from miasma (bad or polluted air), had many advocates. Miasma was believed to come from decomposition in the ground, and attack weak individuals and occasionally cause disease outbreaks [1]. In "The History of Epidemics in Britain (1891-1894)", the well known British epidemiologist, Charles Creighton, tried to prove the miasmatic theory [2]. When his works were published, England and the rest of the world had just suffered from an influenza pandemic more severe than its predecessors. It was named Asiatic or Russian influenza, as the first reports of incidences came from a small village in the Asian part of Russia. It was detected in May 1889, reached St Petersburg in October and spread all over the world within a year.

On 4 February 1890, the Swedish Society for Medical Doctors assembled to discuss how the Russian influenza, at that time peaking in Sweden, was disseminated. The Society decided to undertake an epidemiological study to find the answer. A survey in the form of postal cards and a questionnaire were sent to all Swedish doctors. The results were collected by Dr Klas Linroth (1848-1926), Sanitary Inspector in Stockholm, in cooperation with Dr Curt Wallis and Dr Fredrik Warfvinge. Linroth analysed the results and concluded that the disease was spread along the communication network from person to person and not by miasma [3]. Linroth's main conclusions concerning the disease are presented in Table 1, to provide a general background for the geographic analyses made in this study.

Using Linroth's data this work intends to show how modern GIS technology can be used to add a geographic dimension to epidemiological analysis in order to facilitate the evaluation of hypotheses, conclusions and decisions. We looked into the geographical aspects of Linroth's carefully conducted epidemiological surveillance and explored the GIS methods in combining epidemiological data with geographic and census data to extract new information. The use of standard formats

**Table 1**

**Main findings of the epidemiological study conducted by K. Linroth during the Russian influenza epidemic in Sweden in 1889-90**

| Duration | 3 months (end November 1889 - end February 1890) |
|---|---|
| Incubation time | 1-3 days |
| Duration of the disease | 2.3 – 9.4 days |
| Proportion of the population affected (men; women) | 60.7% (60.0%; 61.1%) |
| Proportion of infected by age-groups (in years) | |
| <1 | 36.2% (153/450) |
| 1-10 | 59.8% (4,938/2,956) |
| 11-20 | 65.3% (3,379/5,170) |
| 21-40 | 61.5% 6,162/10,014) |
| 41-60 | 62% (2,896/4,666) |
| >60 | 47.2% (837/1,770) |
| Excess mortality, Stockholm | 0.13% (300/235,000 inhabitants, Stockholm) |

and techniques ensures that the results obtained can easily be shared between the authorities and communicated to the public. To understand the nature of a pandemic it is essential to study its progress in both space and time. Modern GIS has all the tools necessary to make this happen.

## Methods and results

### The data sets

The request from the Swedish Society of Medical Doctors to all Swedish doctors contained two forms; the first one was a postcard, with three questions:

1. When was the first influenza case detected in your district?
2. When do you consider that the epidemic in your district reached its peak?
3. How large, according to your opinion, was the percentage infected by the influenza?

The postcards (study 1) were returned by 398 doctors. From the answers a table was compiled and a map was drawn in 1890, indicating when the influenza first appeared at different locations. To support the contagiousness theory an analysis of the railway network was done in relation to the onset of the outbreak. In the first week in December 1889, 12 of the 13 affected places outside Stockholm had railway stations. In another table Linroth demonstrated that by 20 December, 82% of reporting places with a railway station and 47% without one had been affected. Sea ports with daily communications to and from Stockholm were also attacked early. There is, however, an uncertainty concerning these results, as it was never quite clear to the respondents whether it was the first locally infected patient or the first infected individual arriving by train or by boat that should be regarded as the first case in a particular location.

The second form (study 2) was designed to assess the number, age and sex of patients infected in the doctors' districts. The doctors were asked to fill in a questionnaire for each household they visited, providing information on the number, sex and age of all persons in the household and of those that had had influenza. They were also asked to communicate any observation that could add to the understanding of the characteristics and the spread of the influenza. In total 126 forms were returned with information on 32,683 individuals (0.68% of the total population in Sweden at the time) and 42 of the doctors added personal notes. Separately, Linroth received 115 letters with additional information. Linroth used these answers to compile a table giving detailed information on the development of the influenza at 69 locations.

### GIS data and analysis

In the GIS study, both Linroth's tables were converted into Excel format. For unknown reason the first data set is in 5 days intervals, whereas the second (the more comprehensive one) is in one week intervals (Table 2). The tables were checked for inconsistencies and some of the place names were changed to the spelling of today, to enable interactive geocoding (giving geographical coordinates to address information). In ArcGIS (GIS software from ESRI Inc., US) the Excel tables could be spatially joined to a Multinet (geographic data from Tele Atlas NV, The Netherlands) layer, using the place names. New point layers were thus created to link Linroth's data to places with geographical coordinates.

A background map showing land and water and the communication network was obtained. In 1890, the railway network was the main communication system of the country. By courtesy of the Railway Museum in Gävle we received a digital copy of a map of the Swedish railroads of 1890. As the geometrical quality of this

**Table 2**

**Part of K. Linroth's table with data on Russian influenza epidemic in 69 Swedish localities (study 2), converted into Excel format using ArcGIS**

| Object ID | Name | Before 1889/12/01 | Week 89/49 | Week 89/50 | Week 89/51 | Week 89/52 | Week 90/01 | Week 90/02 | Week 90/03 | Week 90/04 | Week 90/05 | Week 90/06 | Week 90/07 | Week 90/08 | Week 90/09 | After 1990/03/01 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 6 | Karlskrona | | | 4 | 10 | 21 | 31 | 30 | 21 | 7 | 14 | 3 | | | | |
| 7 | Eksjö | 1 | | 1 | 5 | 13 | 27 | 25 | 7 | 1 | 3 | 2 | 2 | 3 | 2 | |
| 8 | Jönköping | | | 1 | 1 | 7 | 4 | 4 | | 1 | | | | | | |
| 9 | Västervik | | | 2 | 2 | 30 | 73 | 67 | 42 | 18 | 6 | 6 | 1 | 2 | 3 | |
| 10 | Värnamo | | | | | 81 | 50 | 12 | 3 | | 3 | 1 | 3 | | 2 | |
| 11 | Söderåkra | | | | 1 | 9 | 13 | 12 | | | | | | | | |
| 12 | Marstrand | 1 | 2 | 5 | 7 | 23 | 36 | 27 | 6 | 2 | | 1 | 4 | | | 4 |
| 13 | Halmstad | | 1 | 8 | 13 | 39 | 61 | 57 | 20 | 12 | 6 | 4 | 2 | 3 | 3 | 1 |
| 14 | Göteborg | | | 12 | 123 | 526 | 3525 | 2226 | 767 | 279 | 138 | 75 | 39 | 28 | | |
| 15 | Skövde | | | | | 20 | 24 | 29 | 3 | | | | | | | |
| 16 | Hjo | | | 7 | 13 | 54 | 133 | 169 | 102 | 5 | 23 | 16 | 11 | 2 | | |
| 17 | Motala | | | | 12 | 44 | 59 | 58 | 2 | 2 | | 2 | | | | |
| 18 | Linköping | | | | 18 | 65 | 95 | 36 | 13 | 2 | 3 | | | 4 | | 1 |
| 19 | Eskilstuna | | | 1 | 23 | 60 | 299 | 171 | 115 | 14 | | | | | | |
| 20 | Bettna | | | 1 | 6 | 13 | 33 | 32 | 3 | 2 | 1 | | | | | |
| 21 | Österlövsta | | | 2 | 25 | 86 | 58 | 14 | 10 | 1 | 5 | 1 | 2 | | | |
| 22 | Västerås | | | 3 | 8 | 18 | 16 | 2 | 2 | 2 | | | | | | |

map is not very good, we decided to use the modern, geometrically satisfactory, railroad data from Tele Atlas instead. From the current (as of 2007) Tele Atlas railroad layer all railroads not existing in 1890 were manually deleted, using the museum map as master.

Using data from study 1 (onset of first cases) as well as the railway network, maps were created to show, in five-day intervals, how the epidemic spread in the country.

Similar maps were created on the basis of study 2 data. For each of the 15 weeks in the spread sheet (Table 2) from Linroth's second study, GIS layers were created, showing the number of infected persons at each location. These layers can be displayed in many different ways. Figure 2 shows places in Sweden where influenza was reported with the weekly incidences indicated by the size of the dots.

The map series in Figure 1 (with data from Linroth's first study) can be compared with the map series in Figure 2. The time intervals are slightly different but comparing the two map series one can still see the same pattern along the railways.

Using the same technique as was demonstrated in a previous study [4], time layers can be organised to create video sequences. This was done for both data sets and video clips can be supplied upon request to the authors.

We also managed to create maps with bar charts showing in one map (Figure 3) the progress of the disease week by week. This gives an understanding, in one single map, of how, where and when the influenza was spread.

Other researchers [5] have chosen to visualise local peaks of influenza in maps where each specific colour represents a specific time period (typically a month). Compared to this technique our method has the benefit of showing peaks that may vary in length of time.

**Modelling of spread, combining population and the epidemiological data**

The available data on the number of infected persons (study 2) refer only to 69 locations and the 32,683 infected individuals. There is no information on the spread of the epidemic to other places. Thus, it is not known when and how many people in total were infected in Sweden.

A geodatabase of the Swedish population per parish and year has been created for another study, not yet published. The population data was gathered by Professor Lennart Andersson-Palm at the University of Gothenburg. By importing the parish boundaries along with population figures from 1890 into this study, the local impact of the influenza was geographically assessed, based on reported data from 69 locations.

Thereafter, to understand how the influenza was disseminated to all other places, the so called Thiessen (or Voronoi) polygons [6] were created around each of the 69 studied locations. All positions within a polygon are closer to the point location, around which the polygon was created, than to any other of the remaining 68 location points (Figure 4).

Furthermore, we assumed that influenza spread in the same way in all parishes.

Using a GIS tool called "Select by Location" [7] we can see, for example, that the Thiessen polygon surrounding Västerås has 90 parishes (the small polygons in the map in Figure 4) with their centroids within the surface of the polygon.

The 90 parishes were then merged into a new polygon. We called this polygon a "Thiessen area". This was repeated for each of the 69 Thiessen polygons, covering all of Sweden. It is important to note that the shorter the distances between the observation points are, the smaller the Thiessen areas get.

We then created an Excel sheet with the numbers of infected persons per Thiessen area (Table 3).

Next, this Excel sheet was spatially joined with the Thiessen area layer. Time layers were then extracted for each week. These layers were colour coded to show the magnitude of the influenza, per Thiessen area, for the week layer displayed (Figure 5). Using the ArcGIS Animation Manager [8], video sequences were created for the 15 weeks the influenza lasted. The video sequences can

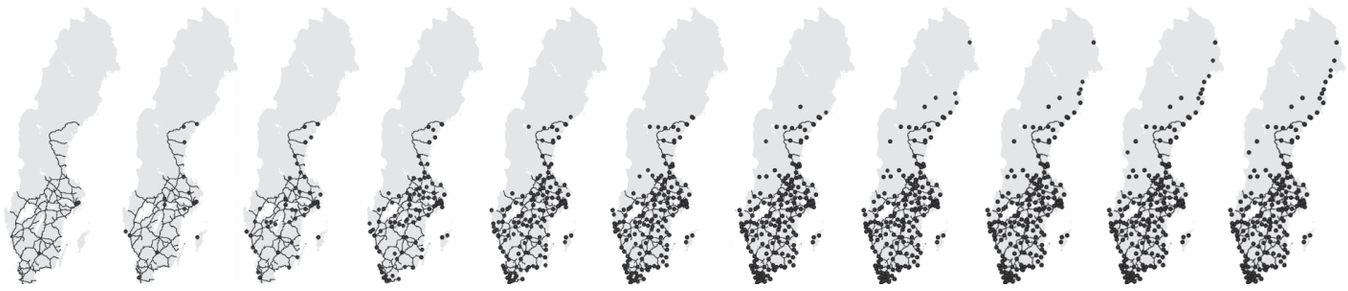
**Table 3**

**Part of a spread sheet containing calculated numbers of infected persons per Thiessen area for each week during the influenza epidemic. Analysis of Russian influenza in Sweden in 1889-90**

| Name | Before 1889/12/01 | Week 89/49 | Week 89/50 | Week 89/51 | Week 89/52 | Week 90/01 | Week 90/02 | Week 90/03 | Week 90/04 | Week 90/05 | Week 90/06 | Week 90/07 | Week 90/08 | Week 90/09 | After 1990/03/01 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Vaxholm | 0 | 2292 | 24062 | 8021 | 1146 | 1146 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Visby | 0 | 2973 | 811 | 4865 | 8649 | 9730 | 2973 | 2162 | 1351 | 811 | 270 | 0 | 0 | 0 | 0 |
| Vrigstad | 0 | 0 | 0 | 5386 | 6155 | 20005 | 10003 | 3847 | 2308 | 0 | 0 | 0 | 0 | 0 | 0 |
| Vålberg | 0 | 0 | 0 | 0 | 0 | 3553 | 7105 | 21316 | 3553 | 0 | 0 | 0 | 0 | 0 | 0 |
| Värnamo | 0 | 0 | 0 | 0 | 21737 | 13418 | 3220 | 805 | 0 | 805 | 268 | 805 | 0 | 537 | 0 |
| Västervik | 0 | 0 | 283 | 283 | 4252 | 10345 | 9495 | 5952 | 2551 | 850 | 850 | 142 | 283 | 425 | 0 |
| Västerås | 0 | 0 | 5051 | 13469 | 30305 | 26937 | 3367 | 3367 | 3367 | 0 | 0 | 0 | 0 | 0 | 0 |
| Åmål | 0 | 272 | 0 | 272 | 1630 | 2445 | 7879 | 8694 | 4075 | 2445 | 1087 | 0 | 0 | 272 | 0 |
| Örebro | 342 | 856 | 1883 | 9242 | 10782 | 10611 | 4792 | 2054 | 1540 | 685 | 856 | 342 | 342 | 342 | 171 |
| Österlövsta | 0 | 0 | 311 | 3890 | 13383 | 9026 | 2179 | 1556 | 156 | 778 | 156 | 311 | 0 | 0 | 0 |
| Överkalix | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 40 | 437 | 800 | 701 | 368 | 209 | 80 | 35 |
| TOTAL | 4908 | 33825 | 123653 | 190456 | 435529 | 718155 | 575811 | 348406 | 185867 | 111512 | 70479 | 44338 | 14825 | 5943 | 7786 |

**Figure 1**

**Onset of Russian influenza epidemic in Swedish localities (data derived from study 1). Analysis of Russian influenza in Sweden in 1889-90.**



Dots represent places where first infected cases had been reported to date, maps correspond to five-day intervals, starting from the last week of November 1889 (left) until 21 January 1890 (right). The railroad network is shown.

**Figure 2**

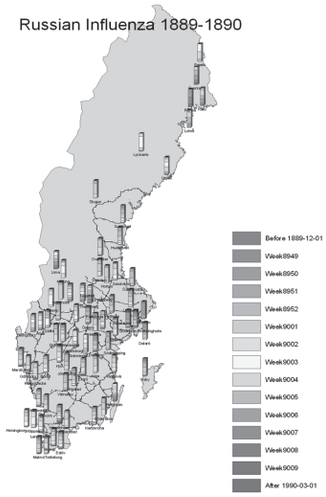
**The numbers of infected patients reported by the local doctors within one-week intervals (study 2). Analysis of Russian influenza in Sweden in 1889-90.**


The dots indicate the number of cases; each map represents one week, starting from the last week of 1889 (upper left) and ending with the week of 1 March 1890 (lower right). The railroad network is shown.

For higher resolution colour maps, see: http://www.eurosurveillance.org/public/public_pdf/GIS_colour.pdf

**Figure 3**

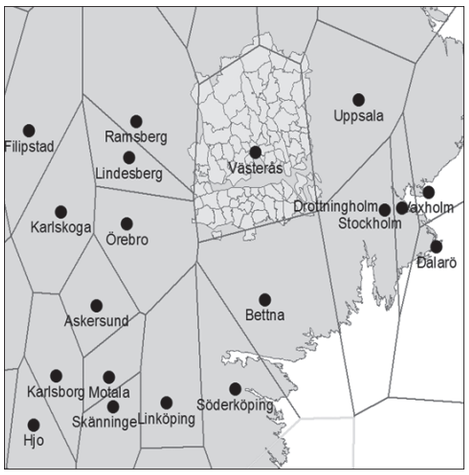
**A map with bar charts showing the intensity of the pandemic, week by week. Analysis of Russian influenza in Sweden in 1889-90.**




One colour (shade) represents one week. The heights of the respective coloured segments represent the relative intensity at each time period and location. The largest segments represent the local peaks in intensity. To interpret this map correctly it needs to be magnified at least 100%.

**Figure 4**

**Thiessen polygons surrounding each of the observation points where cases had been reported by local doctors. Analysis of Russian influenza in Sweden in 1889-90.**




The small polygons represent parishes.

**Figure 5**

**The estimated cumulative total numbers of infected persons in Thiessen areas indicated by colour-coding. Analysis of Russian influenza in Sweden in 1889-90.**


Darker colours mean more infected persons, varying from 0 to 50,000. The time difference between maps is one week, starting with the week of 1 December 1890 (upper left) and ending with the week of 1 March 1990 (lower right). The railroad network is shown. With fewer observation points in the north, the Thiessen areas there are accordingly larger in comparison with the southern parts of Sweden.

be displayed in ArcMap or saved as video clips that may easily be distributed.

Using the above material it is possible to estimate how many infected persons there were for each of the 2,390 Swedish parishes (according to the administrative division of the country at that time) during the whole period of the Russian influenza epidemic in Sweden.

For example, the 90 parishes surrounding Västerås had a total population of 143,105 persons in 1890. Taking into account Linroth's figures on the extent of the epidemic, the incubation time, dissemination speed and mortality, we conclude that 60% of the population of these parishes (85,683 persons) should have been infected by the Russian influenza and the number of deaths was approximately 260 (0.3% mortality).The epidemic was present in this area between week 50 of 1889 and week 4 of 1890, with a peak in the last week of 1889

## Discussion

One of the basic reasons for K. Linroth and his colleagues to start their study was to determine whether the influenza was a miasmatic or a contagious disease. Many of the respondents commented on that issue. Linroth claimed that the disease basically followed the transportation network (the railways and, in some cases, the seaways) in "a way typical for contagious diseases" (Linroth's words) but also for those diseases that were called contagious-miasmatic. Linroth does not define the meaning of contagious-miasmatic. He could be referring to Peter Ludwig Panum who in 1847 discussed the possible miasmatic-contagious character of measles [9].

The dissemination was very fast and the local epidemics developed at a pace that in some cases were described as explosive. Due to the general susceptibility, the short incubation time and the difficulty to detect the very first cases, more proof were needed to scientifically verify that the influenza was indeed contagious. Linroth was however of the opinion that the many individual testimonies describing how the infection was transferred directly from infected persons justified the hypothesis: Influenza is a contagious disease.

The effects and the spread of the Russian flu were studied in many countries [10] and it can be regarded as a watershed in the history of influenza. It was the first influenza epidemic after the breakthrough of bacteriology. The miasmatic theory was thereafter not much heard of for many years. Modern studies on bioaerosols, however, to some extent support the theory of contagious-miasmatic disease dissemination. A new influenza virus was found in bioaerosols in 1968 and large quantities of influenza virus are suggested to be spread over the world as a bioaerosol, and El Nino is said to have influenced the quick spread of bioaerosols in the atmosphere [11]. We believe that climate changes may influence the spread of influenza over the world, though this remains to be proven. Had GIS existed in the times of Linroth, addition of weather information to the study could have helped in verifying the theory of influenza being a contagious disease. Other modern explanations of the spread of influenza [12] claim that pathogens may be seeded in humans over a longer period of time and that host susceptibility varies in cycles.

Unfortunately it has not been possible for us to get access to the original data and answers from which Linroth compiled his tables. Also, there are obvious weaknesses in the Linroth study itself. There were no clear definitions of the cases. The doctors answered voluntarily and in some cases off the top of their heads. There was an obvious lack of control system and data provided by the doctors was not verified. Still, the fact that they made so many home visits gave them a very good insight into the effects of the influenza.

The various applications of our GIS model illustrate visually what had previously been difficult or impossible to demonstrate. Concerning the spread model, it can of course be disputed whether the same temporal pattern can really be applied to all of the parishes with their centroids within a certain Thiessen polygon. Linroth collected evidence that locally the disease first appeared close to the place containing the railway station. The population living at distant houses and farms were infected at a later stage. It is our opinion though, that ours is the best assumption you can make based on the existing data, as there is no reliable information on where the influenza started in each region (polygon). Thiessen polygons are frequently used in several GIS applications. One example is the empirical modelling of government health service use by children with fevers in Kenya [13]. Another technique for generalisation is Kriging as described in T. Sakai et al. [5].

Additional advantage of the method presented is that GIS analyses can be made on site. This study has been performed using desktop GIS. There are many ways to make the established geodatabase public. Using a GIS web server, maps and data can easily be accessed and interrogated from web readers over Internet or an Intranet. Interactive questions can then be put to the geodatabase via the map interface by anyone (authorised) having access to a web reader.

In our time, influenza data from sentinel doctors are continuously collected at the Swedish Institute for Infectious Disease Control [14]. No more than 500-1000 patients are reported yearly. So far this has been regarded as too little for GIS work on monitoring and prediction. This year however, an evaluation of the usefulness of data collected for GIS analyses will be performed. In an ongoing study, 3,500 persons from all over Stockholm (positions are defined with zip code) continuously submit self-reports on influenza-like symptoms using a web application or over the phone via an interactive voice response [15]. The number of reporting individuals will be extended in 2009. We believe that GIS applications as described in this article will be extremely useful not only for visualisation of spread, but also for prediction and for identification of still unknown factors that may contribute to influenza epidemics.

## Conclusion

Combining epidemiological data with additional available geographically oriented data clearly shows the power of GIS in epidemiological research, also in a historic perspective. In the study presented in this paper it made it possible to display the spatial patterns in time in tables, in video sequences and in single map documents showing temporal variations. Using the interactive map as an interface to the epidemiological data we were able to extend the analysis to a level where everyone involved in disease prevention and crisis management may have easy access to vital information, presented in an intuitive way. This can also be applied in case of information to the public. The techniques we used in this study can of course be used in other studies in epidemiology or related disciplines. How to implement these methods in crisis management should be further studied.

Note: Lars Skog works for ESRI S-Group Sverige AB, provider of GIS and mapping software.

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# Increased number of *Clostridium difficile* infections and prevalence of *Clostridium difficile* PCR ribotype 001 in southern Germany

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In recent years, *Clostridium difficile* infection (CDI) has emerged as an increasing problem, both in in- and outpatients. In a rural region of southern Germany, the annual number of *C. difficile* toxin (Tcd)-positive patients has increased from 95 to 796 in the period from 2000 to 2007. Simultaneously, the proportion of positive tests among all Tcd examinations has risen from 7.0% to 12.8%, indicating that the higher number of affected patients was not solely due to an increase in the number of assays. Elevated numbers of CDI have recently been associated with outbreaks of the ribotype 027 strain, particularly in North America. This strain has also been isolated in Europe, including in Germany. Ribotyping and PCR testing for binary toxin genes of *C. difficile* strains isolated from in- and outpatients demonstrate a predominance (59%) of *C. difficile* ribotype 001, which exhibits antibiotic resistance to erythromycin, ciprofloxacin, and moxifloxacin, but lacks binary toxin genes. In summary, in our region of Germany, the number of patients affected by CDI has increased, probably due to spread of *C. difficile* ribotype 001.

## Introduction

Numbers of *Clostridium difficile* infections (CDI) are increasing in- and outside of Europe [1-5]. CDI in North America and partly also in western Europe have often been attributed to outbreaks caused by the hypervirulent strain NAP1/027 containing the binary toxin genes cdtA and cdtB [1,3,6]. Recently, this strain has also been isolated from patients in western Germany [7]. Different *C. difficile* strains are isolated in different European countries, suggesting a prevalence of particular strains in local settings [8-10].

CDI is usually regarded as a nosocomial infection that can be minimised by robust infection control practices and good antibiotic stewardship. In some hospitals in Europe it has become the most frequent nosocomial disease and consequently, analyses of *C. difficile* epidemiology were restricted to hospital outbreaks [11]. However, community-acquired cases of CDI have been observed for a few years now [12,13]. Interestingly, *C. difficile* strains associated with CDI in hospitalised patients were different from the ones isolated from community cases [13].

Our laboratory is located in a rural area in southern Germany. In this region, CDI is noticed as a growing nosocomial problem with sporadic fatal cases. However, the available information about the real extent of this apparent increase in CDI is limited. Furthermore, no studies have been done on distinct *C. difficile* strains in Germany or defined regions in Germany. We therefore collected data on the number of patients known to excrete *C. difficile* toxin (Tcd) in stool and on the number of patients analysed for Tcd. PCR was performed on *C. difficile* isolates from outpatients and from patients treated in two hospitals located in southern Germany, in order to gain knowledge about the epidemiological background of these regional strains.

Here we present data about the prevalence of a quinolone- and erythromycin-resistant *C. difficile* ribotype 001 strain in southern Germany.

## Patients and methods

### Laboratory and hospitals setting

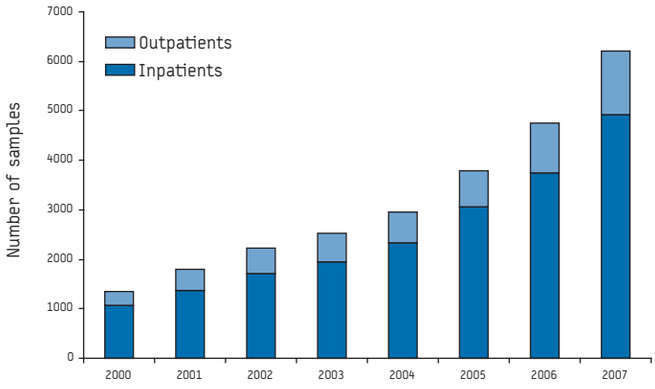
The Synlab Medical Care Service analyses laboratory samples from about 40 hospitals and more than 2,000 physicians serving outpatients. In 2006 a total of 161,000 microbiological samples were examined. *C. difficile* was isolated from Tcd-positive stool samples from patients diagnosed at two hospitals (A and B) and from outpatients. Hospital A is a primary health care hospital with 270 beds comprising two tertiary university hospital facilities (cardiology, gastroenterology). In 2006, 10,793 patients were admitted to that hospital (74,146 patient days). Hospital B is a primary health care hospital with 135 beds, and 4,886 patients (34,811 patient days) were admitted to that clinic in 2006.

### Epidemiolologic analysis of *C. difficile* in South Germany

Numbers of Tcd-positve stool samples and numbers of Tcd-positive patients were evaluated by the Hybase system (Cymed AG, Bochum, Germany) linked to the laboratory data system "promed open" (mcs, Eltville, Germany). Hybase (http://www.cymed.de/download_hy.php) is a computer programme that

**Figure 1**

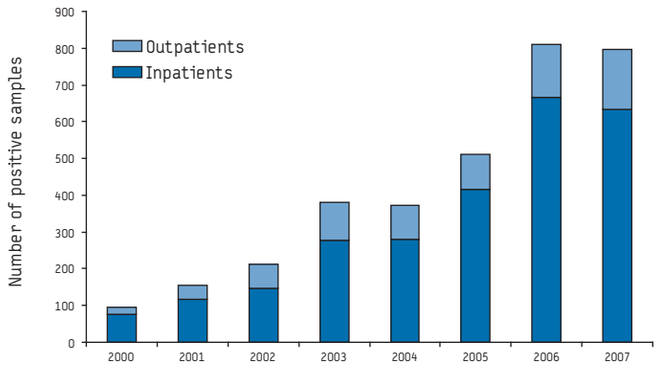
**Number of patients examined by Tcd ELISA, southern Germany, 2000–2007**




Tcd: *C. difficile* toxin; ELISA: enzyme-linked immuno-sorbent assay.

**Figure 2**

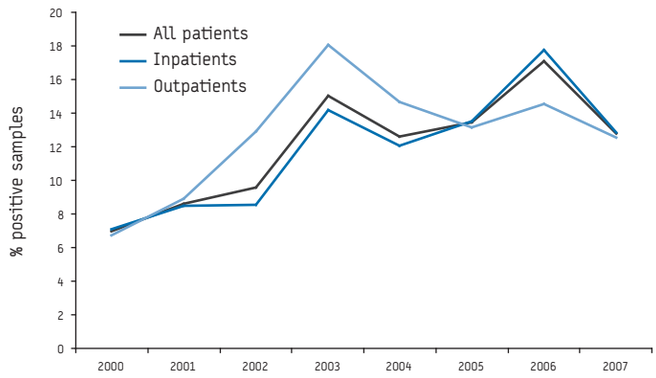
**Number of Tcd-positive patients, southern Germany, 2000–2007**




Tcd: *C. difficile* toxin.

**Figure 3**

**Percentage of Tcd-positive patients, southern Germany, 2000–2007**




Tcd: *C. difficile* toxin.

supports the surveillance of bacterial pathogens, e.g. calculation and documentation of the number of notifiable bacterial species.

Data (age, sex, outpatients versus inpatients, taking into account where they were treated) from patients with Tcd-positive stool in 2006 were documented.

### *C. difficile* toxin analysis, culture and antibiotic susceptibility testing

Stool samples from inpatients of hospitals A and B were collected between May 2006 and March 2007 and tested for *C. difficile*. Samples from outpatients were collected between March and April 2007.

Tcd was examined by using an enzyme-linked immunosorbent assay (ELISA) detecting toxin A and B (R-Biopharm AG, Darmstadt, Germany). Bacterial cultures were grown on *C. difficile*-selective agar containing cefoxitin and cycloserin (Heipha, Eppelheim, Germany; www.heipha.de/db/files/209e.pdf) under anaerobic conditions.

Identification of *C. difficile* was performed on rapid ID 32 A system (identification system for anaerobes, Biomerieux, Nürtingen, Germany). Antibiotic susceptibility was tested using ATB ANA strips (susceptibility test for strict anaerobic bacteria, Biomerieux, Nürtingen, Germany) according to the manufacturer´s instructions or alternatively in an E-test procedure (for erythromycin, ciprofloxacine, moxifloxacine, cefotaxime; AB-Biodisk, Solna, Sweden). E-test results were confirmed at the German consiliary laboratories for *C. difficile* (Mainz) or gastrointestinal infections (Freiburg). Presence of binary toxin genes was examined at the German consiliary laboratory for *C. difficile* (Mainz) according to Stubbs et al. [14].

### Ribotyping of *C. difficile* strains

Ribotyping was performed at the German consiliary laboratory for gastrointestinal infections (Freiburg). PCR ribotyping was performed according to he protocol of Bidet et al. [15] resulting in so-called "ribotype Freiburg". In previous comparative analyses, representative isolates of each ribotype Freiburg had been sent to the Anaerobe Reference Laboratory in Cardiff for re-typing according to the "Cardiff" PCR ribotyping library in order to establish the correlation between ribotype Freiburg and the commonly used ribotype nomenclature of Stubbs et al. [16]. It was therefore possible to relate local PCR results not only to "ribotype Freiburg" but also to European C. difficle ribotypes.

## Results

Over the past years, reported numbers of patients affected by *C. difficile* infection (CDI) have increased markedly in Germany [4]. Figures 1-3 show a comparison of the number of stool samples tested for *C. difficile* toxin (Tcd) with the number of Tcd-positive stool samples in the period between 2000 and 2007.

The number of patients analysed for Tcd increased by 458% (from 1,358 to 6,214; Figure 1), but the actual number of Tcd-positive samples increased by 838% in the same period of time (from 95 to 796; Figure 2). The percentage of Tcd-positive patients increased from 7.0% in 2000 to 12.8% in 2007, with two peaks in 2003 (15.0%) and in 2006 (17.1%; Figure 3). As demonstrated by Figure 3, the peak in 2003 predominantly resulted from a high proportion of Tcd-positive outpatients (18.0%). In contrast, the peak in 2006 was caused by Tcd-positive inpatients (17.8%).

In summary, these data indicate that the increasing numbers of CDI in this region are real and not simply a result of increasing analysis efforts. Furthermore, not only hospitalised patients but also non-hospitalised patients were affected by CDI.

Previous reports have identified high age as an important risk factor for contracting CDI [4,5]. A representative list concentrating on the age and sex distribution of patients who had Tcd-positive stools in 2006 is shown in Table 1.

A total of 784 patients were registered in our database, 17.3% of which were outpatients. Looking at the median age, the majority were elderly patients. Interestingly, the median age of outpatients (69 years) was lower than that of inpatients (77 years). In addition, Tcd-positive women tended to be older than Tcd-positive men.

To assess the cause for the increasing numbers of Tcd-positive patients via spread of hypervirulent *C. difficile* 027, ribotyping of *C. difficile* was performed on isolates from Tcd-positive stool samples previously collected from outpatients and from patients treated in two different hospitals in southern Germany (Hospitals A and B).

As shown in Table 2, at least seven different *C. difficile* ribotypes could be identified. While *C. difficile* ribotype 001 was isolated from 11 patients, other types were only isolated once from a single patient. *C. difficile* ribotype 001 was isolated from inpatients of both hospitals and was also common in outpatients indicating a predominance of this strain in this region.

Ribotype 001 *C. difficile* lacked the binary toxin genes but was resistant to quinolone antibiotics (ciprofloxacine, moxifloxacine) as well as to erythromycin, cefotaxime (MIC >16 µg/mL) and clindamycin. However, ribotype 001 strains were susceptible to ampicillin/sulbactam, piperacillin, piperacillin/tazobactam, imipenem, vancomycin and metronidazole.

## Discussion

Worldwide – as well as in Germany – there is a discussion about increasing case numbers of CDI-affected patients [1-5]. In this study we demonstrate that the number of Tcd-positive patients increased markedly in southern Germany in the period between 2000 and 2006. It was assumed that this might be a result of intensified examination efforts, as from 2000 to 2007, the total number of stool samples examined for Tcd per year increased, too. However, the percentage of Tcd-positive patients also increased markedly during this period (from 7.0 to 12.8%) showing a maximum in 2006 (17.1%). This higher ratio indicates that the increased number of Tcd-positive patients is a real phenomenon and not solely due to the fact that examination efforts were stepped up.

Between 2006 and 2007, the number of CDI-affected patients remained constant, although the number of patients examined for CDI increased. This finding suggests that the intensified infection control measures may have been successful in preventing the nosocomial spread of *C. difficile*. However, the possibility to separate between nosocomial and community acquired CDI is limited by the lack of patient data.

In agreement with earlier studies [4,5], Tcd-positive stool samples were mainly obtained from elderly patients. The fact that 136 of 784 Tcd-positive patients (17.3%) in 2006 were outpatients clearly shows that CDI was not restricted to hospitalised patients. On the other hand, the median age of Tcd-positive inpatients was higher than that of Tcd-positive outpatients, an indication that CDI in younger people has a milder course and does not require hospital admission.

**Table 1**

**Number and characteristics of in- and outpatients with Tcd-positive stool samples, southern Germany, 2006**

| | Outpatients | Inpatients (all hospitals) | Hospital A | Hospital B |
|---|---|---|---|---|
| Number of patients | 136 | 648 | 45 | 34 |
| CDI per 1,000 admissions | | | 4.2 | 6.1 |
| CDI per 10,000 patient days | | | 7.0 | 9.0 |
| Proportion of positive Tcd analyses (%) | 14.6 | 17.8 | 16.4 | 13.9 |
| Age distribution | | | | |
| Age of patients, median (mean) | 69.0 (62.3) | 77 (73.1) | 75 (72.6) | 80.5 (76.6) |
| Number of patients <6 years | 6 | 4 | 0 | 1 |
| Number of patients <21 years | 8 | 14 | 1 | 0 |
| Number of patients 21-79 years | 79 | 325 | 25 | 15 |
| Number of patients >79 years | 35 | 258 | 18 | 11 |
| Number of patients >89 years | 8 | 47 | 1 | 7 |
| Sex distribution | | | | |
| Number of female patients | 77 (56.6 %) | 373 (57.6 %) | 27 (60 %) | 19 (55,9 %) |
| Age females, median (mean) | 68 (62.3) | 79.0 (76.1) | 79 (75,48) | 81 (82.0) |
| Number of male patients | 59 (43.4 %) | 275 (42.4 %) | 18 (40.0 %) | 15 (44.1 %) |
| Age males, median (mean) | 69,0 (63.3) | 73.0 (69.2) | 71 (68.39) | 75 (69.8) |

Tcd: *C. difficile* toxin

The *C. difficile* 027 strain was detected in Germany for the first time in 2007 [7]. However, the ribotyping results presented here reveal that this strain was not prevalent in northern Bavaria. In contrast, multi-resistant *C. difficile* 001 were frequently found. For this analysis, *C. difficile* were cultured from Tcd-positive stool samples from in- and outpatients. The hospitalised patients had been treated at two hospitals located about 200 km apart. Since *C. difficile* type 001 was also isolated from outpatients, it is obvious that this strain is predominant in southern Germany.

All tested ribotype 001 *C. difficile* proved to be resistant to erythromycin and moxifloxacine in the antibiotic susceptibility testing, a feature commonly observed for ribotypes 001, 027 and 106 [6,17]. Ribotyping and binary toxin gene analysis showed that all of these *C. difficile* strains were different from the NAP1/027 strain. Recently, it has been discussed whether ribotype 027 strains could be more virulent than other ribotypes [11,18]. Only scarce clinical information - reported anecdotally - is available about the death of several patients. Nevertheless, it is clear that severe courses of CDI in our region are not limited to ribotype 027 isolates.

Ribotyping further revealed that more than 50% of *C. difficile* isolates exhibited identical features, a possible indication of clonal spread within the local population. In the case of increased CDI case numbers due to admission of affected patients bearing predominantly ribotype 001, proven clonality of *C. difficile* isolates by ribotyping might erroneously suggest nosocomial spread. Under the given circumstances of many *C. difficile* isolates being clonally related, this typing method therefore provides only limited information for outbreak analyses in a defined hospital. Consequently, use of more discriminatory typing methods, e.g. multi-locus variable-number tandem repeat analysis (MLVA), may be better suited for future epidemiological studies, at least if ribotype 001 or other frequently occuring ribotypes are involved [19].

In summary, the present study shows an increase of Tcd-positive patient numbers in southern Germany. Multi-resistant *C. difficile* ribotype 001 is prevalent in southern Germany, and this strain is thought to be responsible for severe, if not fatal, cases of CDI. In due course, more discriminatory methods may be able to improve our understanding of the epidemiology of this successful strain.


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**Table 2**

**Characterisation of *C. difficile* isolates obtained from Tcd-positive stool samples, collected between May 2006 and March 2007 in southern Germany**

| Source, Age, Sex | Typing | | Binary toxin genes | | MIC (µg/ml) | | |
|---|---|---|---|---|---|---|---|
| | Ribotype Cardiff | Ribotype Freiburg | *cdtA* | *cdtB* | Ery | Moxi | Cipro |
| A, 87, m | 001 | 45 | - | - | >256 | >32 | >32 |
| B, 73, f | 001 | 45 | - | - | >256 | >32 | >32 |
| A, 83, m | 001 | 45 | - | - | >256 | >32 | >32 |
| B, 78, f | 001 | 45 | - | - | >256 | >32 | >32 |
| B, 81, f | 001 | 45 | - | - | >256 | >32 | >32 |
| A, 75, m | n.d. | n.d. | + | + | n.d. | n.d. | n.d. |
| A, 66, m | 078 | 40 | + | + | 0.75 | 2 | >32 |
| A, 73, m | 049 | 22 | - | - | 1.0 | 1 | >32 |
| A, 67, f | 014 | 1 | - | - | 0.5 | 1,5 | >32 |
| B, 14, f | 015 | 8 | - | - | 0.75 | 1 | >32 |
| B, 75, f | 001 | 45 | - | - | >256 | >32 | >32 |
| B, 88, f | n.d. | n.d. | - | - | >256 | >32 | >32 |
| A, 83, f | n.d. | n.d. | - | - | 0.75 | 1,5 | >32 |
| A, 89, f | 001 | 45 | - | - | >256 | >32 | >32 |
| Out, 63, m | 042 | 21 | - | - | 0.5 | 1,5 | >32 |
| Out, 89, f | 001 | 45 | - | - | >256 | >32 | >32 |
| Out, 64, m | 001 | 45 | - | - | >256 | >32 | >32 |
| Out, 56, f | 081 | 16 | - | - | 0.5 | 1 | >32 |
| Out, 31, f | n.d. | n.d. | - | - | >256 | 1 | >32 |
| Out, 77, f | 001 | 45 | - | - | >256 | >32 | >32 |
| Out, 82, m | 001 | 45 | n.d. | n.d. | >256 | >32 | >32 |
| U | 001 | 45 | n.d. | n.d. | >256 | >32 | >32 |

Patients had been treated either at hospital A or B or had been outpatients (Out). Minimal inhibitory concentrations (MIC) of erythromycin (Ery), ciprofloxacine (Cipro) and moxifloxacine (Moxi) were determined by E-test. Only two isolates exhibited binary toxin genes (*cdtA*, *cdtB*). One strain obtained from a university hospital in south-western Germany (U) was also included.
n.d. = not determined; Tcd: *C. difficile* toxin.
Ribotype Cardiff represents the European ribotype.

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# Prioritisation of infectious diseases in public health - call for comments

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In order to allocate rationally resources for research and surveillance of infectious diseases at the level of the German public health institute (RKI), we prioritised pathogens by public health criteria. After screening the relevant literature we developed a standardised methodology including a three-tiered scoring system for selected pathogens. The pathogens were rated in four categories containing a total of 12 criteria: burden of disease including incidence, severity, mortality; epidemiologic dynamic including outbreak potential, trend, emerging potential; information need including evidence on risk factors/groups, validity of epidemiologic information, evidence for pathogenesis; international duties and public attention; health gain opportunity including preventability, treatability. For each criterion a numerical score of +1, 0 or -1 was given and each criterion received a weight by which the numerical score of each criterion was to be multiplied. The total weighted scores ranged from +22.7 (influenza) to - 64.4 (cholera) with the median being -22.9 (rubella). Relevant changes were observed between weighted and unweighted scores. The chosen approach proved to be feasible and the result plausible. However, in order to further improve the methodology we invite experts to give feedback on the methodology via a structured web-based questionnaire at www.rki.de/EN > Prevention of infection > Infectious Disease Surveillance > Pathogen prioritization. Results of this survey will be included in a modification of the methodology.

## Background

One of the challenges of public health is that infectious disease control covers a wide range of pathogens requiring diverse methods for prevention and control. Furthermore, infectious diseases vary greatly in occurrence, severity and other factors that make it difficult to compare the public health importance of the underlying pathogens. Resources for research, surveillance and other public health activities are limited; it is therefore of major importance to allocate rationally these resources by using public health criteria. The agendas of institutions in the field of public health and infectious diseases, however, are fragmented and experts are increasingly specialised, making it difficult to find institutions or individuals who would be able to prioritise a broad range of infectious diseases without being biased by individual professional focus on one hand or lack of specific pathogen-related knowledge on the other.

In the past decade a number of efforts have been made to prioritise systematically infectious diseases by public health criteria resulting in different outcomes depending on the objectives and methodology used [1-5]. But even prioritisation schemes with similar objectives have applied different sets of criteria as illustrated in Table 1.

In 2004 the department for infectious disease epidemiology of the Robert Koch Institute (RKI), the national public health institute in the portfolio of the German federal ministry of health, initiated a prioritisation exercise to guide the research and surveillance strategies of the department [6]. Initial findings were presented at three international scientific conferences in 2006 and 2007 [7-9].

After this a publication in a nationwide non-scientific journal [10] elicited considerable and unexpected interest from the general public and the scientific community. Therefore, as part of updating and improving the current prioritisation methodology, we would like to present this methodology also to the broader international public health community outside the RKI and Germany to collect suggestions for improvement. In the following we describe and evaluate the methodology of the prioritisation previously conducted by the RKI to provide the background information necessary for comment on our approach. We cordially invite comments on the proposed methodology via a web-based questionnaire accessible at http://www.rki.de/EN > Prevention of infection > Infectious Disease Surveillance > Pathogen prioritization.

## Methodology

While preparing our exercise we analysed prioritisation efforts over the past decade by searching the literature in Medline using the search terms prioritisation OR priority AND (surveillance OR infectious diseases OR public health) and based on presentations from the EAN workshop on "New Tools for early Warning" that took place in Lyon on 6 and 7 February 2004, [1-5,18,19]. A flow chart of our methodology is presented in Figure 1.

A list of pathogens was compiled based on one or more of the following criteria: notifiable according to German law [11], reportable within the European Union according to European regulations [12], listed as chapters in selected established manuals and textbooks on infectious diseases [13-15], causative agent in outbreaks reported to RKI in the past 10 years, agent with potential for deliberate release [16]. In the following we list the pathogens but also refer to the related diseases in humans.

Every pathogen was rated according to the 12 criteria listed in Table 2. For each criterion a numerical score of +1, 0 or -1 was given as defined in Table 2. The score of +1 represented high and a score of -1 low importance with respect to a criterion. A score

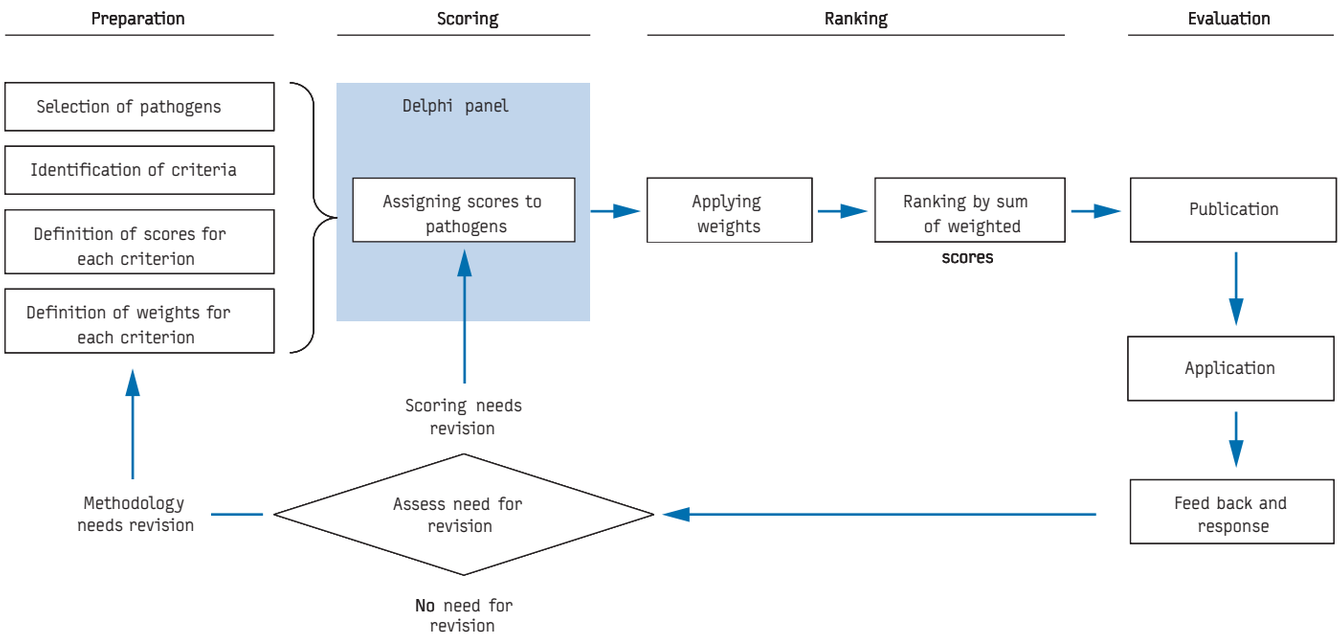
**TABLE 1**

**Comparison of the evaluation criteria of different schemes for prioritisation of infectious diseases (the prioritisation by Reseau National de Santé Publique, 1995, France, is not included as it contained categorisation principles rather than criteria) between 1995 and 2008**

| Reference | Rushdy & O'Mahony 1998 (3) | Weinberg *et al* 1999 (20) | Doherty 2000 (1) | Horby *et al* 2001 (2) | Institute de Veille Sanitaire (InVS) 2001 (5) | World Health Organisation 2003 (4) | Krause *et al.* 2008 (6) |
|---|---|---|---|---|---|---|---|
| Country | United Kingdom | European Union | Canada | United Kingdom | France | South East Europe | Germany |
| Group of criteria | Specific name of criteria (as used in respective publications) | | | | | | |
| International aspects and public concern | - public concern<br>- public health laboratory service (PHLS)-added value | - international surveillance programmes | - international consideration<br>- risk perception<br>- potential to drive public health policy<br>- other sector interest | -public concern | - not applied - | - not applied - | - international duties and public attention |
| Occurrence | - not applied - | - not applied - | - incidence | - not applied - | - epidemiology | - not applied - | - incidence |
| Epidemiologic dynamic | - potential threat | - not applied - | - potential spread<br>- changing patterns | - potential threat | - not applied - | -potential threat<br>-long term effects on communicable diseases | - outbreak potential<br>- trend<br>- emerging potential |
| Burden of disease | - burden of ill health | - not applied - | - severity | - burden of ill health | - not applied - | -disease impact<br>-present burden of ill health | - severity<br>- mortality |
| Health gain opportunity | - health gain opportunity | - not applied - | - preventability | - health gain opportunity | - prevention and control measures | -low incidence only maintained by public health activities<br>- health gain opportunity<br>- necessity for immediate public health response | - preventability<br>- treatability |
| Socioeconomic aspects | - social/ economic impact | - collective economic impact | - socioeconomic burden | - social/ economic impact | - not applied - | -social/economic impact | - not applied - |
| Information need | - not applied - | - not applied - | - not applied - | - not applied - | - not applied - | - not applied - | - evidence for risk factors/ groups<br>- validity of epidemiologic information<br>- evidence for pathogenesis |
| Other | - not applied - | - not applied - | - not applied - | - not applied - | - veterinary public health | - not applied - | - not applied - |

**FIGURE 1**

**Work flow for prioritisation, Robert Koch Institute, 2008**



of 0 referred to pathogens with average importance or pathogens, for which lack of knowledge or opinion of the participants in the working group did not allow a decision for one of the other two scores.

Each criterion received a weight by which the numerical score of each criterion was to be multiplied. Hence for each pathogen a sum of the unweighted and a sum of the weighted scores was generated. The weight of each criterion was determined before and independently of the categorisation for each pathogen: all participants were asked to put the 12 criteria in a sequential order with 12 being the most important and one being the least important criterion. An average was computed for each criterion, defining its weight. The total weighted score was defined as the sum of the weighted scores of all 12 categories per pathogen. These were finally normalised to the spectrum of the unweighted total scores to allow comparisons. We demonstrate the effect of weighting by presenting detailed data on the highest, lowest and median ranking pathogen as well as for the two pathogens with adjacent ranks to the median rank.

## Results

The overview of prioritisation exercises in Table 3 shows that objectives, methodological approaches and especially the level of standardisation differed considerably in these efforts. Partly due to different objectives of the prioritisation, also the number and type of criteria varied. Categories used by most groups are incidence, burden of disease and opportunity for health gain [1-5], which are included in our exercise.

The working group on prioritisation consisted of eleven senior epidemiologists and infectious disease specialists at the department for infectious disease epidemiology at RKI. They categorised a list of 85 pathogens shown in Table 4.

The distribution of the normalised ranks is presented in Figure 2 and detailed scores for selected diseases are shown in Table 5. The total weighted scores ranged from +22.7 (influenza) to - 64.4 (cholera) with the median being -22.9 (rubella). The spectrum found in the total unweighted scores contained 12 possible ranks ranging from +2 to -9. Table 5 demonstrates the differences obtained from weighting for some selected pathogens.

**Table 2**

**Criteria and definition of the respective scores for the prioritisation of pathogens, Robert Koch Institute, 2008**

| Criteria | Values | | |
|---|---|---|---|
| | -1 | 0 | 1 |
| **Burden of disease** | | | |
| Incidence | <1/100.000 | 1/100.000-20/100.000 | >20/100.000 |
| Severity | hospitalisation is very rare, work loss less than 2 days, no persisting handicaps | hospitalisation is rare, work loss of more than 5 days is rare, very rarely persisting handicaps | hospitalisation is frequent, work loss of more than 5 days is frequent, persisting handicaps do occur |
| Mortality* | <50 deaths/year in Germany | between 50 und 500 deaths /year in Germany | more than 500 deaths /year in Germany |
| **Epidemiologic dynamic** | | | |
| Outbreak potential | outbreaks are very rare | outbreaks with 5 or more cases are rare | outbreaks with 5 or more cases are frequent |
| Trend | diminishing incidence rates | stable incidence rates | increasing incidence rates |
| Emerging potential | disease already endemic or very unlikely to be introduced to Germany | disease has the potential to be introduced to Germany sporadically | disease is likely to emerge in Germany in a relevant way |
| **Information need** | | | |
| Evidence for risk factors /groups | risk factors and risk groups are identified based on scientific evidence | risk factors and risk groups are basically known but scientific evidence is missing | risk factors and risk groups are not known |
| Validity of epidemiologic information | epidemiologic situation is well known and scientifically valid | epidemiologic information exists but is scientifically not very valid | epidemiologic information is insufficient |
| International duties and public attention | no international duties or political agenda, minor public attention | no international duties but informal political expectations, moderate public attention | international duties or explicit political agendas, high public attention |
| Evidence for pathogenesis | information on pathogenesis and transmission routes is available and well supported by scientific evidence | information on pathogenesis and transmission routes is basically available but not well supported by scientific evidence | information on pathogenesis and transmission routes is hardly available |
| **Health gain opportunity** | | | |
| Preventability | there are hardly any possibilities for prevention or there is no need for prevention | concepts for prevention are established but there is need for further research to improve its effectiveness | strong need for further research on preventive measures because need for prevention is clear but concepts for prevention are missing |
| Treatability | medical treatment is rarely necessary or effective treatments are available to positively influence the burden of disease or the prognosis | medical treatment is frequently indicated but medical treatments only have a limited influence on the burden of disease or the prognosis | medical treatment is desirable but currently there is no treatment available that positively influences the burden of disease or the prognosis |
| **Proposed alternative to mortality** | | | |
| Case fatality rate* | <0,01% | 0,01- 1% | > 1% |

**Table 3**

**Distribution of pathogens by total weighted and un-weighted scores during prioritisation, Robert Koch Institute, 2008**

| Reference | Anonymous 1995 (19) | Rushdy & O'Mahony 1998 (3) | Weinberg *et al* 1999 (20) | Doherty 2000 (1) | Horby *et al* 2001 (2) | Institute de Veille Sanitaire (InVS) 2001 (5) | World Health Organisation 2003 (4) | Krause et al. 2008 |
|---|---|---|---|---|---|---|---|---|
| Year | 1995 | 1997 | 1997 | 1998 | 1999 | 2000-2001 | 2002 | 2005 |
| Country | France | United Kingdom | European Union | Canada | United Kingdom | France | South East Europe | Germany |
| Organisation | Reseau National de Santé Publique (RNSP) | Public health laboratory service (PHLS) Overview of Communicable Diseases Committee | Charter group of European Commission (EC) | Canadian Advisory Committee on Epidemiology | Public health laboratory service (PHLS) Overview of Communicable Diseases Committee | Institute de Veille Sanitaire (InVS) | Dubrovnik Pledge / World Health Organisation | Robert Koch Institute |
| Prioritisation objective | select diseases for surveillance | programme initiatives in infectious disease control | select diseases for surveillance in | select diseases for surveillance | programme initiatives in infectious disease control | prevention of non-food-borne zoonotic diseases | select diseases for surveillance | epidemiological research and surveillance |
| Number of diseases | 84 | 33 (+8 generic disease groups) | 26 | 43 | 58 (+11 generic disease groups) | 37 | 53 | 85 |
| Number of criteria | 3 principles | 5 criteria | 9 criteria | 10 criteria | 5 criteria | > 5 criteria | 8 criteria | 12 criteria |
| Scoring system | No | 5-tiered | 5-tiered | 3-, 4-, and 6-tiered | 5-tiered | not quantifiable | 5-tiered | 3-tiered |
| Score-specific definition | no | no | no | yes | no | no | no | yes |
| Weighting applied | no | no | no | implicitly | no | no | no | systematically |
| Methodology of collecting opinion | Delphi | survey | Delphi | Delphi | survey | working group | Delphi | Delphi |
| Number of participants | over 50 | 194 | 14 | 6 | 518 | 10 | not published | 11 |
| Type of participants | interministerial and regional experts | experts in communicable disease control and public health laboratory service (PHLS) | heads of national institutions with responsibilities for communicable diseases surveillance | provincial epidemiologists | different health care professionals | interministerial and regional experts | participants of World Health Organisation workshop (not published) | epidemiologists at national public health institute (RKI) |

**Table 4**

**List of pathogens selected for prioritisation, Robert Koch Institute, 2008**

| | | | |
|---|---|---|---|
| Adenovirus | *Escherischia coli*, shigella toxin producing (STEC/HUS) | *Leishmania* spp. | *Salmonella* typhi |
| *Babesia microti* | *Echinococcus granulosus* | *Leptospira interrogans* | *Shigella* spp. |
| *Bacillus anthracis* | *Echinococcus multilocularis* | *Listeria monocytogenes* | *Staphylococcus aureus*, methicillin resistant (MRSA) |
| *Bartonella* spp. | *Ehrlichia chaffeensis* | Measles virus | *Staphylococcus aureus*, toxigenic |
| *Bordetella pertussis* | *Entamoeba histolytica* | *Microsporum* spp. | *Streptococcus* spp. other than *Str. pneumoniae* |
| *Borrelia burgdorferi* | Epstein-Barr virus | Molluscipoxvirus | *Streptococcus pneumoniae* |
| *Brucella abortus* | *Francisella tularensis* | Mumps virus | *Toxoplasma gondii* |
| Bovine Spongioform Encephalitis (BSE)/ variant Creutzfeldt Jakob Disease (vCJD) | *Giardia lamblia* | *Mycobacterium* Leprae | *Treponema pallidum* |
| *Campylobacter jejuni* | *Haemophilus influenzae* | *Mycobacterium tuberculosis* | *Trichinella spiralis* |
| Central European tickborne encephalitis virus | Hanta virus | *Mycobacterium*, other (non-tuberculous) | *Trichomonias vaginalis* |
| *Chlamydophila pneumoniae* | *Helicobacter pylori* | *Mycoplasma* spp. | Varicella virus |
| *Chlamydophila psittaci* | Hepatitis A virus | *Neisseria gonorrhoeae* | Variola virus |
| *Chlamydia trachomatis* | Hepatitis B virus | *Neisseria meningitidis* | *Vibrio cholerae* |
| *Clostridium botulinum* | Hepatitis C virus | Norovirus | Viruses, others causing hemorrhagic fevers |
| *Clostridium tetani* | Hepatitis D virus | Parvovirus B 19 | West Nile virus |
| *Corynebacterium diphtheria* | Hepatitis E virus | *Plasmodium* spp. | Yellow fever virus |
| *Coxiella burnetii* | Herpes simplex virus (HSV) | Polio virus | *Yersinia enterocolitica* |
| *Cryptosporidium parvum* | Human immunodeficiency virus (HIV) | Rabiesvirus | *Yersinia pestis* |
| *Cyclospora cayetanensis* | Human papilloma virus (HPV) | Rota virus | *Yersinia pseudotuberculosis* |
| Cytomegalovirus | Human T-cell lymphotrophic virus (HTLV) | Rubellavirus | |
| Dengue virus | Influenza virus | *Salmonella* spp. (non typhi non paratyphi) | |
| *Escherischia coli*, enteropathogenic (non STEC/HUS) | *Legionella pneumophila* | *Salmonella* paratyphy | |

## Discussion and conclusions

The described methodology builds on the experiences of similar efforts [1-5,18, 19] and attempts to increase the level of standardisation and transparency in prioritising pathogens based on public health criteria. In comparison to the cited prioritisation efforts, our approach may appear overly standardised. We believe, however, this ensures transparency and reproducibility, which are important, especially as prioritisation may easily affect funding and policy issues. Furthermore, our methodology allows for adaptations if certain conditions change e.g. if a vaccine becomes available or if the incidence changes significantly.

The result of the prioritisation at RKI shows a multi-modal distribution with the majority of scores below 0 indicating that, with a given definition of scores and a list of diseases to prioritise, participants tended to opt more frequently for lower scores. Therefore, we propose to replace the criterion of mortality by case fatality, as presented in Table 2, because mortality is implicitly dependant on incidence, whereas case fatality is another criterion for burden of disease complementing the criterion of severity. Among the selected diseases presented, the proposed exchange would somewhat lower the score for influenza but it does not seem to result in a relevant change of ranking.

A five-tiered scoring system as used in the overview of communicable diseases or in the Dubrovnik pledge could allow for a more differentiated scoring than the three-tiered system we used [2-4]. However, the challenge to generate clear definitions for each score increases with the number of scores. For many diseases and criteria information may not be available in the detail needed to permit such a differentiated approach.

The examples in Table 5 demonstrate that some diseases that were far apart in the unweighted scaling moved close together after weighting had been applied. This makes it obvious that weighting is important and that it may result in changes in both directions. There is reason to believe that the objectiveness of the procedure is increased if weighting is done independently of, and prior to,

**Figure 2**

**Distribution of pathogens by total weighted and unweighted scores during prioritisation, Robert Koch Institute, 2008**

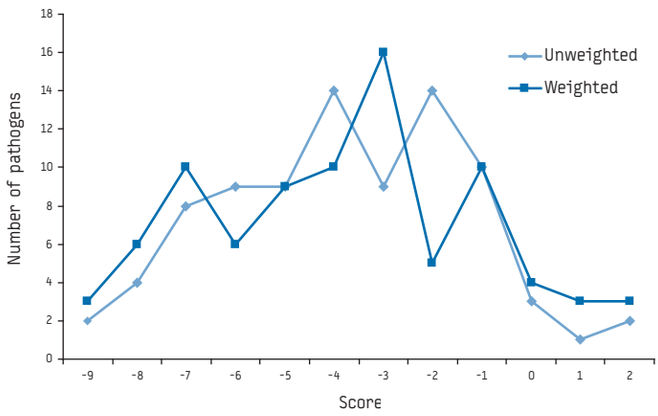



**Table 5**

**Prioritisation scores for five selected pathogens out of 85, Robert Koch Institute, 2008**

| | | Crude weighted scores | | | | |
|---|---|---|---|---|---|---|
| | | Maximum | | Median | | Minimum |
| Disease | Weight | Influenza | Rotavirus | Rubella | Cyclosporiasis | Cholera |
| **Burden of disease** | | | | | | |
| Incidence | 10.7 | 10.7 | 10.7 | 0 | -10.7 | -10.7 |
| Severity | 10.3 | 0 | -10.3 | -10.3 | -10.3 | 0 |
| Mortality | 8.4 | 8.4 | 0 | -8.4 | -8.4 | -8.4 |
| **Epidemiologic dynamic** | | | | | | |
| Outbreak potential | 10.1 | 10.1 | 10.1 | 10.1 | 0 | -10.1 |
| Epidemiologic trend | 7.7 | 0 | 0 | 0 | 0 | -7.7 |
| Emerging potential | 5.4 | -5.4 | -5.4 | -5.4 | 0 | 0 |
| **Information need** | | | | | | |
| Evidence for risk factors /groups | 5.5 | -5.5 | -5.5 | -5.5 | 5.5 | -5.5 |
| Validity of epidemiologic information | 5.4 | -5.4 | -5.4 | 0 | 5.4 | -5.4 |
| Political agendas, public awareness | 5.2 | 5.2 | 0 | -5.2 | -5.2 | 0 |
| Evidence for pathogenesis | 3.4 | -3.4 | -3.4 | -3.4 | 0 | -3.4 |
| **Health gain opportunity** | | | | | | |
| Preventability | 8.0 | 8 | -8 | 0 | 0 | -8 |
| Treatability | 5.2 | 0 | -5.2 | 5.2 | 0 | -5.2 |
| **Total weighted score (crude)** | | 22.7 | -22.8 | -22.9 | -23.7 | -64.4 |
| **Total unweighted score** | | 1 | -5 | -4 | -2 | -9 |
| **Total weighted score (normalised to a scale from +2 to -9)** | | 2 | -4 | -4 | -4 | -9 |

scoring. This is a way to avoid individual preferences of participants biasing the process. The advantage of quantitatively determining the weight for each individual criterion is that other institutions may choose to apply different weights to adapt the ranking to their respective mission. This increases the flexibility of the system and allows it to be used for different applications. For example the Eurostat task force on human health issues related to food safety has recently adopted a number of our criteria and also our concept of weighting in an attempt to identify the top 20 diseases from the inventory of food safety related diseases in Europe. (Ana Martinez, Eurostat, personal communication)

### Call for comments

For an upcoming update of our prioritisation methodology we plan to include the views from experts from various fields and institutions outside the RKI.

While suggesting that a structured prioritisation approach similar to the one presented here is useful, there are still a number of questions that we plan to re-assess before going through such a procedure again:

- Does the list contain all relevant pathogens?
- Do the 12 criteria cover the relevant characteristics for prioritisation and are they not redundant or strongly dependant on each other? If other categories are missing, would the available information suffice to allow scoring based on defined scores?
- For which categories would a five-tiered scaling be a major improvement and if so would it be feasible to generate clear definitions for each scale?
- Are the existing definitions for the three scores for each criterion clear and plausible? Can they be applied? Are they valid to detect differences?
- Is the weighting of the criteria plausible?
- How large should the group of participating experts be and how should it be composed?

We invite suggestions, feedback and answers to the questions above through a structured web-based questionnaire available from http://www.rki.de/EN > Prevention of infection > Infectious Disease Surveillance > Pathogen prioritization. This may initiate a fruitful discussion in the scientific community and provide some guidance on how to improve our prioritisation scheme and maybe that of other institutions. Ultimately, we hope this will in return contribute to rational allocation of attention and resources in the control and prevention of infectious diseases.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# Developing the Community reporting system for foodborne outbreaks

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Investigating and reporting of foodborne outbreaks became mandatory with Directive 2003/99/EC. In 2006 and 2007 the Community reporting system for foodborne outbreaks was further developed in an interdisciplinary approach, which is described in this paper. This involved experts on investigating and reporting foodborne outbreaks as well as experts on communicable diseases in addition to the European Food Safety Authority (EFSA) Task Force for Zoonoses Data Collection, the European Centre for Disease Prevention and Control (ECDC) Advisory Forum and representatives of ECDC, the World Health Organization (WHO), the World Organization for Animal Health (OIE) and the European Commission. European Union Member States participated in a survey regarding their national reporting systems and the needs for information on foodborne outbreaks at the Community level. The acceptability, the functionality and the data quality of the current reporting system were evaluated. The results were used to propose new variables on which data should be reported. Pick-lists were developed to facilitate reporting and better integration of the Community system with Member States' reporting systems. The new system is expected to yield better quality data on foodborne outbreaks relevant for risk assessment and risk management while reducing the work load for Member States.

## Introduction

Protection of human health against diseases and infections transmissible directly or indirectly between animals and humans (zoonoses) is of paramount importance. In order to assess the priorities for preventive action against zoonoses in the European Community, the European Union (EU) Member States have been obliged since the end of 1993 to collect data on the trends and sources of zoonotic infections in the human population and on the occurrence of zoonotic agents in animals, food, and animal feed [1].

Foodborne outbreaks, if thoroughly investigated, provide the possibility to identify the pathogen, the food vehicle involved and the factors in the preparation and handling of food that contributed to the outbreak. Therefore, it was considered appropriate to make provision for such investigations and for close cooperation between the various authorities when a new "Zoonoses directive" was developed in 2003. The Directive 2003/99/EC of the European Parliament and of the Council on monitoring of zoonoses and zoonotic agents [2] requests the EU Member States to investigate foodborne outbreaks and to transmit each year to the Commission a summary report of the results of the investigations carried out. The European Food Safety Authority (EFSA), who is assigned the task to collate, analyse and report the data collected, developed a reporting system for foodborne outbreaks in 2003. When the reporting of foodborne outbreaks became mandatory in 2005, EFSA with the assistance of its Foodborne Outbreak Contractor, the Bundesinstitut für Risikobewertung (Federal Institute for Risk Assessment, BfR), and in collaboration with the European Centre for Disease Prevention and Control (ECDC) further developed the Community reporting system for foodborne outbreaks.

This report describes the activities undertaken in this context and summarises their results.

## Methods

A survey was conducted with the aim to investigate the national reporting systems for foodborne outbreaks currently in place in the Member States and to establish the need for collecting further information on foodborne outbreaks at the Community level.

Following this, the current Community reporting system for foodborne outbreaks was evaluated regarding its acceptability, data quality and sensitivity.

The results of the questionnaire survey and the evaluation were used in further developing the Community reporting system for foodborne outbreaks.

### Questionnaire survey

Two questionnaires were prepared for the survey. In the first questionnaire, the recipients were asked to describe the structure of their national reporting system for foodborne outbreaks, how foodborne outbreaks were investigated and results of those investigations reported. In the second questionnaire, the recipients were asked to prioritise proposed objectives of the improved Community reporting system for foodborne outbreaks and to list other objectives they considered important. They were also requested to prioritise possible new parameters on which data should be reported to the Community level through the improved Community reporting system.

Both questionnaires were sent to representatives of the EU Member States and other European countries participating in the EU data collection (30 countries in total were contacted). They were asked to further distribute the questionnaires among the relevant institutes and persons in charge of the reporting of foodborne outbreaks in their country, and to return the completed forms. In

addition, representatives of the European Commission, a number of international organisations and networks, and EFSA scientific panels were asked to complete the second questionnaire only.

Completed questionnaires were analysed at the BfR. The absolute and relative frequencies were calculated for all response options given in the questionnaires. In addition, for each of the general objectives and parameters of the improved reporting system given in the second questionnaire on information needs, scores were calculated by multiplying the frequency with which an objective had been assigned a priority level with the rank of the priority level ("High priority" = 2, "Low priority" = 1, "No need" = 0).

### Evaluation of the reporting system

The current reporting system was evaluated by the Foodborne Outbreak Contractor (BfR) by assessing the data on foodborne outbreaks occurring in 2005 and submitted by Member States to the system before August 2006. The acceptability of the system was evaluated by calculating the overall participation rate of Member States, the submission rate for the respective reporting forms of the system and the completeness (represented by non-blank data fields) for all fields of the reporting forms. Data quality was evaluated by assessing the validity and the completeness of data submitted through the reporting forms. The sensitivity of the system relative to the sensitivity of national reporting systems was estimated for a subset of countries by comparing data on foodborne outbreaks submitted to the Community reporting system with data on foodborne outbreaks published in national bulletins, national annual reports and peer-reviewed journals.

### Developing the reporting system further

A working group on foodborne outbreaks was set up by EFSA with experts on food safety and public health as well as representatives of ECDC, the World Health Organization (WHO), the World Organization for Animal Health (OIE) and the Directorate General for Health and Consumer Protection of the European Commission. Its task was to identify the need for reporting more information on foodborne outbreaks at the Community level and the availability of this data in the national reporting systems in Member States. The working group also analysed the results and the functionality of the current Community reporting system for foodborne outbreaks.

Based on their work, as well as the results of the questionnaire survey and the evaluation of the reporting system, a proposal for the improved reporting system was drafted. Subsequently, both the Task Force on Zoonoses Data Collection of EFSA and the Advisory Forum of ECDC were consulted and provided their comments, and the draft document was accordingly adjusted.

## Results

### Questionnaire survey

Twenty-six countries (response rate 87%) provided information on current national reporting system of foodborne outbreaks through the first questionnaire (27 systems were described, as one country provided information on two systems). In addition, 32 pick-lists of possible entries for a range of variables used in the national reporting systems were provided by 13 countries [3].

Thirty-five completed copies of the second questionnaire on information needs were received from representatives of 26 countries and two international bodies.

#### *Foodborne outbreak reporting systems in place in the countries*

All respondents confirmed that their country operated a reporting system for foodborne outbreaks, including waterborne outbreaks. All countries covered outbreaks caused by bacteria, viruses and parasites (n=26). Information on outbreaks caused by toxins were collected by 22 systems and data on outbreaks caused by chemicals by 10 systems. The majority of the national reporting systems were complex and involved several authorities. Eight countries claimed that there was close co-operation between public health and food safety/ veterinary authorities while five countries reported the establishment of national commissions or platforms for foodborne outbreaks aiming at improving the exchange of information and collaboration between the public health, veterinary and food safety authorities on zoonoses and, specifically, on foodborne outbreaks. Most countries recorded information on the number of human cases, the number of hospitalisations and deaths related to the outbreak. Many of them also differentiated between laboratory-confirmed and epidemiologically linked human cases and included age and gender of the cases (Table 1).

The incriminated food item could be reported as a free text in 17 of the systems. Five systems provided a default list with food items or categories from which the appropriate item could be picked and five systems offered both options. Most systems recorded the place of consumption and the place of preparation of the incriminated food, while the methods of food processing and food preparation were registered less frequently (Table 2). The most frequently stated shortcomings of the national reporting systems were the varying depths of outbreak investigations and the difficulties in tracing back the incriminated food.

#### *Information needs at the Community level*

The three objectives for data collection that received the highest overall score from all respondents were the identification and the monitoring of the food vehicles, the causative agents and the risk factors of foodborne outbreaks. Altogether 29 variables on which data should be collected through the improved Community foodborne outbreak reporting system were offered for prioritisation.

**Table 1**

**Information on human cases involved in foodborne outbreaks covered by the national reporting systems (n=27)**

| Variable | Systems | |
|---|---|---|
| | n | % |
| Number of human cases in the outbreak | 27 | 100 |
| Number of deaths caused by the outbreak | 25 | 93 |
| Number of cases hospitalised | 24 | 89 |
| Number of laboratory confirmed human cases in the outbreak | 22 | 82 |
| Number of epidemiologically confirmed cases in the outbreak | 18 | 67 |
| Age of the person affected | 18 | 67 |
| Gender of the persons affected | 18 | 67 |
| Number of persons at risk | 17 | 63 |
| Number of laboratory confirmed clinical* cases in the outbreak | 14 | 52 |
| Number of laboratory confirmed asymptomatic cases in the outbreak | 7 | 26 |
| Number of person-days-in-hospital caused by the outbreak | 3 | 11 |

n = number of national reporting systems collecting data on the variable
% = percentage of all reporting systems
* = symptomatic

Among the variables related to human cases, the following were considered to be most important: the number of human cases and deaths, the beginning and the end date as well as the location of the outbreak and the type of the outbreak. Of the variables related to the food vehicle, the identification of the food vehicle, its origin, the evidence for incriminating the food vehicle, the places of food preparation and consumption, the origin of the contamination of the food vehicle, the factors contributing to its contamination as well as the results of the laboratory analysis of the food vehicle were considered to be the most relevant variables (Table 3).

**Evaluation of the reporting system**

The web-based reporting system for foodborne outbreaks developed by EFSA in 2003 and used until 2007 provided a table form to capture information on the total number of outbreaks per year, the number of human cases and deaths in these outbreaks, the causative agents of the outbreaks, the foodstuffs implicated as vehicles of the causative agents, the location of exposure of the human cases to the contaminated food vehicle and the contributory factors, i.e. the factors contributing to the contamination of the incriminated food. In addition, a text form was provided by the web-based reporting system to capture information on the national system in place for identification, epidemiological investigations and reporting of foodborne outbreaks, the types of outbreaks covered by the system, the national evaluation of the reported outbreaks with respect to relevance of the different causative agents, food categories and the agent/food category combinations and an evaluation of the severity and clinical picture of the human cases, the description of single outbreaks of special interest and on the control measures or other actions taken to improve the situation. All data fields except those for the information on the 'causative agent', which could be chosen from a pick-list with variable degrees of detail (speciation and subtyping information), were free text fields.

By August 2006, of the 26 countries eligible for reporting (25 EU Member States plus Norway), 24 countries (23 EU MS and Norway) submitted data on foodborne outbreaks which had occurred in 2005, resulting in 92% participation rate. The table form was used by 21 EU MS and Norway (n=22, 85%), whereas the text form was submitted by 19 EU MS and Norway (n=20, 77%). In all, 972 table-form reports were submitted, the majority of which contained information on individual outbreaks (n=826, 85%), whereas in less than one-fifth of the reports (n=146, 15%) information on more than one outbreak was aggregated.

Information on the causative agent at the genus-level was provided in all aggregated and all individual reports. All reports also contained information about the type of outbreak ("general outbreak" or "family outbreak"). The number of human cases was given in 99% of individual and 96% of aggregated outbreak reports. Data on the vehicle of the outbreak, that is the foodstuff incriminated for causing the outbreak, was available in 92% of the individual outbreak reports but only in 78% of the aggregated outbreak reports. Information on the "location of exposure" was

**Table 2**

**Information on factors regarding the incriminated food item collected by the national reporting systems (n=27)**

| Variable | Systems | |
|---|---|---|
| | n | % |
| Place of consumption | 26 | 96 |
| Place of food preparation | 23 | 85 |
| Factors contributing to contamination of the food | 23 | 85 |
| Factors contributing to survival/multiplication of the agent in the food | 21 | 78 |
| Origin of contamination of the food | 20 | 74 |
| Origin of incriminated food (i.e. imported or national product) | 18 | 67 |
| Method of food preparation | 15 | 56 |
| Method of food processing | 14 | 52 |
| Reasons not allowing identification of origin of food contamination | 12 | 44 |

n = number of national reporting systems collecting data on the variable
% = percentage of all reporting systems

**Table 3**

**Prioritisation of objectives for the Community foodborne outbreak reporting system by the respondents (n=35)**

| Objective | High priority | | Low priority | | No need | | Other | | Score |
|---|---|---|---|---|---|---|---|---|---|
| | n | % | n | % | n | % | n | % | |
| Gather information on and monitor the vehicles of food-borne outbreaks | 31 | 88 | 3 | 9 | 1 | 3 | 0 | 0 | 65 |
| Gather information on and monitor the agents causing food-borne outbreaks | 31 | 88 | 3 | 9 | 1 | 3 | 0 | 0 | 65 |
| Gather information on and monitor risk factors* for food-borne outbreaks | 30 | 85 | 4 | 12 | 1 | 3 | 0 | 0 | 64 |
| Monitor trends in agents causing food-borne outbreaks | 28 | 79 | 6 | 18 | 1 | 3 | 0 | 0 | 62 |
| Identify new agents causing food-borne outbreaks | 29 | 82 | 3 | 9 | 2 | 6 | 1 | 3 | 61 |
| Provide comparable data on food-borne outbreaks | 26 | 74 | 8 | 23 | 1 | 3 | 0 | 0 | 60 |
| Evaluate the impact of control measures taken | 25 | 71 | 9 | 26 | 1 | 3 | 0 | 0 | 59 |
| Identify new vehicles of food-borne outbreaks | 24 | 69 | 9 | 26 | 2 | 6 | 0 | 0 | 57 |
| Monitor trends in vehicles involved in food-borne outbreaks | 22 | 62 | 12 | 35 | 1 | 3 | 0 | 0 | 56 |
| Gather information on and monitor special risk groups of consumers for food-borne outbreaks | 19 | 56 | 13 | 35 | 3 | 9 | 0 | 0 | 51 |

n= number of respondents assigning the objective to the priority level; %= percentage of all respondents; score= number of respondents assigning the objective to a given priority level multiplied with the rank of the priority level ("High priority" = 2, "Low priority" = 1, "No need" = 0); * risk factors = host factors and factors contributing to the contamination of the incriminated food

given in 95% of the individual and 75% of the aggregated outbreak reports respectively (Table 4).

Most countries provided some information on their reporting systems, on the evaluation of the national situation regarding foodborne outbreaks as well as a description of the types of outbreaks covered by their reporting systems (between 80 to 90% completeness) through the text form.

The quality of the submitted data was assessed separately for data submitted through the table form and for data submitted through the text form. In the individual outbreak reports submitted through the table form, most of the data provided on the type of evidence and the location of exposure were submitted under the corresponding field of the table (96 and 90% of the relevant entries). In contrast, only 70% of the information on the food vehicle of the outbreak was submitted under the corresponding field ("Source"), and only slightly more than half of the information on contributing factors was reported under the field "Contributing factors" (Table 5).

For all 146 aggregated outbreak records submitted in the table form, whenever information on the incriminated food vehicle was given it was entered in the corresponding field of the table. The same applies to the information submitted on the location of exposure. In contrast, only 76% of the information on contributing factors was provided under the corresponding field.

A large proportion of the 20 completed text forms contained the requested information on the authorities and institutions involved in investigating and reporting foodborne outbreaks, on their roles and responsibilities, and on mandatory and voluntary activities in this field (75 to 80%). Approximately half of the text forms contained the requested information on the relevance of the agents involved in the reported foodborne outbreaks (60%) and the types of outbreaks covered by the system (50%). Information on the trends observed in the number of outbreaks and cases, the relevance of the places of food production and preparation as well as the evaluation of the severity and clinical pictures of the human cases was provided less frequently (range 5-35% completeness).

**Table 4**

**Completeness of outbreak records submitted in the table forms (n=972)**

| | All outbreak records (n=972) | | Aggregated outbreak records (n=146) | | Individual outbreak records (n=826) | |
|---|---|---|---|---|---|---|
| Data field | No. non-blank fields | Completeness (%) | No. non-blank fields | Completeness (%) | No. non-blank fields | Completeness (%) |
| Causative agent | 972 | 100 | 146 | 100 | 826 | 100 |
| Causative agent species | 797 | 82 | 120 | 82 | 677 | 82 |
| Causative agent Subtype | 304 | 31 | 49 | 34 | 255 | 31 |
| Outbreak type | 971 | 100 | 146 | 100 | 824 | 100 |
| Number of persons ill | 959 | 99 | 140 | 96 | 819 | 99 |
| Number of persons who died | 653 | 67 | 112 | 77 | 541 | 65 |
| Number of persons in hospital | 732 | 75 | 112 | 77 | 620 | 75 |
| Source* | 878 | 90 | 114 | 78 | 764 | 92 |
| Level of confirmation of source* | 784 | 81 | 79 | 54 | 689 | 83 |
| Type of evidence | 576 | 59 | 63 | 43 | 513 | 62 |
| Location of exposure | 897 | 92 | 110 | 75 | 787 | 95 |
| Contributing factors | 382 | 39 | 21 | 14 | 361 | 44 |
| Comment | 80 | 8 | 24 | 16 | 56 | 7 |
| Footnote | 212 | 22 | 16 | 11 | 196 | 24 |

*source = implicated food

**Table 5**

**Distribution of information of individual outbreak records (n=826) in corresponding and non-corresponding fields of the table form**

| Thematic area | Requested information provided in corresponding field | | Requested information provided in other field | | Requested information provided total |
|---|---|---|---|---|---|
| | n | % | n | % | n |
| Source* | 712 | 70 | 303 | 30 | 1015 |
| Location of exposure | 713 | 90 | 80 | 10 | 793 |
| Type of evidence | 279 | 96 | 11 | 4 | 290 |
| Contributing factors | 146 | 55 | 119 | 45 | 265 |

*source = implicated food

***Sensitivity analysis***

The sensitivity of the reporting system was assessed for a subset of countries (Denmark, France, Germany, Ireland, Norway, Sweden, United Kingdom) by comparing individual records of outbreaks occurring in 2005 and reported to EFSA (EFSA dataset, n=229) with reports on individual foodborne outbreaks occurring in 2005 and published in national bulletins, annual reports or peer-reviewed journals (national dataset, n=124). Information on the causative agent and the type of outbreak was complete in both data sets. There was little difference between the levels of completeness for the number of human cases (97% in the EFSA and 96% in the national data set), the place of exposure (85% and 84% respectively), the incriminated food (54% and 63% respectively), the type of evidence (40% and 37% respectively) and the food processing information (26% and 28% respectively). Information on the number of deaths and the number of hospitalisations was more complete in the EFSA data set with 43% and 34% respectively as compared to the national data set with a completeness of 8% each. The national data set was more complete than the EFSA data set with regards to subtyping information (85% as compared to 65%), species information for food of animal origin (23% as compared to 18%), and contributing factors (15% as compared to 8%).

Thirty-nine identical outbreaks were identified in the EFSA and the national dataset through matching of the information on the parameters "reporting country", "causative agent", "number of cases" and "food vehicle". For most of these outbreaks the level of detail of the information provided on the species for food of animal origin, on the place of exposure and on processing of incriminated food was the same in the EFSA and the national dataset (92%, 87% and 82% respectively). 50% of the reports contained information on the type of evidence only in the EFSA data set, whereas for 37% of the outbreak records information on contributing factors was reported exclusively in the national data set [4].

**Developing the reporting system further**

Taking into consideration the results of the survey and the evaluation of the current system, a proposal for a new foodborne outbreak reporting system was drafted. This proposal was subsequently accepted by the participating countries through the EFSA Task Force on Zoooses Data Collection and the ECDC Advisory Forum. The system has been used in May 2008 to report data from 2007.

Its main objectives are to assess the trends in the number and size of foodborne outbreaks and the share of outbreaks related to different causative agents [5]. It should also collect information on the severity of disease in the human cases involved; the importance of different food categories as vehicles of foodborne outbreaks and the risk factors contributing to the occurrence of foodborne outbreaks. The scope of the new system has been set to cover foodborne outbreaks caused not only by zoonotic agents, but by any virus, bacterium, algae, fungus, parasite, and their products, such as toxins and biological amines (e.g. histamine) as well as foodborne outbreaks where the causative agent remains unknown. Foodborne outbreaks caused by chemical agents are, however, not covered at this stage. Outbreaks caused by ingestion of drinking water are also considered foodborne since drinking water is defined as food in Regulation 178/2002/EC. An additional table form capturing the number of foodborne outbreaks, distinguishing between possible and verified foodborne outbreaks, has been introduced. Possible foodborne outbreaks are outbreaks compatible with descriptive epidemiological evidence alone including also outbreaks where the causative agent is unknown. Their number should be reported by causative agent, including the option "unknown agent", in the new table. The original table form should only be used to report details on verified outbreaks, i.e. outbreaks compatible with descriptive epidemiological evidence and laboratory detection of the causative agent in implicated food or analytical epidemiological evidence or both. The table has been modified by adding pick-lists for most of the variables. In addition to selecting the implicated foodstuff category from a pick-list, a free text field can be used to define the foodstuff in more detail, e.g. to submit details on the animal or plant species the food was made from and the treatment of the food. Two new variables have been added to the table to collect information on the place where the contamination or the mishandling of the implicated food occurred ("place of origin of problem") and on the origin of foodstuff, e.g. whether the implicated foodstuff originated from the domestic market, from intra-community trade or was imported from outside the EU. A comprehensive manual containing definitions of all terms included in the pick-lists as well as examples has been prepared to facilitate reporting. In April 2008 EFSA, in collaboration with ECDC, organised a training course in the new system for relevant officers of the countries participating in reporting.

## Discussion and conclusion

The responses received through the questionnaire survey show that the vast majority of the national foodborne outbreak reporting systems in the EU provide the information that is requested pursuant Article 9 (1) of the Zoonoses Directive (Annex IV, E) [2]. In fact, many of the national systems collect complementary information on a number of variables. It is particularly encouraging to note that already many national systems collect detailed data on the incriminated food vehicles, on the causative agents, on the human cases and on the contributing factors. This could contribute to reaching the objectives of the Community reporting system considered most important by the survey respondents, i.e. the identification and the monitoring of the vehicles, the causative agent and the risk factors involved in foodborne outbreaks. However, when interpreting the results of the questionnaire on information needs it should be taken into account that the responses might have been influenced at least partially by the countries' capacities to collect the respective data. For example, the fact that collection of data on the method of food processing or on the origin of the food contamination ranked relatively low on the priority list is probably related to difficulties in tracing back the origin of foodstuffs and establishing this kind of information.

The evaluation of the Community reporting system revealed that its acceptability in general was very high as reflected by the high rates of participation and submission as well as the high proportion of completeness of most data fields. Also the sensitivity assessment indicated that the Community systems captured almost all foodborne outbreaks reported in national reports or peer-reviewed journals and it collected sufficient detail of information available on most variables. With regard to subtyping information, which was less frequently captured by the EFSA system, it might be useful to consider whether reporting this type of data could be further simplified in the EFSA system. However, the results of the sensitivity assessment should be interpreted with some caution as the countries included in this evaluation have well established foodborne outbreak reporting systems and might not be representative for all EU Member States.

The fact that a considerable fraction of the requested information is not reported in the corresponding data field of the current system makes the analysis of the reported data difficult. This is further aggravated by the occurrence of spelling variations (e.g. "restaurant" versus "restarant") and synonyms (e.g. "kindergarten" vs. "day care center") inherent in text data reporting. While spelling variations and the use of synonyms can be obviated by introducing list fields instead of free-text fields, the frequent misplacement of information in another than the intended field also indicates that clearer instructions and further explanations might be needed on the kind of information requested in each field of the reporting form.

The Community foodborne outbreak reporting system was developed further taking into account the existing structures, variables and pick-lists of Member States' national systems as well as other reporting systems, such as the WHO surveillance system for control of foodborne infections and intoxications in Europe [6].

This should not only harmonise, but also make the reporting of foodborne outbreaks easier for Member States. Another move into this direction is the introduction of the possibility to upload national data in bulk using XML-format. Through the differentiation between possible and verified foodborne outbreaks in the new system the quantity of data to be reported should be less, as detailed information is only requested for verified outbreaks. The data on verified outbreaks will be used to characterise the nature of foodborne outbreaks in the Community and to carry out in depth-analysis of the involved food vehicle-causative agent combinations. At the same time, the system should allow to study the overall extent and impact of foodborne outbreaks in the Community by additionally capturing the number of possible outbreaks. Detailed definitions for all variables have been established. They have been agreed upon by experts from both veterinary and public health. The introduction of pick-lists for most variables will facilitate both the manual inputting of data as well as the uploading of data in bulk. Together with the introduction of definitions, this will lead to a harmonisation of reporting and ease the analysis of the reported data. Possible problems with misunderstanding the meaning of the values in the pick-list should be minimal because of the provision of comprehensive explanations and examples in the reporting manual and extensive online-user-guidance provided by the web-based system.

Because of its higher level of integration with other existing reporting systems, its increased simplicity and, therefore, higher acceptability the new Community foodborne outbreak reporting system is expected to yield better quality data on foodborne outbreaks. This will hopefully increase the availability of relevant data for food safety risk assessment critical for identifying priorities for control and monitoring programmes.


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# Meeting reports

## Ethical and legal issues related to health access for migrant populations in the Euro-Mediterranean Area

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The Institut National d'Hygiène (Morocco) and the Instituto de Salud Carlos III (Spain) are involved as a consortium in a project called "Impact of migration on HIV and TB Epidemiology in the Mediterranean Area", funded by the Sixth Framework Programme for research of the European Commission. The project started in May 2007 and is intended as a specific support action to promote international research cooperation in the Euro-Mediterranean area. In particular, its objective is to improve the capacity of the countries around the Mediterranean Basin for obtaining quality epidemiological information on human immunodeficiency virus (HIV) and tuberculosis (TB) among migrants, while taking into consideration ethical and legal issues related to health in migrant populations. To this end, the project proposed to hold two workshops to bring together all the relevant stakeholders: delegates of international and national non-governmental organisations (NGOs) concerned with the process, experts and health professionals, researchers, representatives of the United Nations Agencies and other decision makers.

The first workshop was dedicated to reviewing epidemiological and laboratory issues and was held in Rabat (Morocco) in November 2007. An account of the main issues covered at this workshop has been published [1].

France, Italy, Mauritania, Morocco, Portugal, Spain and Tunisia were represented at the second workshop, which took place in Madrid from 25 to 27 June 2008. This second workshop was intended to provide an overview on the ethical and legal issues related to health in migrant populations, contributing to the Euro-Mediterranean dialogue on the situation of migrants. In addition, it aimed at determining the specific requirements to be taken into consideration when trying to improving the epidemiologic surveillance of HIV and TB in migrant populations.

The workshop was organised around four main topics:

a) Migrants and health: ethical and legal issues;
b) Access of migrants to prevention and care for HIV and TB;
c) Stigma and discrimination;
d) The way forward: role of different stakeholders in improving health care and health information in migrants.

A summary of the discussions on those topics during the Madrid meeting are provided below.

### Migrants and health: ethical and legal issues

The right to health, regardless of the legal status of individuals, is recognised widely in the different legislative frameworks, both at international and national level.

In the international context, the most important regulations on immigration and health matters are: the WHO Constitution (1946) [2], the Universal Declaration on Human Rights (1948) [3], the International Convention on the Protection of the Rights of All Migrant Workers and Members of Their Families (1990) [4], the International Labour Organization (ILO) Conventions 97 and 143 [5], the Declaration on the Human Rights of Individuals who are not Nationals of the Country in which they Live (1985) [6], the Convention relating to the Status of Refugees (1951) [7] and the Guiding Principles on Internal Displacement (1998) [8]. Those conventions have been ratified by most countries in the world, including those belonging to the European Union (EU) and the Mediterranean region. In addition, national legislation recognising the right to health as a fundamental human right exists in Euro-Mediterranean countries. Furthermore, the Council of Europe has recognised the right of everyone to attainable standards of physical and mental health and the right to receive health care in the event of sickness and pregnancy. Moreover, any legislation or practice that denies the provision of medical assistance to foreign nationals within Europe, even if they are undocumented, is contrary to the European Social Charter [9].

Given the legal framework, policy options that contravene the United Nations and European conventions should not be pursued in the Euro-Mediterranean area, and current legislation should be enforced and implemented. Nevertheless, it is unclear how immigrants, especially undocumented, receive health care in case of need in many countries of the area.

### Access of migrants to prevention and care for HIV and TB

Early detection and treatment of HIV and TB in foreign-born individuals in the host country has proved to have an enormous potential public health benefit.

The rationale for treating people living with HIV/AIDS (PLWHA) with highly active anti-retroviral therapy (HAART) or TB patients with anti-TB drugs is based both on human rights and public health protection grounds. HAART decreases HIV-related morbidity

and mortality, enabling infected people to remain socially and economically active, and reduces the infectivity of PLWHA, thus becoming an important prevention instrument for HIV transmission [10, 11]. Likewise, treating TB patients, apart from the individual beneficial impact, is the most important TB control measure [12].

The provision of health services for TB and HIV patients is obviously subject to certain economic considerations. For this reason, cost-effectiveness analyses of HIV and TB treatment have been carried out in different settings, concluding that both interventions are cost-effective.

There are great variations in self-perceived health and utilisation of health services both among migrant populations coming from other countries and migrant groups from different parts of the same country. Thus, migrant populations are heterogeneous and should not be considered as one entity. In addition, undocumented migrants may be less likely to regularly attend health services for fear of legal actions against them. In this respect, some studies have shown that, contrary to many health professionals' and the general public's perception, migrants tend to utilise healthcare services less than nationals [13].

Access of migrants to health services might be particularly difficult in the event of incarceration. Some countries like Spain have shown that it is possible to implement prevention programmes for TB and HIV, including syringe exchange programmes, in prisons. Health policies should guarantee that prisoners and non-prisoners receive equal conditions regarding prevention and healthcare services provided, and that migrants have access to them on the same basis as nationals.

On the subject of HIV and TB screening, additional considerations must be taken into account, since affected individuals can be exposed to stigma and ostracism that might be compounded by compulsory health screening. Screening of migrants for TB and HIV is carried out in many countries, but the evidence base in support of this policy is weak. Compulsory screening is expensive in terms of both start-up and recurring costs and, once implemented, is difficult to halt. Resources allocated to compulsory screening might be more effectively directed into providing better health care and preventive services [14, 15].

NGOs operating in the Euro-Mediterranean area include among their activities free healthcare services for migrants such as screening, counselling, information on healthcare access and prevention services. The vulnerability of migrant populations has been stressed by these NGOs. Migrants need to be reached and constructively engaged into community activities, taking into account their social and cultural characteristics. It is necessary to remove all the obstacles that migrants face when it comes to accessing prevention and health care.

### Stigma and discrimination

Migrant populations around the world are likely to experience stigma and discrimination, in particular illegal migrants.

In addition, there is evidence indicating a growing "feminisation" of the migration phenomenon. Many women are forced to migrate due to discrimination and lack of opportunities in their countries of origin and those who are in low-skilled jobs or working illegally, especially in unregulated sectors such as domestic employment, are at a greater risk to suffer from violence, poor working conditions, long working hours, sexual exploitation and poor reproductive health. In Spain, migrant women from low-income countries have the worst health indicators, according to the "First Report on Inequalities and Health in Andalusia" [16]. To tackle social and gender discrimination, a coherent and integrated approach through health and social policies should be implemented.

Discrimination and stigmatisation is one of the dramatic consequences PLWHA have to face and a major obstacle to prevention and care. Fear of discrimination and stigma causes people to avoid testing and prompts those infected with and affected by HIV/AIDS to remain silent, depriving them from essential treatment and social care. These problems are perhaps magnified by the existing taboos regarding sexuality, affecting more intensively women.

### The way forward: role of different stakeholders in improving health care and health information in migrants

The unprecedented scale of migration to Europe for reasons of protection, employment and family reunion poses many opportunities and challenges. This is an area of policy making which is moving fast and involves many different stakeholders at the international, national and local level. In that respect, NGOs play an important role in providing socio-sanitary assistance to populations with difficult access to healthcare and in the emergency and humanitarian reception of undocumented migrants.

Public administrations in the host country must find solutions to cope with growing migration and arising needs, adapting existing health systems to the new situation [17]. For this purpose, it is important to study the health status, health needs and healthcare service utilisation of migrant populations. Similarly, it is necessary to know health professionals' perceptions and needs regarding the provision of healthcare to these populations.

At the international level, the International Organization for Migration (IOM) is the leading inter-governmental organisation in the field of migration and is dedicated to promoting humane and orderly migration. It does so by providing services and advice to governments and migrants. IOM acts at a political and operative level, working to achieve consistent immigration policies, to reduce vulnerability and improve migrants' health.

At the local level, health professionals play a fundamental role in improving healthcare and health information for migrants. Apart from difficulties in healthcare access, some concerns related to healthcare provision have been reported, such as language and cultural barriers, administrative problems and difficult diagnosis, treatment and follow-up. Institutional support is needed to improve this situation. The role of cultural mediators is particularly important in order to facilitate the relationship between nationals and migrants and promote reciprocal knowledge.

Europe is witnessing increases in migrant-associated TB and HIV, and these are important public health challenges. Migration cannot be avoided as long as economic differences prevail between the industrialised and the poor countries. The strongest policy instruments should be used to tackle this truly global issue at the appropriate levels. An example of best practice that should be built on across the EU and the Mediterranean region would be the provision of HIV and TB healthcare and preventive services to

migrants, documented or undocumented, on the same basis as to nationals of the host countries. Networking of people working on migrant issues and development of common definitions and procedures is necessary to improve knowledge on the subject.

The workshop was organised by the HIV/AIDS Epidemiology Unit of the Secretariat of the National Plan on AIDS/Instituto de Salud Carlos III, Madrid and funded by DG RESEARCH of the European Commission. The speakers were: Rajae El Aouad (project coordinator, National Institute of Hygiene, Morocco), Mercedes Diez (project co-researcher, HIV/AIDS Epidemiology Unit of the Secretariat of the National Plan on AIDS/Instituto de Salud Carlos III, Spain), Enrique Acín García ( General Directorate of Health in Prisons, Spain), Josefina Alventosa (Asociación deJuristas del Sida (JURISIDA), Spain), Delphine Antoine (Institut de Veille Sanitaire, France), Henrique Barros (Coordenação Nacional para a Infecção VIH/sida, Portugal), Eddy Beck (UNAIDS, Switzerland), Aziza Bennani (National programme for HIV, Morocco), Nadia Bezad (Pan African Organization Against Aids, Morocco), José Chamizo (Defensor del Pueblo, Andalucía, Spain), Almudena Echevarría (Cruz Roja Española, Spain), Amine Ezzahri (Ministry of Health, Morocco), Mariam Fadlou- Allah (Association de Lutte Contre le Sida, Morocco), Diego Gracia Guillén (Universidad Autónoma de Madrid, Spain), Jamila Lamani (Association de Lutte Contre le Sida, Morocco), Rogelio López-Vélez (Tropical Medicine & Clinical Parasitology. Hospital Ramón y Cajal, Spain), Tona Lizana Alcazo (Catalonian Health Department), Claudia Natali (International Organization for Migration, Switzerland), Paola Pace (International Organization for Migration, Switzerland), Tullio Prestileo ( ANLAIDS, Italy), Anna Rodés Monegal (Tuberculosis Programme, Generalitat de Catalonia, Spain), Elena Rodríguez (Universidad del País Vasco, Spain), Ali Sadiq (Department of immigration and border surveillance, Morocco), Ahmed Zekri (Mohammed V University Agdal, Morocco. More information available at: http://www.sante.gov.ma/Departements/INH/WorkshopTBHIV/index.htm).


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# Letters

## Influenza vaccination coverage in England, 2000-2008

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Dear Editor,

We read with interest the article by P Blank and colleagues on "Trends in influenza vaccination coverage rates in the United Kingdom over six seasons" published on 23 October 2008 [1].

The authors' study sets out to measure vaccine uptake over six seasons, with a secondary objective to ascertain motivations to be vaccinated. Between 2001 and 2007, they undertook annual household surveys by telephone interview in the UK. In their results, the authors describe a significant decline in vaccine uptake in those 65 years and more in age from 78.1% to 65.3% with evidence of year to year variation.

For several years, the Health Protection Agency on behalf of Department of Health has undertaken routine annual uptake monitoring of the seasonal influenza vaccination programme in England in order to provide an annual estimate of uptake in targeted groups. Data are now collected on registered patients in all general practices in England using a web-based reporting system. Many practices use automated extraction procedures based on standard queries. A detailed description of the methods used to collect the data is available [2].

In 2007-8, 95% of 8,375 GP practices in England took part in data collection. The sample size (registered patients, aged 65 and over) was 8,071,671). The national mean uptake in those 65 years and above in England was 74%, approaching the WHO target of 75%. Uptake levels have reached a plateau after the steady increase from 65% when the over-65-year-old programme was introduced in 2000 (Table 1). Data are published [3].

What might be the explanation for the discordance in the trend findings? As Blank et al discuss, their telephone study did have a large number of non-respondents (6% response rate), with the consequent potential for selection bias. In addition, the approach of self-reporting vaccination status can result in misclassification bias. Finally, the relatively small annual sample for the 65+ subgroup (<400 persons) will result in power limitations.

Telephone survey data can be a useful study tool. Our direct data allows the authors to validate the accuracy of their findings. There are discrepancies, indicating care needs to be taken when interpreting these results.

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**Table**

**Influenza vaccine uptake for 65 years and over by survey year, England, 2000-2008**

| Survey year | Number of participating GPs/total GPs | Total number of persons aged 65 years and over vaccinated | Total number of persons aged 65 years and over in registered population | Vaccine uptake (%) |
|---|---|---|---|---|
| 2000-1 | N/A | 4,820,239 | 7,373,157 | 65% |
| 2001-2 | N/A | 5,232,826 | 7,726,992 | 68% |
| 2002-3 | N/A | 5,487,645 | 8,008,299 | 69% |
| 2003-4 | 8,484/8,611 (99%) | 5,788,875 | 8,157,671 | 71% |
| 2004-5 | 8,147/ 8,675 (94%) | 5,621,381 | 7,870,212 | 71% |
| 2005-6 | 8,318/8,527 (98%) | 6,122,744 | 8,131,513 | 75% |
| 2006-7 | 7,860/8,464 (93%) | 5,779,145 | 7,815,298 | 74% |
| 2007-8 | 7,988/8,375 (95%) | 5,934,370 | 8,071,672 | 74% |

Note: Uptake figures include only those GP practices who have returned confirmation to the survey and reflect data for individuals vaccinated at these premises
Data Source: Influenza Immunisation Uptake Monitoring Programme HPA/DH

# Letters

## Authors' reply: Influenza vaccination coverage in the United Kingdom

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Dear Editor,

In response to our study investigating influenza vaccination coverage rates in the United Kingdom over six seasons with a telephone-based methodology [1], Pebody and colleagues note differences between our results and those reported by the Health Protection Agency (HPA) on behalf of Department of Health for registered patients in general practices in England. In their letter, Pebody and colleagues focus on our finding of a decline in coverage from 78.1% to 65.3% in the population aged 65 or over between 2005-6 and 2006-7.

At a closer look, our coverage results for this age group and those reported by the HPA are quite similar for 2003-4 (70% vs. 71%, respectively) and 2005-6 (78% vs. 75%, respectively) while our coverage results for 2004-5 and 2006-7 are indeed substantially lower. Given confidence interval widths, as shown in Figure 2 of our report, chance alone does not appear to be a likely explanation for this discrepancy.

With respect to selection effects, it should be noted that our survey methodology was designed to capture a representative sample of the population, even in the presence of low response rates. We are aware, however, that the increasing use of answering machines, voicemail systems, caller IDs and mobile phones creates an emerging challenge for telephone surveys [2,3]. Given that, we clearly cannot exclude selection bias as a partial explanation. However, as our methodology remained the same during the entire time period covered, it is unclear why selection bias should have occurred in two of the influenza seasons covered, but not in the other two.

The interviews were generally conducted between December and February as most individuals get (typically) vaccinated between September and November. The time lag between the vaccination and the fieldwork period was kept small in order to minimise incorrect recall of vaccination status. However, if the time distribution of vaccination episodes is atypical, as may have been the case in the 2006-7 influenza season due to delayed availability of the vaccine, this approach to survey timing may potentially lead to underreporting.

It should also be noted that our household surveys do not study the same population as the HPA. While our methodology covers the entire population that can be reached by a (landline) telephone connection, the HPA approach is restricted to those persons who are registered with a general practitioner and hence will, on average, differ in health status and perhaps other respects. Our knowledge of the British health system is not detailed enough to make a judgement on the potential implications of this difference.

In order to avoid double counting, we primarily studied only those persons aged 65 or over who did not have a chronic illness and were not working as health care professionals, i.e. we did not include all persons aged 65 or over, as was the case in the HPA analyses. Lower coverage rates would be expected for the resulting lower risk group, while in those aged 65 or over and with a chronic disease, whom we analysed separatly , distinctly higher coverage rates were indeed seen.

In conclusion, it is not obvious, in our opinion, what gave rise to the differences noted by Pebody and colleagues. There are several possible explanations which are not mutually exclusive. The different approaches to studying influenza coverage rates may be complementary rather than contradictory.

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# Outbreak of *Salmonella* enterica serovar Typhimurium in Switzerland, May – June 2008, implications for production and control of meat preparations

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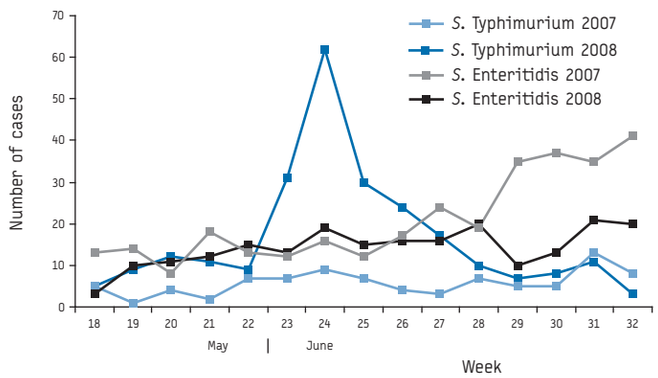
An increased number of *Salmonella* Typhimurium cases were reported in Switzerland between May and June 2008. Investigations involved 72 cases. Results of PFGE typing identified several outbreak strains, the dominating one present in 43 of the 72 isolates. Strains affecting one third of the cases were also found in animal samples, in particular pork. However, no specific food source could be identified. Outbreaks described in this paper highlight the importance of food safety regulations such as those on minced meat and meat preparations issued by the European Commission and adopted by Switzerland into the national law.

## Introduction

A sharp and countrywide increase of the number of reported *Salmonella* Typhimurium isolates was observed in May 2008 starting in week 19 and peaking in week 24 (Figure 1). Between early May to late June (weeks 19 – 27), 205 cases (2.70 cases / 100,000 inhabitants) were recorded compared to 44 (0.58 / 100,000 inhabitants) in the same period of the preceding year. In week 28, the number of cases returned to the level of 2007.

**Figure 1**

**Number of reported *Salmonella* Typhimurium and *Salmonella* Enteritidis cases by week of reception of the stool sample in the laboratory, Switzerland, weeks 18 – 32, 2007 and 2008**




## Methods

A total of 72 patient isolates with dates of isolation extending from week 17 to 27 were subjected to molecular analysis using Pulsed Field Gel Electrophoresis (PFGE) [1] by the National Centre of Enteropathogenic Bacteria (NENT) and the Institute for Food Safety, University of Zurich. Minimal inhibitory concentrations for antimicrobial susceptibility testing of representative strains were determined on Mueller-Hinton agar (Becton Dickinson, Sparks, USA) using E test strips (AB Biodisk, Solna, Sweden).

When a private food quality assurance laboratory reported the isolation of *S.* Typhimurium in pork samples, the cantonal authorities of official food control were asked to intensify the sampling and testing activity of meat products and to submit all *Salmonella* isolates from food analyses to the NENT. Subsequently, four official laboratories of food control (Zurich, Vaud, Fribourg, Liechtenstein) analysed 38 samples of raw meat and meat preparations from pork and 15 samples of raw meat and meat preparations from poultry for the presence of *Salmonella*. Furthermore, 55 samples of ready-to-eat raw meat sausages were tested.

Moreover, 24 patients were interviewed by phone between June 25 and July 7, 2008, using a standardised questionnaire. They were asked about food consumed three days before the onset of illness and travel history during the week before the onset of illness.

## Results

### Epidemiological data

The cases were located in 22 of the 26 Swiss cantons (203 cases) and in the Principality of Liechtenstein (two cases) (Table 1). The distribution of the cases by age (Table 2) in weeks 19 – 27 showed a shift towards the teenage group (23.4% of cases aged 10-19 years) when compared with the period 2000 - 2007 (13.5%). At the same time, children below the age of five years were much less represented during the outbreak (12.7%) than in the preceding eight-year period (28.0%). The sex ratio male / female seemed to be more even during the outbreak (50.2% / 46.8%) compared to the period 2000 - 2007 (54.0% [range: 49.1-56.9%] / 42.5% [range: 40.0-44.6%]).

**Table 1**

**Number of cases of *Salmonella* Typhimurium and incidences per 100,000 inhabitants in the cantons of residence of the patients, Switzerland, weeks 19 – 27, 2008**

| Canton | Number of cases | Population | Incidence |
|---|---|---|---|
| Nidwalden | 4 | 40,287 | 9.9 |
| Grisons | 11 | 188,762 | 5.8 |
| Uri | 2 | 34,989 | 5.7 |
| Appenzell Ausser Rhoden | 3 | 52,654 | 5.7 |
| Lucerne | 19 | 363,475 | 5.2 |
| Basel-Stadt | 8 | 185,227 | 4.3 |
| Bern | 39 | 962,982 | 4.0 |
| Schaffhausen | 3 | 74,527 | 4.0 |
| Zug | 3 | 109,141 | 2.7 |
| Basel-Land | 7 | 269,145 | 2.6 |
| Zurich | 31 | 1,307,567 | 2.4 |
| Solothurn | 6 | 250,240 | 2.4 |
| Neuchatel | 4 | 169,782 | 2.4 |
| Fribourg | 6 | 263,241 | 2.3 |
| Aargau | 13 | 581,562 | 2.2 |
| Geneva | 9 | 438,177 | 2.1 |
| St. Gallen | 10 | 465,937 | 2.1 |
| Thurgau | 4 | 238,316 | 1.7 |
| Valais | 5 | 298,580 | 1.7 |
| Vaud | 11 | 672,039 | 1.6 |
| Jura | 1 | 69,555 | 1.4 |
| Ticino | 4 | 328,580 | 1.2 |
| Total | 203 | 7,593,494 | 2.7 |

Note: The Principality of Liechtenstein regularly reports to the Federal Office of Public Health on a voluntary basis. Regarding the outbreak presented here, Liechtenstein reported 2 additional cases, reflecting an incidence of 5.7 cases / 100,000 inhabitants.

**Table 2**

**Age distribution of cases of *Salmonella* Typhimurium in the outbreak in weeks 19 – 27 of 2008, and of all cases of *S.* Typhimurium reported in 2000 – 2007**

| Age group (years) | Percentage of cases in the outbreak weeks 19-27, 2008 | Percentage of all cases reported in 2000-2007 |
|---|---|---|
| 0-4 | 12.7 | 28.0 |
| 5-9 | 9.8 | 14.6 |
| 10-19 | 23.4 | 13.5 |
| 20-29 | 14.6 | 9.2 |
| 30-39 | 6.3 | 8.5 |
| 40-49 | 7.8 | 6.8 |
| 50-59 | 6.3 | 7.1 |
| 60-69 | 5.4 | 5.3 |
| 70+ | 13.7 | 5.9 |

**Laboratory investigations**

The PFGE typing identified several outbreak strains (Figure 2).

The dominating type, designated "strain 2", was found in 43 of the 72 isolates. It appeared for the first time in week 23 and was obviously responsible for the main phase of the outbreak (Figure 3). However, no matching strains from food isolates have been found. None of the 108 samples of raw meat and meat preparations and ready-to-eat raw meat products analysed by four official laboratories of food control revealed *Salmonella* isolates. Other control laboratories reported no *Salmonella* isolations from foods prior and during the outbreak period within their routine testing programs.

"Strain 1" (11 isolates) was present at the beginning of the outbreak and remained up to week 24. "Strain 3" (six isolates) appeared only in weeks 25 and 26. Both strains matched with isolates from pork samples taken from a meat producer/distributor.

Two further pork-related strains were found in some patients. A strain identified in a spare rib sample from Germany (strain pm - processed meat), was found in three patients with an indistinguishable pattern. A strain identified in a sample taken from a pig at a slaughterhouse (strain sl) was isolated from two patients. Strain sl showed a PFGE profile very similar to that of the outbreak strain 3. In fact, one large band appeared to have been split in two smaller ones by a single genetic difference (Figure 2). Strains 3 and sl might therefore be considered two variants of a single clone.

Finally, seven patient isolates yielded PFGE patterns that were different from each other and from all other strains (although one in week 20 resembled strain 1), and can therefore be regarded as sporadic cases. In total, the pork-related strains 1, 3, sl and pm represented 34% (22/65) of the human cases which were not considered sporadic.

The most prevalent PFGE profiles, yielded by strains 1 to 3, were compared to international databases of Enter-Net, Salm-gene/Pulse-Net [2]. All three types matched profiles in the databases (Table 3). For example, strain 1, indistinguishable from JPXX01.0038, was found in seven patients and three non-human specimens (beef and turtle) in 2008 in the United States [personal communication by P. Gerner-Smidt, Centers for Disease Control and Prevention, US]. In Europe, a very similar profile, but with an extra band at 150 kb, was represented by 34 Pulse-Net entries. Strain 2, the dominant Swiss outbreak clone, was found among European data only once. This single entry in the Salm-gene database was submitted as a human isolate of page type DT 193 by German authorities in 2002. Strain 3 was represented three times in the Pulse-net database [personal communication by J. Threlfall and M. Hampton, Health Protection Agency, United Kingdom]. Interestingly, none of the Swiss outbreak strains corresponded to *S.* Typhimurium U292 which is responsible for a large current outbreak in Denmark.

The outbreak strains 1 to 3, as well as strains sl and pm were fully susceptible to the used panel of antimicrobials (ampicillin, ceftazidime, chloramphenicol, nalidixic acid, streptomycin, tetracycline, and trimethoprime/sulfamethoxazole). In contrast, one randomly chosen isolate from a sporadic case (18/022351) was resistant to ampicillin, chloramphenicol and tetracycline (data not shown).

### Interview results

Eight of the 24 interviewed patients were found to be infected with pork-related strains 1, 3 or sl. Six of these patients confirmed having eaten pork, one denied it and one was uncertain. The latter two, however, reported that they had eaten chicken and had taken part in a barbecue event where different sorts of meat were grilled, whereby the possibility of cross contamination should be taken into consideration. In further 15 patients among those interviewed the main outbreak strain 2 was found. Eleven of these reported having eaten pork, nine had consumed beef, six had eaten chicken and seven other kinds of meat (lamb, horse), and four participated in a barbecue. Only one patient reported having travelled (to Germany) in the seven days before onset of illness and having fallen ill while travelling, but this patient was among the sporadic cases.

Interviews were not suggestive of any food item other than those mentioned as a possible common source of infection. The variety of mentioned food items and the variety of identified strains favour the possibility that several outbreaks occurred simultaneously.

## Discussion

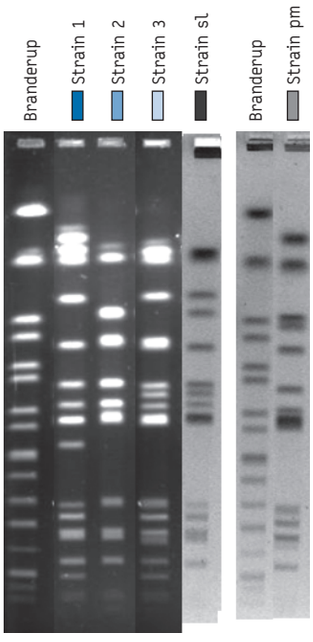
The steep rise in cases of *S.* Typhimurium infections in May 2008 was detected by the mandatory reporting system of the Federal Office of Public Health (FOPH) in the context of infectious diseases surveillance in Switzerland. Within a period of nine weeks, the number of registered cases exceeded almost fivefold those of the preceding year. The investigations in collaboration with the National Centre for Enteropathogenic Bacteria (NENT) and the Institute for Food Safety of the University of Zurich confirmed the ongoing of a countrywide outbreak or – more likely – several simultaneous outbreaks caused by different strains of *S.* Typhimurium. On the other hand, microevolution seems to have already gone on, since strains 3 and sl were differentiated by only one or two bands (Figure 3). Therefore, these two strains could be considered two variants of a single clone.

The findings gathered through the patient interviews showed that there was a median delay of six days between onset of disease and date of reception of the stool sample at the laboratory. In addition, a median delay of 10 days was brought about by the elapsed time between reception of the stool sample at the primary diagnostic laboratory and reception of the notification at the FOPH. In total, two to three weeks could have elapsed between the onset of disease and the registration of the infection. This shows that reducing the statutory notification period (currently one week) to 24 hours would improve the timeliness of patient interviews and of potential public health interventions.

About 34% of the human cases were infected with strains which were also demonstrated in quality control samples of pork from a particular company, on a pig carcass from a slaughterhouse and in an imported (from Germany) spare rib sample. Therefore, the evidence by PFGE analysis of human and food isolates, partly

**Figure 2**

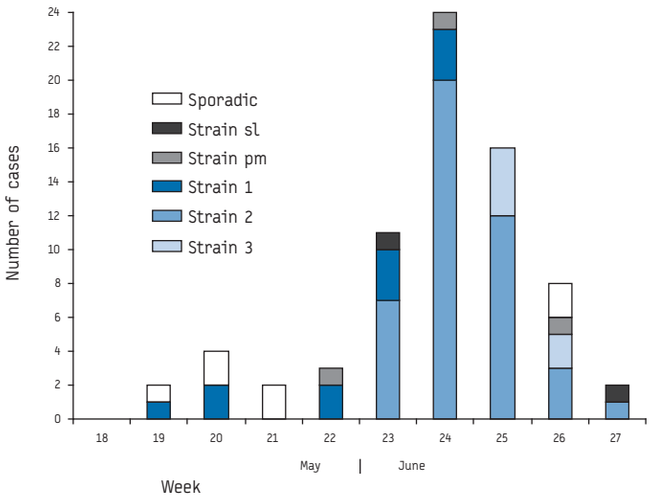
**PFGE profiles of the relevant *Salmonella* strains, Switzerland, 2008**




Strain 1 (patient 18/027428); Strain 2 (patient 18/027416); Strain 3 (patient 18/027772); Strain sl (slaughter house); Strain pm (processed meat); Braenderup (S. Braenderup (H9812) DNA, restricted with XbaI, and used as a size marker [1])

**Figure 3**

**Number of *Salmonella* Typhimurium isolates belonging to different PFGE types, Switzerland, weeks 18 – 27, 2008 (n=72)**




Strains 1, 2, 3 are different outbreak strains; Strain sl (slaughter house) was identified in a pig; Strain pm (processed meat) was found in a meat sample

**Table 3**

**Relatedness of outbreak strains 1 to 3 identified in Switzerland and other *Salmonella* Typhimurium strains deployed in international PFGE databases [2]**

| Swiss strain | USA[a] | SalmGene / PulseNet Europe[b] | Denmark[c] |
|---|---|---|---|
| Strain 1 | JPXX01.0038 | STYMXB.0103 | JPXX01.0178.DK |
| Strain 2 | no match | STYMXB.0134 | JPXX01.0020.DK |
| Strain 3 | no match | STYMXB.0214 | JPXX01.0022.DK |

a) Courtesy: P. Gerner-Smidt; b) Courtesy: J. Threlfall, M. Hampton; c) Courtesy: S. Ethelberg and R.F. Petersen

supported by patient interviews, allowed the conclusion that about one third of the observed outbreak cases was caused by contaminated pork.

However, in 108 market samples of raw pork and poultry meat, meat preparations and sausages, no *Salmonella* could be isolated. These findings indicated that contamination levels of market products with *Salmonella* must have been low or that the contaminated products were no longer present in the market.

Strain 2 was dominant in the weeks with the majority of cases (43 of 72 cases analysed by PFGE, that may be extrapolated to some 120 of the total 205 cases), but could not be linked to a specific food item. This same profile matched a contemporary cluster of 13 human isolates obtained in Denmark, but was clearly different from strains identified in the large ongoing Danish *S.* Typhimurium U292 outbreak [personal communication by *S.* Ethelberg and R. F. Petersen, Statens Serum Institute, Denmark]. It also matched at least 18 human isolates in France [personal communication by J. de Valk, Institut de veille sanitaire, France]. In France as well as in Switzerland, this strain was found to be fully susceptible to all tested antimicrobials [3].

The pork-related strains 1 and 3 also found their matches in Denmark where strain 3 represented "a rather common profile". Infection through contaminated pork products is also the main hypothesis for the U292 and other *S.* Typhimurium outbreaks that occurred this year in Denmark [4].

## Conclusions in the context of food safety legislation

In outbreaks where a large spectrum of foods, such as meat and meat preparations are potential sources of infection, it is more or less accidental to trace a targeted pathogen successfully with a reasonable number of samples. In the present case, market samples were analysed at the end of the outbreak which possibly was too late. The company which found *S.* Typhimurium in several samples of pork in the context of quality control actions launched a large environmental screening for *Salmonella* in their facilities. These investigations clearly revealed that the strain isolated from pork samples was not persistent in the factory but was introduced by pork imported from other European countries. The contaminated meat was processed into products used for barbecue such as pork sausages. The hypothesis that such products contributed to the outbreak is supported by the fact that younger people were overrepresented among the infected persons. In this age group barbecue parties during the summer months are very popular and frequently practiced. Considering this particular risk, FOPH published a fact sheet on hygienic rules to be applied in barbecue events on its website [5].

To prevent outbreaks such as described in this paper, measures have to be taken at the meat production level as well. The faecal carriage of foodborne pathogens among livestock animals at slaughter is strongly correlated with the hazard of carcass contamination. In order to reduce the risk represented by *Salmonella*, the maintenance of slaughter hygiene is consequently of central importance in meat production. *Salmonella* sampling on carcasses is regulated in view of slaughter hygiene monitoring in the European Commission Regulation (EC) No 2073/2005 [6]. In the same regulation, microbiological criteria are decreed for *Salmonella* in minced meat and meat preparations from poultry meat intended to be eaten cooked and minced meat and meat preparations from other species than poultry intended to be eaten cooked (absence in 10 g; n=5; c=0) [6]. This regulation was adopted by Switzerland into the national law [7]. For companies, there remains in fact only one option to deal with the new requirements, namely the use of *Salmonella*-free raw materials for certain final products. There are two ways to reach that target. Either only meat that comes from *Salmonella*-free herds is processed or raw meat is analysed with rapid test for the presence of *Salmonella* prior to further processing. If imported meat is used, the producer has to make it clear to the importing company that only *Salmonella*-free meat is accepted. In this way, a certain pressure will build up on farmers and it is there that the problem has to be addressed. For decades, raw meat has been considered unsafe for consumption since it could contain pathogenic bacteria. With the new EU-regulation which demands the absence of *Salmonella* in minced meat or in meat preparations a change of paradigm occurred. There is no doubt that the practical implementation of this regulation will be a costly and long lasting challenge for all involved stakeholders, in particular the livestock keepers who must make efforts to reduce *Salmonella* prevalence.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# Excess of infections due to a multi-drug sensitive *Salmonella* enterica serotype Typhimurium in France in June 2008

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An unusually high number of cases of *Salmonella* Typhimurium was reported in France in June 2008. In the course of epidemiological investigations 112 cases were ascertained, of whom 75 were interviewed. Subtyping by PFGE and MLVA identified a strain named "majority profile". Subtyping results were available for 45 interviewed cases, 30 of whom (majority below 15 years of age) were found to be infected with the majority profile strain. Evidence suggested the occurrence of an outbreak due to a monoclonal *S.* Typhimurium strain with the single PFGE profile XTYM-50. Cases with identical PFGE profile were also detected in Switzerland but no link with outbreaks occurring in the same period in Denmark and in the Netherlands was found. Contamination of a product distributed nationally was suggested as the cause of the outbreak but investigations did not reveal any specific food source.

## Introduction

In the middle of June 2008, several community-based medical laboratories reported an unusually high number of *Salmonella* Typhimurium infections to the French Institute for Public Health Surveillance (Institut de Veille Sanitaire). The laboratories were scattered throughout France and most cases were not linked to each other by a common meal. At that time, national and regional outbreak detection thresholds were not exceeded. Initial sub-typing at the French National Reference Centre for *Salmonella* (Centre National de Référence *Salmonella*, CNR *Salmonella*) revealed that several isolates recently received were susceptible to all antibiotics and exhibited an identical Pulsed Field Gel Electrophoresis (PFGE) and Multiple Loci Variable Number of Tandem Repeats Analysis (MLVA) profile. During the investigation, this profile was then named "majority profile". In the same period, *S.* Typhimurium outbreaks were reported in Denmark [1,2], Switzerland [3] and the Netherlands [4].

We carried out an epidemiological and microbiological investigation in order to confirm the occurrence of an outbreak and, if so, to assess its extent, and to identify a potential link between cases in terms of food or other exposure. We also investigated possible links between notified French cases and the Danish and Swiss outbreaks.

## Methods

A case was defined as a person from whom *S.* Typhimurium was isolated in June or July 2008. Cases were identified by contacting all major laboratories in districts where an increase of cases was reported. Patients were interviewed via telephone using a standardised trawling questionnaire on possible exposures including questions on food consumption (dairy, meat, fish, vegetable, pastry and chocolate products), occurrence of other cases in the family, meals in restaurants or other facilities, and animal contacts in the three days preceding the onset of symptoms. Medical laboratories were asked to send their isolates to the CNR *Salmonella* for PFGE [5] or MLVA sub-typing [6].

The French Food Safety Agency (Agence Française de Sécurité Sanitaire, AFSSA) sub-typed by PFGE the *S.* Typhimurium food isolates that were fully susceptible to all antibiotics and had been received through routine collection since January 2008.

We reviewed point-source food-borne outbreaks due to *S.* Typhimurium that were reported through the mandatory notification system during the period investigated.

We carried out a case-case comparison study among individuals who were interviewed and for whom the strain subtype was available. Cases were individuals infected with the *S.* Typhimurium majority profile strain. Controls were selected among individuals who, during the same period as the cases, were infected with a strain of *S.* Typhimurium with a non-majority profile. One individual for each non-majority profile strain was selected, in order to ensure the highest possible heterogeneity of strain profiles among controls [7]. Selected controls were therefore individuals infected with strains presenting different non-majority profiles.

Data were analysed using Stata 9.2 (College Station, Texas). We calculated univariate odds ratios and their exact 95% confidence intervals to examine the risk associated with each exposure. Differences in categorical variables were compared using the $\chi 2$ Fischer exact test.

## Results

The number of *S.* Typhimurium isolates received by the CNR *Salmonella* in June 2008 was twice the mean number of those received in June of the previous four years (312 isolates versus 115 mean isolates in 2004-2007). With reference to the date of first laboratory diagnosis, the number of cases started increasing in the first week of June 2008, peaked (95 isolates) in the following week, and gradually returned to the expected seasonal values in the second week of July (Figure 1).

A total of 112 cases were ascertained in districts reporting an excess of cases between June and July 2008. Seventy-five were interviewed.

The CNR *Salmonella* sub-typed 90 isolates received between April and July 2008. Fifty-two isolates presented the MLVA "majority profile": 42 isolates with profile STTR3, number of repeats 11 (500 bp), STTR5, number of repeats 17 (282 bp), STTR6, number of repeats 9 (317 bp), STTR9, number of repeats 4 (171 bp), STTR10, no amplification, and 10 isolates with a single difference either in the locus STTR5 or in the locus STTR6. Isolates with the "majority profile" were fully susceptible to the most commonly used antibiotics [5], showed a Xba-I PFGE profile XTYM-50 and had a different PFGE profile than the DT104 *S.* Typhimurium profile. The remaining 38 isolates presented 31 different MLVA profiles.

The isolated strain was sub-typed for 45 interviewed cases. Thirty cases were infected with the majority profile strain and diagnosed between 3 and 22 June 2008; 15 cases were infected with 13 different MLVA profile strains ("control cases") and diagnosed between 13 May and 21 June 2008.

Among the 30 majority profile strain cases, 24 (80%) were below 15 years of age, all, except one child of 1 month of age, were between 1 and 14 years. Age distribution below 15 years was higher in majority profile strain cases, when compared with *S.* Typhimurium cases recorded at the CNR *Salmonella* in the years 2004-2007 (62%), a difference that was very close to statistical significance (p = 0.057). Male/female ratio among the majority profile strain cases was 1.1. Twelve majority profile strain cases (34%) were residents in one district of region Centre. Two further cases were resident in another district of the same region, and eight cases were living in three neighbouring districts of regions Ile-de-France and Haute-Normandie. The other eight majority profile strain cases were scattered in fours different districts of France (Figure 2).

The French majority profile strain corresponded to the dominant Swiss outbreak strain [3], but did not correspond to the Dutch outbreak strain in August 2008 [4]. Neither the majority profile strain nor any other non-majority profile strain sub-typed during this investigation matched with the Danish outbreak profile [1,2].

**Figure 1**

**Comparison of weekly number of *Salmonella* Typhimurium isolates received in 2008 with mean number for the years 2004-2007, by date of first isolation of the strain, CNR Salmonella, Pasteur Institute, Paris, France**

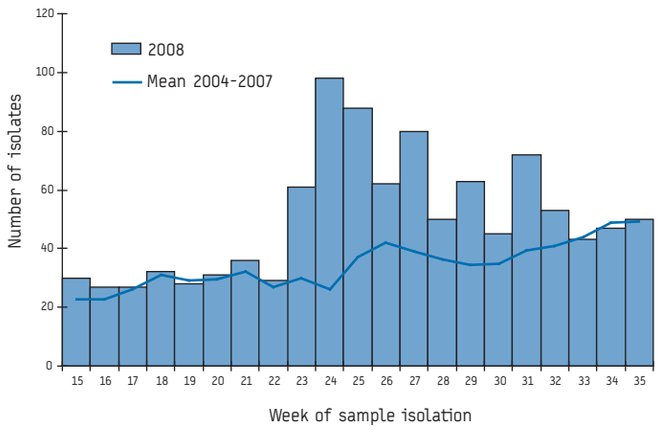
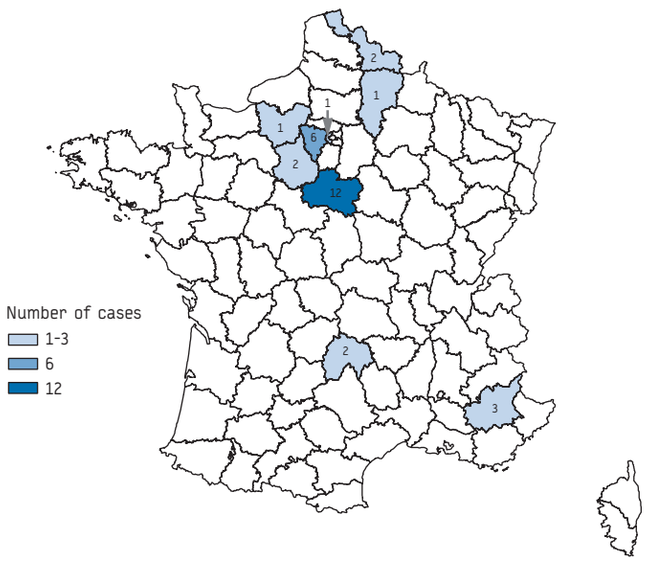



**Figure 2**

**Cases infected with the *Salmonella* Typhimurium majority profile strain PFGE profile XTYM-50, by district of residence, France, June 2008 (n =30)**



We identified one notified point source food-borne outbreak due to the *S.* Typhimurium majority profile strain involving two cousins. However, assessments of family food consumption did not permit identification of any exposure that could be incriminated as source of contamination.

The case-case comparison study was carried out on the 30 majority profile strain cases and 13 controls. Cases and controls did not significantly differ in age, symptoms and hospitalisation rate. No food product or other exposure was significantly associated with the majority profile strain infection.

AFSSA sub-typed 22 *S.* Typhimurium food isolates received through routine collection since January 2008. None of these corresponded to the PFGE profile XTYM-50 (majority profile strain) or to the Danish outbreak profile [1,2].

## Discussion

Available information strongly suggested the occurrence of an outbreak due to a monoclonal *S.* Typhimurium strain with the single PFGE profile XTYM-50 in France in June 2008. This strain may have affected a younger than usual population. Although the majority of cases infected by this strain were concentrated in three regions, other cases were scattered in other French regions, suggesting the contamination of a product distributed nationally. Cases with identical PFGE profile were also found in Switzerland [3], but microbiological assays indicated no link with the outbreaks occurring in the same period in Denmark [1,2] and in the Netherlands [4].

Despite extensive epidemiological and microbiological investigations, we were not able to identify any specific food or other exposure as possible vehicle or way of contamination which could explain the occurrence of this outbreak. Hence no specific control measures could be proposed following this investigation. In July the number of human *S.* Typhimurium isolates reported at the CNR *Salmonella* returned within the expected values for the season.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Centre hospitalier, Mantes-la-Jolie: Florence Richardin.


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# Large outbreaks of *Salmonella* Typhimurium infection in Denmark in 2008

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An outbreak of *Salmonella* Typhimurium phage type U292 has been ongoing in Denmark since 1 April, with 1,054 cases registered until 23 October 2008. Extensive investigations including hypothesis-generating interviews, matched case-control studies, cohort studies in embedded outbreaks, shopping list analyses, analyses of food samples from patient's homes, trace-back analyses and extensive microbiological analysis of products have not provided clear indications of a specific source of infection but the main hypothesis is that the vehicle of the outbreak are different pork products. In addition to the large U292 outbreak, at least four other *S.* Typhimurium outbreaks (caused by phage types U288, DT120, DT3 and DT135) have been investigated in Denmark in 2008.

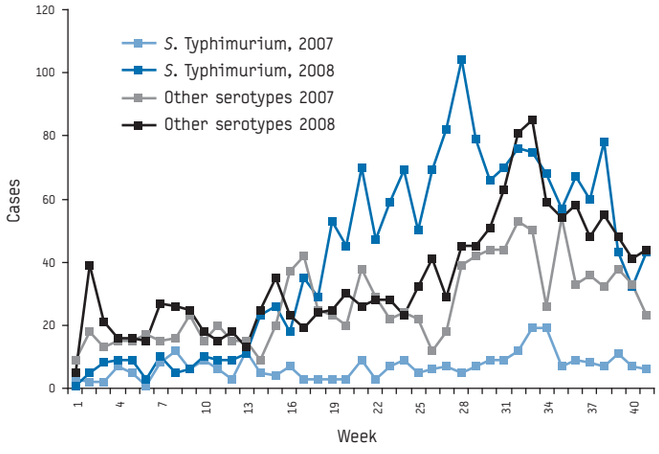
## Introduction

The outbreak caused by *Salmonella* enterica serotype Typhimurium phage type U292 which was detected in April 2008 [1] is still ongoing and the source has not been found. The outbreak includes 1,054 patients as of 23 October 2008, thus being the largest outbreak of salmonellosis in Denmark recorded since 1980 when the present surveillance system became active.

The total number of laboratory-confirmed infections with *S.* Typhimurium (phage type U292 and other phage types) was 1,652 as of 12 October 2008; at the same time in 2007 the cumulative annual number of *S.* Typhimurium infections was 285 (Figure 1). In comparison, the number of *Salmonella* Enteritidis infections registered up to this time of the year (i.e. end of week 41) was 557 in 2008, 473 in 2007 and 497 in 2006 [2].The high number of *S.* Typhimurium infections in 2008 include several distinct outbreaks in addition to the U292 outbreak. This report gives a brief account of the present status of the investigations of the U292 outbreak and presents basic epidemiological facts of the other recent *S.* Typhimurium outbreaks.

**Figure 1**

**Number of cases of *Salmonell*a Typhimurium and other *Salmonella* serotypes registered by Statens Serum Institut in Denmark for 2007 and 2008, by week of submission of stool sample to the laboratory (weeks 1-41)**




## Methods

In Denmark clinical microbiology laboratories are required, within one week, to notify Statens Serum Institut (SSI) of findings of salmonella from patient samples. In addition strains are sent to the SSI and further characterised. Currently, all strains of serotype Typhimurium are subtyped using Multiple Loci Variable Number of Tandem Repeats Analysis (MLVA) as a means of detecting outbreaks [3]; furthermore *S.* Typhimurium strains are phage typed and tested for resistance, and selected strains are typed by Pulsed Field Gel Electrophoresis (PFGE). Clusters of patient-isolates with identical MLVA types are investigated as potential outbreaks. The case definition in the outbreaks described here is by MLVA type.

Investigation of the U292 outbreak has been performed using a number of different methods which include the following: 1) Patient interviews performed using telephone-administered trawling questionnaires, focus group interview and home visits, the latter including recently conducted interviews of cases occurring at the Faroe Islands (which are part of the Danish kingdom). 2) Three separate matched case-control investigations with 29/83, 21/41 and 30/35 case/control sets respectively. 3) Investigations into point source sub outbreaks occurring among groups of people in closed settings, including two outbreaks where it was possible to perform cohort studies with 15/8 and 46/24 ill/healthy respondents respectively. 4) Two rounds of comparative analyses of patients'

shopping lists obtained from supermarket computers with 126 cases invited out of whom data were collected for 41 cases. 5) Case-case analyses of interviewed *S.* Typhimurium cases of different phage types. 6) Early visits to homes of suspected *S.* Typhimurium patients in order to collect and analyse samples of food items which might have been eaten prior to onset of symptoms. 7) A large number of trace-back analyses of suspect food products, trade patterns and connections between herds in addition to geographical analyses. 8) Comparative molecular subtyping of patient-isolates with isolates obtained from food, animals and slaughterhouses in Denmark. 9) And finally, investigations, including sampling and microbiological analyses, into many domestic food production facilities and slaughterhouses of which some were selected based on epidemiological leads and some following a structured risk ranging approach.

## Results

### Outbreak of *S.* Typhimurium phage type U292

The first cases of the U292 outbreak reported onset of illness in February. Over the following three months the weekly number of cases increased and since May has stayed at the level of 30-60 cases per week (Figure 2). The age distribution is skewed towards younger age groups; the median age is 15 years. For comparison 70% of *S.* Typhimurium cases registered in previous years had been older than 15 years of age. The gender distribution is almost even, with 53% female cases. Cases have occurred in almost all parts of the country, but are not evenly distributed among the regions. Nine persons infected with the outbreak strain are known to have died; however, these patients had severe underlying illnesses. The strain is fully susceptible to all antibiotics in the test panel and does not appear to cause severe symptoms; the hospitalisation rate is between 15 and 20%.

Close to 500 cases have been interviewed as part of the different investigations. No vegetarians or persons specifically reporting never to eat pork have been identified in the course of these interviews. Judging by the names of patients, among those who have not been interviewed we have not been able to identify any persons originating from countries where people are predominantly Muslim. The outbreak appears to be confined to Denmark; U292 is a rare phage type and clusters of cases have not been reported from other countries. Less than 10 cases (not counting 14 cases from the Faroe Islands) from outside of Denmark have been detected; they originated from Norway, Sweden and Canada and all, except one, had become infected while staying in Denmark for more than one week.

The analytical epidemiological investigations have largely been inconclusive and not been able to provide a clear indication of the source. Restaurant outbreaks or cases associated with canteens or similar facilities have not been detected, but four distinct embedded outbreaks are known and there are several occurrences of multiple cases within families. The outbreak strain has been found in pork from a major Danish slaughterhouse, in clinically ill calves or cows at three separate farms and at a broiler farm, in addition to food products of pork origin obtained from the home of a case family, but under circumstances that did not allow for epidemiological conclusions to be drawn. *S.* Typhimurium U292 with the same resistance pattern (fully susceptible) and same PFGE pattern (using XbaI), but with a MLVA type differing in two loci, has been found in a number of Danish pig herds within recent months.

### Outbreaks of other *S.* Typhimurium phage types

In addition to the large U292 outbreak, at least four other *S.* Typhimurium outbreaks have been investigated in Denmark in 2008 (Figure 3). Outbreak 1 was caused by a strain of phage type U288. It comprised 37 cases and occurred from March to May. Cases were predominantly living near Denmark's second largest town, Århus, and epidemiological investigations showed a clear link to a group of kebab restaurants located in Århus. The precise mechanism of transmission of the infections was not found. U288 is a rare phage type in humans in Denmark, but is known to have been present for many years among pig herds in Denmark.

The three other outbreaks were not geographically restricted. Outbreak 2 was caused by a strain of phage type DT120. There

**Figure 2**

**Cases of *Salmonella* Typhimurium U292, with the outbreak MLVA type, by week of submission of stool sample to the laboratory, Denmark 2008, (n=1,054 as of 23 October)**

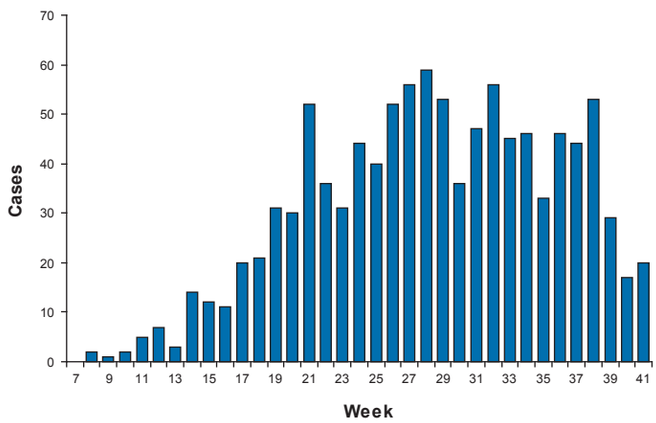
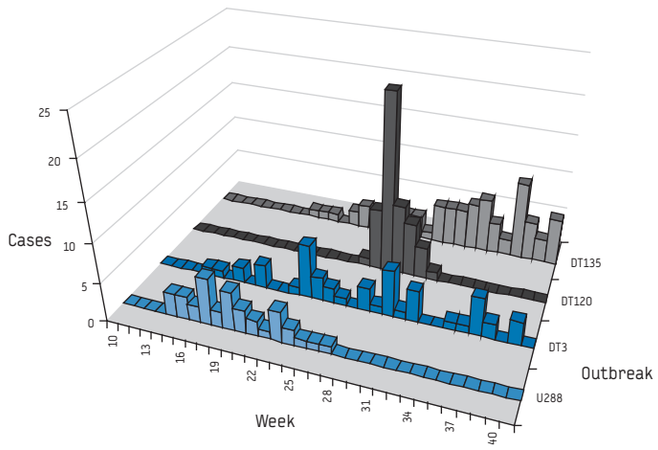



**Figure 3**

**Registered cases of *Salmonella* Typhimurium associated with four different outbreaks (U288, DT120, DT3 and DT135), by week of submission of stool sample to the laboratory, Denmark 2008 (n=214, as of 12 October)**



were 55 cases predominantly in June and July. As a side-result of investigations into the U292 outbreak, a Danish-produced smoked ham collected from the refrigerator of a case was found positive for this outbreak strain and hence it is believed that this outbreak was caused by consumption of products of the same brand.

Outbreak 3 is caused by a strain of which the majority of isolates have been found to be of phage type 3. Low numbers of cases have been detected since the beginning of the year and are still occurring; currently a total of 50 cases have been registered. A clear hypothesis as to the source of this outbreak does not exist.

Outbreak 4 caused by a strain of phage type DT135 is ongoing. Up to now 77 cases have been registered, predominantly since June. This outbreak shares a number of the epidemiological characteristics of the U292 outbreak. Investigations into this outbreak are ongoing.

## Conclusions

The results of the investigations into the U292 outbreak indicate that the outbreak is not caused by a single type of food vehicle. The main working hypothesis continues to be that the outbreak originates from pigs, but it should be stressed that an association with pork or pork products has not been proved and that other hypotheses are also being actively investigated.

Circumstantial evidence pointing towards pork as the source of the U292 outbreak include: Very high exposure to pork among interviewed cases, apparent absence of cases that would refrain from eating pork out of religious beliefs or vegetarianism, findings of the outbreak strain in pork and of closely related strains in domestic pig herds and the lack of strong competing hypotheses. A number of large salmonella outbreaks in Denmark have previously been associated with pork [4-8], however, except for one instance, case-control studies have failed to provide evidence for these links [6].

Among the non-U292 outbreaks, the one caused by *S.* Typhimurium DT120 was likely to be associated with Danish produced salted, smoked and cooked ham. It is possible that some of the increased numbers of infections with *S.* Typhimurium observed in Denmark, including the currently ongoing outbreak of *S.* Typhimurium DT135, are also associated with consumption of pork or pork products, which would point to the same general food safety problem. However, due to lack of clear evidence more definite conclusions leading to possible control measures are not possible at this stage of the investigations.


## Acknowledgements

A large number of persons have been involved in these outbreak investigations, including regional food control officers, medical officers, staff from the clinical, typing, food and veterinary laboratories and other members of the National Food Institute, Statens Serum Institut, Veterinary and Food Administration, National board of Health and Regional Veterinary and Food Control Authorities.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# *Salmonella* Typhimurium outbreaks in the Netherlands in 2008

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A large, countrywide outbreak due to multi-resistant *Salmonella* Typhimurium phage type DT104 is ongoing in the Netherlands, with 152 cases as of 20 October. Pilot interviews did not suggest any specific source of infection but a hypothesis pointing to pork products has been formulated and a large case-control study is under way. Earlier this year two other oubtreaks due to *S.* Typhimurium were detected and investigated, the first (DT15A) linked to a particular brand of cream cheese, the other (Dutch phage type ft507) to a local butcher.

## Introduction

In August 2008, a marked increase in the number of reported infections with multi-resistant *Salmonella* enterica serotype Typhimurium phage type DT104 was observed in the Netherlands. The outbreak is still ongoing, with 152 patients included as of 20 October 2008. The outbreak strain is resistant to ampicillin, tetracycline, co-trimoxazol, streptomycin and chloramphenicol and is also less susceptible to ciprofloxacin (minimum inhibitory concentration – MIC 0.25) and nalidixic acid (MIC > 64). Of the patients, more than 20% were hospitalised. Cases are distributed countrywide and no travel-related cases have been reported. The age distribution is similar to that of sporadic *S.* Typhimurium cases and the sex ratio male / female is 1.0. A case-control study is currently being performed. In this report we shortly review the present status of the investigation of the DT104 outbreak and we describe the investigations of two other recent *S.* Typhimurium outbreaks.

## Methods

This outbreak investigation used the Dutch laboratory-based salmonella surveillance at the National Institute for Public Health and the Environment (Rijksinstituut voor Volksgezondheid en Milieu – RIVM) as a source of laboratory data on *S.* Typhimurium DT104 cases and descriptive statistics with regard to age, gender and place of residence of the patients [1]. All strains were subtyped using Multiple Loci Variable Number of Tandem Repeats Analysis (MLVA) and Pulsed Field Gel Electrophoresis (PFGE).

Between 10 and 17 September, trawling interviews with eight recent DT104 cases were performed by telephone using a standardised questionnaire. These interviews covered consumption of different meats, fish, dairy products, vegetables and fruits, establishments where food was purchased and contact with animals in the seven days before onset of illness.

A case control study was started on 22 September. In the case-control study a case was defined as a person in whom *S.* Typhimurium DT104 was isolated after 25 August 2008. Local public health services were asked to contact the cases (after approval of the laboratories and treating physician) to collect their e-mail addresses or, if not available, their home address. Questionnaires were sent to the cases by e-mail using Questback or by post. 240 frequency-matched controls (matched for age, gender and degree of urbanisation) were selected from the Dutch population register and were sent a postal questionnaire. In addition, cases were asked to nominate two controls of the same age (less than 5 years difference) and not living in the same household.

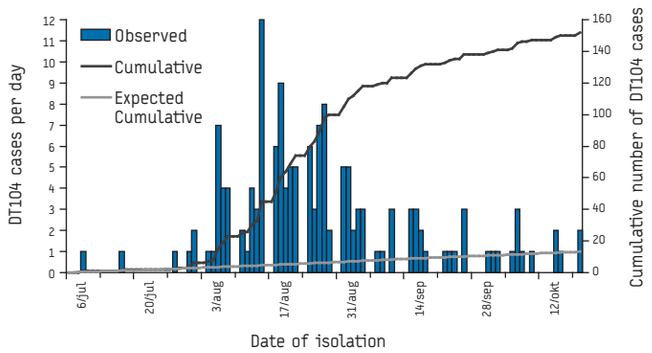
## Results

### Outbreak 1: *Salmonella* Typhimurium DT104

The first cases of the DT104 outbreak were reported in the beginning of August 2008. The number of cases clearly exceeded the expected cumulative number of cases based on a 5-year time series analysis (Figure 1). By September, the weekly number of cases declined, but the outbreak is still ongoing with 5 to 10 cases reported each week. The age distribution is similar to that observed in sporadic cases of *S.* Typhimurium DT104 in the Netherlands and the gender distribution is even (Figure 2). No regional clustering of cases was observed.

**Figure 1**

**Number of *Salmonella* Typhimurium DT104 isolates by date of isolation, the Netherlands 2008 (n=152)**



MLVA typing of 117 strains showed several MLVA types of which type 02-07-12-10-03 dominated: 62 strains had this MLVA type and 36 strains differed only on one locus, of which 20 strains had MLVA type 02-07-12-10-00. These in total 98 strains were considered as related. This MLVA type had not been found in the Netherlands before. All isolates shared the same PFGE profile.

The PFGE profile and the dominant MLVA type were compared to those in databases in other countries. The dominant MLVA type was also found in one patient from Denmark who became ill on the first of August after consumption of sliced ham from a well-known Dutch exporting butcher. Furthermore, in an outbreak in West London in the beginning of August an MLVA type was found that differed on one locus from the dominant MLVA type, but the source of the outbreak was unknown [personal communication with Chris Lane and Tansy Peters, Health Protection Agency, United Kingdom].

The trawling interviews with eight cases did not lead to a clear hypothesis about the possible source of infection, but it appeared that fish and dairy products and contact with animals were unlikely as sources of infection. Subsequently, a case-control study was started to further explore possible sources and to ask detailed questions on food items mentioned frequently in the trawling interviews. In the case-control questionnaire, we reduced the number of questions about consumption of fish and dairy products and contact with animals and we added more detailed questions about other food items, including consumption of sliced ham. In total, 75 cases matched the case definition for the case-control study. So far, 36 cases (48%) have completed the questionnaire and another nine cases have been invited by e-mail. Ten of the 36 cases (28%) had been hospitalised. Of the 240 community controls, 60 (25%) have completed the questionnaire to date. Cases nominated only eight controls and six of them completed the questionnaire. We are awaiting the results of the analysis of the case-control study, which will be done in the following weeks. So far, no clear conclusion could be drawn from the case questionnaires.

In addition to the DT104 outbreak, two other *S.* Typhimurium outbreaks have been investigated in the Netherlands in 2008.

**Outbreak 2: *Salmonella* Typhimurium DT15A**

In March 2008, a countrywide outbreak of *S.* Typhimurium DT15A was detected: 27 cases were identified, whereas only four cases of this phagetype occurred in the past five years. 63% of the cases were below six years of age. Of the cases older than 15 years, 83% were women. Of the 19 interviewed cases, 16 (84%) reported consumption of cream cheese of a brand that is very popular among young children. Instead of comparing with controls in a case-control study, we compared the information of the cases with results from the Food Consumption Survey performed in 2005 and 2006 among 1700 children aged 2-6 years. This supported the hypothesis that cream cheese of a specific brand was the likely source of infection. The Dutch Food and Consumer Product Safety Authority did not find any abnormalities when visiting the producer. The exact methodology of this investigation will be published in more detail in a forthcoming short report.

**Outbreak 3: *Salmonella* Typhimurium (Dutch phagetype ft507)**

In the middle of June 2008, a local outbreak of *S.* Typhimurium (Dutch phagetype ft507) in the south-west of the Netherlands was detected. Patient interviews showed a clear link to a local butcher. The exact vehicle of transmission of the infections remained unknown. The Dutch Food and Consumer Product Safety Authority tested several meat products and environmental swabs for the presence of *Salmonella*, but all were negative. In total, 18 laboratory-confirmed cases were identified between 30 May and 14 June.

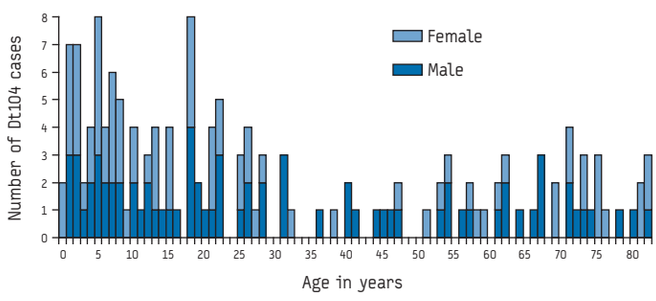
## Conclusion

A large, countrywide *S.* Typhimurium DT104 outbreak is still ongoing in the Netherlands. As the outbreak strain is multi-resistant and has reduced susceptibility to ciprofloxacin, it causes severe symptoms and the hospitalisation rate is high. The outbreak is currently under investigation. Pilot interviews did not lead to a clear hypothesis. However, fish, dairy products and contact with animals were less likely sources of infection. One hypothesis comes from a matching MLVA-type from a patient in Denmark who consumed ham from a Dutch exporting butcher. So far, this is the only lead to a possible source of infection. The case-control study should reveal whether ham is a likely source.

Earlier in 2008, we experienced two other *S.* Typhimurium outbreaks in the Netherlands. A regional outbreak in June was related to a local butcher, but the exact vehicle of infection was not identified. Another nationwide outbreak in March was likely associated with cream cheese of a specific brand. Several other European countries have experienced *S.* Typhimurium outbreaks of various subtypes this year. Denmark faced four outbreaks and is currently experiencing a large-scale nationwide outbreak of *S.* Typhimurium U292. In spite of extensive investigations, the source or sources of infection have not yet been identified, but the main hypothesis is that the source is one or more pork products [2,3]. In February, *S.* Typhimurium U292 was found in a pig in the Netherlands, but no further link with the Danish outbreak was found. Outbreaks in Switzerland and France in May to July shared the same strain [4,5]. The Swiss investigation revealed that pork was the probable source. Microbiological data indicated that the Dutch outbreaks were not related to any of the outbreaks occurring in Switzerland, France and Denmark in the same period.

**Figure 2**

**Age and gender distribution of registered cases in the *Salmonella* Typhimurium DT104 outbreak, the Netherlands, 2008 (n=152)**

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# Low coverage of seasonal influenza vaccination in the elderly in many European countries

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In May 2003, the 56th World Health Assembly (WHA) recommended influenza vaccination for all people at high risk defined as the elderly and persons with underlying diseases [1]. The WHA countries, including all European Union (EU) Member States, also committed to the goal of attaining vaccination coverage of the elderly population of at least 50% by 2006 and 75% by 2010 and to having mechanisms for monitoring the uptake [1]. To date there has been no published survey on how successful European countries have been in implementing this WHA resolution.

According to the Statistical Office of the European Communities (Eurostat), 84.6 million EU citizens, 17.1% of the EU population, are currently aged 65 years or older. It is estimated that by 2010 as many as 86.7 million people will be in this age group. If EU countries are to achieve the 75% vaccination coverage rate, this will correspond to vaccinating approximately 65 million people [2].

The Vaccine European New Integrated Collaboration Effort (VENICE, http://venice.cineca.org/) project was launched in January 2006. Funded by the European Commission and supported by the EU Member States and the European Centre for Disease Prevention and Control (ECDC) it has established a network of experts who work with national immunisation programmes as national 'gatekeepers' in every EU country plus Iceland and Norway. The project carries out several activities, including performing surveys and undertaking scientific research in the field of public health regarding vaccination policies and performance for a number of infections [3] .

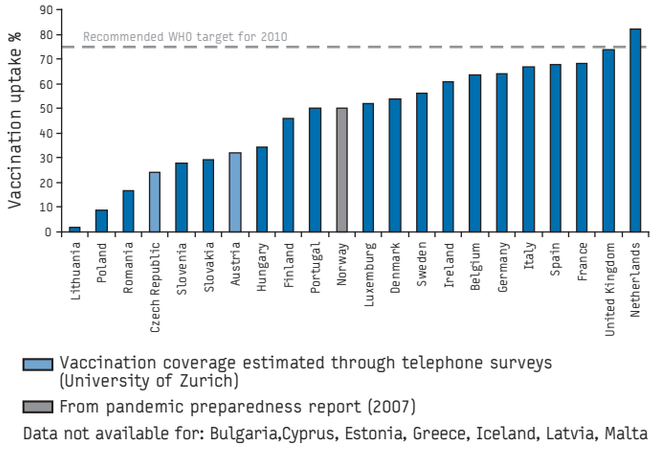
In late 2007 at the request of ECDC the project members undertook a survey of national influenza immunisation programmes, policies and performance in Europe. This was a collaborative study between the ECDC, the VENICE project and the EU and European Economic Area (EEA) countries. Each country had previously identified and enrolled gatekeepers responsible for conducting all VENICE surveys internally within the countries and for liaising with the ministries of health. Data presented in this paper is released ahead of the main reports because the results are relevant to the annual vaccination campaigns in Europe which are presently underway ahead of the 2008-9 winter epidemics with elderly people being the largest target group.*

## Methods

A standardised questionnaire was used to collect information describing seasonal influenza vaccination policies and performance during the 2006-7 influenza season in Europe. The various objectives of the study, the methods and the results are described in detail in an article submitted to Eurosurveillance and in a formal report to be published on the website of the European surveillance network for vigilance against viral resistance (VIRGIL) [4,5]. Some of the data items were collected to obtain the most recent estimates of the levels of seasonal influenza immunisation among the elderly.

**Figure 1**

**Vaccination coverage for seasonal influenza vaccine in the elderly (65 years and older) in EU and EEA countries, season 2006-2007 (data from VENICE survey and other sources, as of March 2008)**



Data were obtained from national sources as made available by the national gatekeepers. Each country then validated the results and ensured that the ministries of health were aware of the overall results by sending them the full report [5].

## Results

Data on influenza vaccine uptake in the elderly were available for 19 countries out of the 29 members of VENICE. The remaining 10 countries reported that they had not collected such data. For seven of these countries, Bulgaria, Cyprus, Estonia, Greece, Iceland, Latvia and Malta, no other sources of data were available. For two, Austria and the Czech Republic, data could be obtained from telephone surveys conducted by the University of Zurich [6]. For Norway data were available from a published national pandemic preparedness self-assessment undertaken with ECDC [7]. As a result, data on immunisation coverage in the elderly were available for 22 European countries (Figure 1).

Only one country, the Netherlands, reached the WHA 2010 target of 75% coverage in the elderly and another, the United Kingdom, was just below this target at 74%. Further nine countries met the 2006 target of 50%. However, the remaining eleven countries (half of those for which data were available) failed to pass the 2006 target of 50% coverage in 2006-7. A number of countries are doing especially poorly, many of them countries that joined the European Union more recently.

## Discussion

The results show that the likelihood that an elderly European citizen is immunised against influenza is related to his or her country of residence.

The reasons for such wide variations in vaccination uptake are not clear. This information was not sought in our study. Further research is needed to determine underlying reasons.

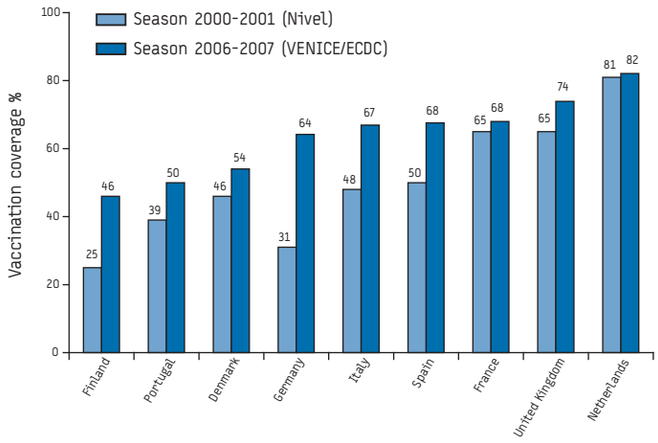
Comparison with an earlier published survey with data from 2000-2001 shows encouraging increases for seven countries over the six years (Figure 2). However it has been suggested that as countries reach higher levels the total rates plateau at or below 75% [8]. This suggestion is supported by the telephone surveys conducted by the University of Zurich using the same methodology for six seasons and five countries: France, Germany, Italy, Spain and the UK [6].

Comparison of our data for 2006-7 with the figures for North America where the United States (US) coverage in the elderly for the same season was estimated to be 65.6% [9] indicates that while some European countries are doing better than the US, Europe as a whole is lagging behind.

It should also be recalled that the 75% target is entirely arbitrary. The immunisation strategy for preventing human seasonal influenza aims at protecting vulnerable individuals rather than trying to achieve herd immunity and reduce transmission in the community [10]. Some groups are more likely to develop severe disease and die as a result of influenza infection and ECDC estimates that at least 40,000 deaths in Europe annually, many of these in the elderly, are attributable directly or indirectly to influenza [11]. With that in mind the only real target for risk groups should be at or approaching 100%. It is of equal concern that while in 2000-1 season, 14 out of 23 European countries could monitor the coverage in the elderly, six years later this number had only increased to 19 out of 29. The fact that ten European countries still do not have any system in place with which to estimate uptake in this high risk group is worrying and suggests that Europe will struggle to achieve the WHA target for 2010 or even to produce good statistics for all its countries.

**Figure 2**

**Reported influenza vaccination uptake among the elderly in nine European Union countries, survey results for seasons 2000-1 (Nivel) and 2006-7 (VENICE/ECDC)**





## Acknowledgments

We would like to acknowledge all VENICE gatekeepers and contact points who contributed to conducting this study, and Patricia Blank, Thomas Szucs and Matthias Schwenkglenks and the Universities of Zurich and Basel for permission to use their data in Figure 1.


*Editors' note: Eurosurveillance has agreed with the authors to publish the data in this rapid communication ahead of a later full-length article covering the study in greater detail, recognising the public health need to have this information in the public domain in the beginning of the annual influenza immunisation season. We believe that this decision conforms to the principles of secondary publications as agreed by the International Committee of Medical Journal Editors (http://www.icmje.org/index.html).

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# HIV/AIDS surveillance in Europe: update 2007

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Human immunodeficiency virus (HIV) infection remains of major public health importance in Europe, with evidence of increasing transmission of HIV in several countries. This article provides an overview of HIV and acquired immunodeficiency syndrome (AIDS) surveillance data, and indicates that since 2000 the rate of newly reported cases of HIV per million population has almost doubled in Europe. In 2007, a total of 48,892 cases of HIV infection were reported from 49 of 53 countries in the Region, with the highest rates in Estonia, Ukraine, Portugal and the Republic of Moldova. In the European Union (EU) and European Free Trade Association (EFTA) countries, the predominant mode of transmission for HIV infection is sex between men followed by heterosexual contact. Injecting drug use is still the main mode of transmission in the eastern part of the WHO European region, while in the central part heterosexual contact is the predominant mode of transmission. In 2007, the reported number of AIDS cases diagnosed decreased in the Region overall, except in the eastern part. HIV/AIDS surveillance data are vital to monitor the trends of the HIV epidemic and evaluate public health responses.

## Introduction

Since January 2008, the European Centre for Disease Prevention and Control (ECDC) and the World Health Organization Regional Office for Europe have been jointly carrying out the HIV/AIDS surveillance in Europe [1]. This article presents the main findings for the whole WHO European Region, the three geographical regions of the WHO European Region (West, Centre and East)* and the European Union (EU) and European Free Trade Association (EFTA) countries.

## HIV case reports in WHO European Region

In 2007, 48,892 newly diagnosed HIV cases (76 per million population) were reported from 49 of the 53 countries in the WHO European Region (no data from Austria, Italy, Monaco and the Russian Federation). In the three parts of the WHO European Region, the rate of newly reported cases of HIV per million population was highest in the East (Table 1); whereas among individual countries, the highest rates were reported in: Estonia (472 per million), Ukraine (285 per million), Portugal (217 per million) and the Republic of Moldova (204 per million). Between

Table 2

**Characteristics of newly diagnosed cases of HIV infection reported in the EU/EFTA countries*, 2007**

| | EU/EFTA countries* |
|---|---|
| Number of HIV cases | 26279 |
| Rate per million population | 64.1 |
| Percentage of cases: | |
| Age 15–29 years | 28% |
| Female | 31% |
| Transmission mode** | |
| Heterosexual*** | 29% |
| Men who have sex with men | 39% |
| Injecting drug users | 9% |

* Missing data: Italy, Austria.
** Transmission group unknown is excluded in the percentages.
*** Excludes persons originating from countries with generalised epidemics (4422 in total).

Table 1

**Characteristics of newly diagnosed cases of HIV infection reported in the WHO European Region and by geographical area, 2007**

| | WHO European Region* | West* | Centre | East* |
|---|---|---|---|---|
| Number of HIV cases | 48892 | 24202 | 1897 | 22793 |
| Rate per million population | 76.4 | 77.0 | 10.1 | 164.8 |
| Percentage of cases: | | | | |
| Age 15–29 years | 33% | 26% | 41% | 40% |
| Female | 33% | 31% | 24% | 36% |
| Transmission mode** | | | | |
| Heterosexual*** | 36% | 29% | 53% | 42% |
| Men who have sex with men | 20% | 40% | 30% | 0.4% |
| Injecting drug users | 32% | 8% | 13% | 57% |

* Missing data: Austria, Italy, Monaco, Russian Federation.
** Transmission group unknown is excluded from the percentages.
*** Excludes persons originating from countries with generalised epidemics (4555 in total; 4540 in West).

2000 and 2007, the annual rate of newly reported cases of HIV per million population has increased from 39 to 75 per million (90% increase) among the 44 countries that have consistently reported.

### HIV case reports in the EU/EFTA

In 28 of the 30 EU/EFTA countries, 26,279 cases of HIV infection (64 per million) were reported in 2007 (Table 2), with the highest rates reported in Estonia (472 per million), Portugal (217 per million) and Latvia (149 per million). The predominant mode of transmission is sexual contact between men (39%), followed by heterosexual contact (29%), when persons originating from countries with generalised epidemics are excluded. Injecting drug use accounted for 9% of newly reported infections. Among the countries that have consistently reported, the rate has increased from 44 per million in 2000 to 58 per million in 2007. Rates of reported HIV infection have doubled in Bulgaria, Czech Republic, Hungary, the Netherlands, Slovakia, Slovenia, Sweden and the United Kingdom.

The number of HIV reports among men who have sex with men (MSM) has increased by 39% between 2003 and 2007 (Figure 1). The number of heterosexually acquired cases has remained fairly stable at around 6,000 cases (although higher numbers were reported in 2004-2006). Further, the number of cases originating from countries with generalised epidemics amongst heterosexually acquired cases varied between 5,000 in 2005 and 4,400 in 2007. The number of HIV reports among injecting drug users (IDUs) has declined by 30% between 2003 and 2007.

### HIV case reports by geographical area

The HIV epidemics across the three geographical areas show remarkable differences (Figure 2).

The data suggest that the HIV epidemic in the western part of the WHO European Region is characterised by a continuing increase in sexual transmission of HIV infection. The distribution of transmission modes largely mirrors that described for the EU/EFTA countries. In 2007, 24,202 new cases of HIV infec¬tion (77/million) were reported from 20 countries (Table 1).

The HIV epidemic in the central part of the WHO European Region remains at low and stable levels (1,897 cases; 10 per million), although there is evidence of increasing sexual (both heterosexual and homosexual) transmission in many countries (Table 1). Heterosexual transmission accounted for 53% of all reported cases, followed by 30% cases reported among MSM and 13% cases among IDUs, data on transmission mode were missing for 33% of cases.

In the eastern part of the WHO European Region, in 2007, 14 countries reported 22,793 new HIV cases (165 per million), of which 58% were from Ukraine. The predominant mode of trans¬mission in this region is through IDUs, accounting for 57% of the reported cases. Between 2000 and 2007, the rate of newly reported HIV infections has in¬creased from 54 per million to 160 per million. However, the numbers in this region are greatly underestimated as no data were reported from the Russian Federation.

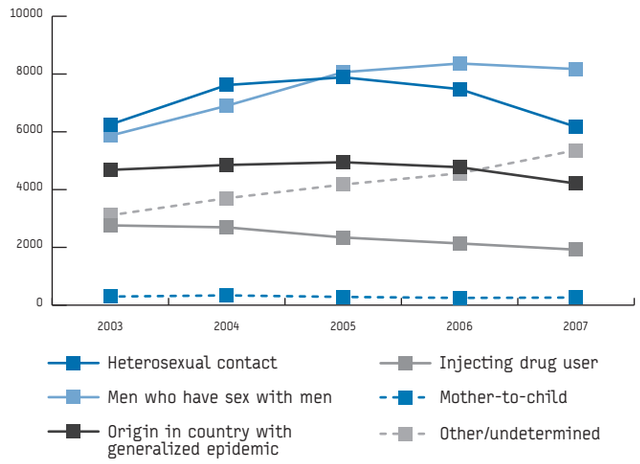
### AIDS diagnoses

In 2007, 5,244 AIDS cases were reported as being diagnosed in 48 of the 53 countries (9 per million) in the WHO European Region (no data from Italy, Kazakhstan, Monaco, Russian Federation and Ukraine). Due to incomplete reporting and no adjustment for reporting delays the total number of AIDS cases is underestimated.

Trends in AIDS diagnoses per million population (Figure 3) have continued to decrease in the WHO European Region overall, from 16 per million in 2000 to 9 per million in 2007, mainly due to decrease in western and central regions probably due to a combination of reporting delay and the effect of highly active antiretroviral therapy (HAART) [2]. However, during the same

**Figure 1**

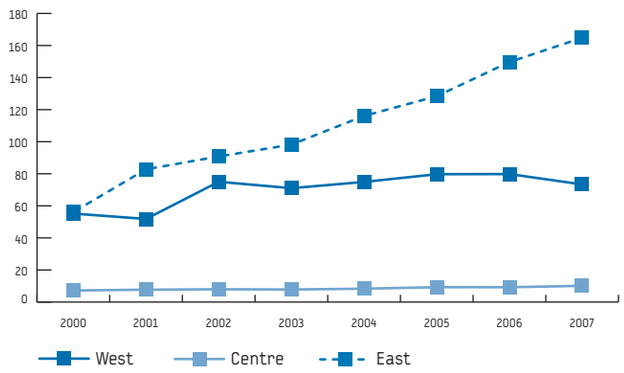
**Number of reported HIV infections by transmission mode, origin and year of notification, EU/EFTA, 2003–2007**




Data were not available for: Austria, Estonia (except for IDU), Italy, and Malta.

**Figure 2**

**HIV cases per million population in geographic areas of the WHO European Region (West, Centre, East) by year of notification, 2000-2007**




Data not included from: West: Andorra, Austria, France, Italy, Malta, Monaco, Spain; Centre: Serbia; East: Russian Federation.

period, the rate increased in 21 (mainly eastern) countries, with the largest increases in Belarus and the Republic of Moldova.

## Discussion and conclusion

HIV infection remains of major public health importance in Europe with a continued increase in the number of HIV cases reported [1,3]. In contrast, the number of AIDS cases diagnosed (not adjusted for reporting delays) has continued to decline, except in the eastern part of the WHO European Region. The data suggest evidence of increased transmission of HIV in many countries. However, the predominant transmission group varies by country and geographical area and the data illustrate the wide diversity in the epidemiology of HIV in Europe.

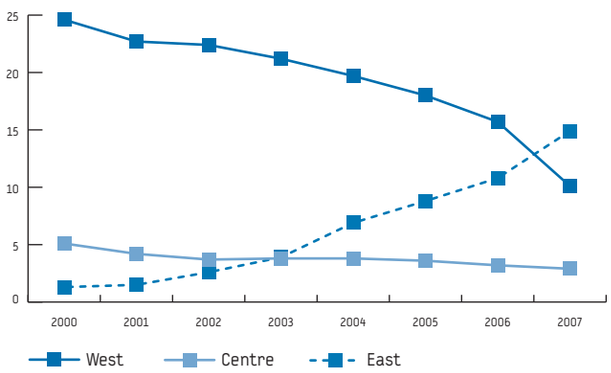
In 2007, in the EU/EFTA countries, also reflecting the western part of the WHO European Region, the highest proportion of HIV cases was reported among MSM. National prevention programmes aimed at reducing HIV transmission within Europe should have a strong focus on MSM [4]. Migrant populations should also be targeted in national prevention programmes and access to treatment and care services should be ensured. Although there seems to be a decline in the number of new diagnoses among IDUs, this is still the predominant transmission group in the Baltic States. In the central part of the WHO European Region, levels of HIV remain low and stable, al¬though there is evidence of increasing sexual transmission in many countries. In the eastern part, the number of HIV cases has increased substantially, mainly driven by an increase in cases acquired through IDU but also by an increase in heterosexually-acquired cases. Interventions to control HIV among IDUs should be the cornerstone of HIV prevention strategies in the eastern part but measures should also be strengthened to prevent heterosexual transmission, especially targeted at those with high-risk partners.

In interpreting the presented data, it should be taken into account that data are incomplete due to non-reporting from a few large countries. Therefore the findings and conclusions are limited to the surveillance data reported by these 49 countries. Had all data from all countries been available, the total number of reported HIV infections could have doubled to almost 100,000 cases in 2007.

Surveillance of HIV/AIDS is essential to monitor the epidemic and evaluate the public health response to control the transmission of infections. Countries in Europe need to ensure that surveillance data is of high quality by implementing case-based reporting systems for HIV and AIDS cases and ensuring its completeness, especially regarding the probable mode of transmission. Achieving full coverage of reporting from all countries in Europe is of utmost importance.

**FIGURE 3**

**Number of diagnosed AIDS cases per million population in the geographic areas of WHO European Region (West, Centre, East) by year of diagnosis, 2000-2007**




Data not included from: West: Andorra, Italy, Monaco; East: Kazakhstan, Russian Federation, Ukraine

*The WHO European Region comprises:

The West, 23 countries: Andorra, Austria (EU), Belgium (EU), Denmark (EU), Finland (EU), France (EU), Germany (EU), Greece (EU), Iceland (EFTA), Ireland (EU), Israel, Italy (EU), Luxembourg (EU), Malta (EU), Monaco, the Netherlands (EU), Norway (EFTA), Portugal (EU), San Marino, Spain (EU), Sweden (EU), Switzerland (EFTA), United Kingdom (EU).

The Centre, 15 countries: Albania, Bosnia and Herzegovina, Bulgaria (EU), Croatia, Cyprus (EU), Czech Republic (EU), Hungary (EU), the Former Yugoslav Republic of Macedonia, Montenegro, Poland (EU), Romania (EU), Serbia, Slovakia (EU), Slovenia (EU), Turkey.

The East, 15 countries: Armenia, Azerbaijan, Belarus, Estonia (EU), Georgia, Kazakhstan, Kyrgyzstan, Latvia (EU), Lithuania (EU), Republic of Moldova, Russian Federation, Tajikistan, Turkmenistan, Ukraine, Uzbekistan.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# Cluster of imported malaria from Gambia in Finland – travellers do not listen to given advice

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Twelve Finnish tourists contracted falciparum malaria from Gambia in the period between 3 and 27 November 2008. The travellers came from different parts of Finland and all except one had booked the trip from the same travel agency. Ten of them had received information about the risk of malaria in Gambia and protection from mosquito bites but none of them had used adequate malaria chemoprophylaxis.

Twelve Finnish tourists contracted falciparum malaria from Gambia in the period between 3 and 27 November 2008. Three of the patients needed intensive care due to complications. In all these cases there was a delay of at least four days in seeking treatment. All patients recovered.

Usually, between 20 and 30 cases of imported malaria are diagnosed every year in Finland (population 5.3 million); most of them are contracted in Africa. In 2007, three cases were imported from Gambia. The cluster of twelve patients described here raises the total number in 2008 to 36 cases to date.

All twelve patients were thoroughly interviewed. The age distribution was 27-66 years, seven patients were male and five were female. The travellers came from different parts of Finland, and they stayed in different tourist resorts in Gambia, approximately 20 km from the capital city Banjul. One traveller resided in the countryside for one month. The other patients stayed in Gambia for one to two weeks.

All except one had booked the trip from the same travel agency. Five travellers had booked a last-minute trip (booking less than five days before departure). One had bought the trip ten days before, and five more than three weeks before the departure. Booking information for the last case was not available.

Ten of the patients had received information about the risk of malaria in Gambia and knew that prophylaxis using anti-malarial drugs was recommended. Six travellers got the information from the travel agency, two from the internet, one had previous information and one was a healthcare professional. Two travellers had not been informed about the risk of malaria; one of them had booked the trip by telephone, the other one on the internet.

None of the patients had used adequate malaria chemoprophylaxis. Three patients had used chloroquine, which is not recommended prophylaxis for tropical Africa. One of them got the prescription from a physician and two of them used chloroquine - against professional advice - stored from a previous trip. Nine travellers did not use any chemoprophylaxis. One of them was prescribed adequate prophylaxis but did not take it because of warnings about side-effects he had read on the internet.

Ten of the patients had either received information on protection from mosquito bites from the travel agency or had found it on their own from various sources. There was wide variation, however, on how the instructions were followed.

The only travel agency organising package trips to Gambia takes approximately 5,000 Finns yearly to Gambia in the period from mid-October to mid-April. The number of travellers has not increased in the last few years. The National Public Health Institute informed clinical practitioners and sent out a press release about the situation on 14 November 2008, which has had wide media coverage. The travel agency discontinued selling last-minute trips to Gambia immediately and has decided to sell trips no later than two weeks prior to departure and to put extra effort into informing the travellers.

The number of Finnish travellers to this region of Africa has not increased in the last few years. Interestingly, there have been similar clusters of falciparum malaria in travellers returning from Gambia also in other European countries; in total, more than 39 travellers were reported to TropNetEurop and GeoSentinel [1]. It is not clear, if this increase in malaria cases is related to a higher malaria activity in Gambia or to a decrease in compliance with protective measures or in risk awareness among travellers purchasing last-minute package tours. In fact, a recently published analysis showed that the risk to acquire malaria in Gambia seems to have decreased significantly between 1999 and 2007 [2]. But this trend could have been changed in 2008. According to unconfirmed information, the rainy season has been longer than usual this year.

Irrespective of what the reasons for this increase in travel-related malaria are, this cluster demonstrates once again the need for adequate chemoprophylaxis and information on protection from mosquito bites for all travellers to West-Africa.

E. Pekkanen has occasionally consulted GlaxoSmithKline and SBL vaccines.

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# European cluster of imported falciparum malaria from Gambia

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A cluster of 56 patients returning from Gambia with falciparum malaria has been noted in several countries of the European Union since September this year. TropNetEurop, the European Network on Imported Infectious Disease Surveillance, collected and reported the cases. Lack of awareness and, consequently, of prophylactic measures against malaria were apparent in the majority of patients.

On 24 November 2008, TropNetEurop, the European Network on Imported Infectious Disease Surveillance (www.tropnet.eu), received information on falciparum malaria cases imported to Denmark. When this information was distributed, further notifications were received from various member sites in the following days. Apparently, the combination of lack of information about their destination and ignorance of potential malaria risks affected travellers throughout Europe.

## Denmark

All Danish travellers bought their journeys at the same travel agency. All stayed at the coast of Gambia at a beach close to the capital Banjul, none of them were travelling around in Gambia.

### Patient 1

A man in his late fifties travelled to Gambia for two weeks and returned home in early November 2008. He did not take malaria prophylaxis. He was admitted to a local hospital ten days later with symptoms suggestive of alcohol withdrawal with tremors, difficulty walking, confusion and fever. Malaria was not suspected until family members mentioned that he had been to Gambia, and the diagnosis was first made two days after admission, when parasitaemia was 5.8%. The patient was transferred to a specialised infectious disease unit and treated for cerebral malaria with artesunate and doxycycline, sedated and put on respiratory support in the intensive care unit. The patient is still closely followed up to date, and neurological deficits still continue.

### Patient 2

A woman in her fifties travelled to Gambia with a friend (patient 3) for one week in early November 2008. She did not take any malaria prophylaxis and was not vaccinated against yellow fever. Onset of symptoms was six days after her return, when she became disorientated and confused. The following days she stayed in bed with fever and headache. Fortunately, a friend visited her at the eight days later and called a doctor who admitted her to a specialised infectious disease unit. She had clinical signs of cerebral malaria and parasitaemia was 2.2%. She was treated with artesunate and doxycycline and discharged on six days after admission with no sequelae.

### Patient 3

A woman in her late forties travelled to Gambia together with patient 2. She did not take any malaria prophylaxis. According to patient 2 she developed fever and diarrhoea about six days after she returned home. Ten days after her return, she was visited in her home by a general practitioner on call and was advised to see her family doctor the next day. The police was notified on the day patient 2 was admitted to hospital, but found patient 3 dead in her home. Autopsy has confirmed that she died from malaria.

### Patient 4

A woman in her late forties travelled with a friend (patient 5) to the Gambia for one week in late October 2008. She did not take malaria prophylaxis. Approximately seven days after she returned home, she got diarrhoea followed by fever and headache. After four days she contacted a local hospital because she suspected she could have malaria. The diagnosis was later confirmed at a specialised infectious disease unit in Copenhagen, with falciparum malaria and <1% parasitaemia. Following treatment, the patient was discharged four days later without sequelae.

**Patient 5**

A woman in her late fifties travelled to Gambia with a friend (patient 4) without taking malaria prophylaxis. She developed fever 6-7 days after she returned home. However, malaria was initially not considered. Two days later she was found in the home with high fever and reduced consciousness. She was admitted on a local hospital and transferred to a department of infectious diseases. Malaria smears showed Plasmodium falciparum with 6% parasitaemia. Insufficient ventilation warranted respiratory support. The patient developed a DIC and necrosis/gangrene of eight fingers and toes.

**Patient 6**

A man in his late sixties travelled to Gambia for one week in November 2008 without any malaria prophylaxis. He was diagnosed with malaria seven days after his return with a parasitaemia of 5%. Following treatment he was discharged seven days later without sequelae.

**Patients 7 and 8**

Two brothers in their late fifties travelled together to Gambia for one week in early November 2008. They did not take malaria prophylaxis, nor were they vaccinated against yellow fever. The first brother was initially treated by his family doctor with penicillin for an upper respiratory tract infection, before he was admitted to a department of infectious diseases and diagnosed with falciparum malaria (3% parasitaemia). Then the police was contacted and a search made for the second brother. He was located in a local hospital to which he had been admitted with cerebral symptoms suggestive of alcohol withdrawal. He was transferred to the same department of infectious diseases and diagnosed with cerebral malaria (5% parasitaemia). Both brothers were treated successfully with parenteral artesunate and oral atovaquone/proguanil.

Following these initial reports from Denmark, further information from other countries was added:

### Finland

Twelve cases were reported between 3 and 27 November 2008, all Finnish travellers returning from Gambia [1]. Five of these patients were female, seven were male. Age distribution was between 27 and 66 years. Their travel destinations were tourist resorts near Banjul. Nine travellers did not take any chemoprophylaxis, three used chloroquine prophylaxis. Ten of the 12 patients had received information about the malaria risk and recommended chemoprophylaxis. The duration of their stay in Gambia was between one and two weeks except for one patient who stayed four weeks. Three had complications that required treatment in intensive care, but all three recovered. For nine patients, the diagnosis was made within three days of onset of symptoms. The three patients with complications had been diagnosed with a delay of up to eight days.

### Norway

Three patients were reported, two men (39 and 71 years-old), and one woman (48 years-old). None of them used chemoprophylaxis during their stay in Gambia. All three patients visited relatives and friends and stayed in and around Banjul. No complications were reported, all patients recovered.

### United Kingdom

Nineteen travellers infected with falciparum malaria in Gambia were reported to the Health Protection Agency's Malaria Reference Laboratory between October and December 2008. Their age was between 21 and 62 years. Six patients were female. The majority of patients travelled as tourists (n=9), two visited friends and relatives in the country, two had migrated from Gambia to the United Kingdom (UK), and the reasons for travel of the remaining six patients were unkown. One traveller used chemoprophylaxis with proguanil, 14 took no chemoprophylaxis, and data for the remaining four were not provided.

### Spain

Two patients with falciparum malaria were noted in Barcelona, Spain. Both were second generation Gambian migrants visiting relatives in Gambia for the first time. They stayed in rural areas in the country for 11 and 13 months, respectively, and did not use chemoprophylaxis. Both were male, one 14 years-old, the other 16 years-old. One of them suffered from uncomplicated malaria and recovered without complications while the other was treated in an intensive care unit due to hypotension, 9.5% parasitaemia, severe anaemia (Hb: 6.5 g/L), low platelet count: 25.000/µl, hyperbilirrubinaemia and somnolence. The infection was treated with quinine and doxycycline, and both recovered without sequelae.

### The Netherlands

In the Netherlands, 10 Dutch tourists were reported with falciparum malaria after returning from Gambia between 21 September and 26 November 2008. The median age was 48 years (range 43-62), six patients were female. Three cases were related (travel companions). The median duration of stay was nine days (range 7-68). Seven travellers did not use malaria chemoprophylaxis, two used homoeopathic drugs (chininum arsenicosum D8) and one tourist stopped atovaquone/proguanil prematurely. The median shortest incubation period was five days (range 0-18). The median interval between the first day of illness and the date of diagnosis was five days (range 0-17). Seven patients were admitted to hospital for treatment. Two patients, aged 45 and 49, died. Both patients had not used chemoprophylaxis. The time to diagnosis was 17 and six days, respectively [3].

### Germany

Two patients were reported in Germany. Both were male, one 15 years-old, the other 54 years-old. They travelled to urban areas in Gambia for one and two weeks, respectively. None of them took chemoprophylaxis. Both recovered after a largely uneventful clinical course. The 54 year-old patient had been returning to Gambia for 10 years, always without prophylaxis. The 15 year-old patient was in Africa for the first time, travelling alone from Turkey, where he was borne.

### Comment

During the comparatively short time period of two months and a half between September and November 2008, TropNetEurop member sites reported 56 patients returning from Gambia with falciparum malaria. Thirty-two of them were male, and 24 female. The age range was 15-71 years.

While the reasons for travel were quite diverse, a striking lack of effective prophylactic measures was apparent in all. Fourty-five patients had not used any malaria chemoprohylaxis. All seven

travellers who indicated that they had taken prophylactic drugs used inadequate or downright wrong ones: two took homeopathic prophylaxis, three used chloroquine only, one used paludrine only, and one stopped taking atovaquone/proguanil too early. No data are available for the remaining four patients.

Thus, despite the documented risk of complicated falciparum malaria from Gambia, virtually all patients chose to use no or inadequate prophylaxis [2]. Several were counselled to take this decision by their travel agency, but in a few cases even by their family doctor.

The cluster underlines the necessity of competent pre-travel information and adequate protection in travellers, in particular at times when malaria appears to be decreasing but still remains a high risk for non-immune travellers [4]. Although there probably is an overuse of chemoprophylaxis against malaria among tourists travelling to Asia and Latin America, chemoprophylaxis is a must for most travellers to African destinations, and in particular to west Africa.

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# Emergence of fox rabies in north-eastern Italy

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Italy has been classified as rabies-free since 1997. In October 2008, two foxes have been diagnosed with rabies in the Province of Udine, north-east Italy. One case of human exposure caused by a bite from one of the foxes has occurred and was properly treated.

On 17 October 2008, the national reference centre for rabies at the Istituto Zooprofilattico Sperimentale delle Venezie of Legnaro in Padova, Italy, identified a rabid red fox (Vulpes vulpes) in the municipality of Resia (Province of Udine, Northeast of Italy) (Figure 1) [1]. The fox had bitten a 69-year-old man on the ankle on 10 October. The victim received first aid assistance and complete post-exposure treatment at the local health unit. The exposed person is currently under active health surveillance.

### Laboratory analysis

A brain sample from the fox initially tested negative in the fluorescent antibody test (FAT) for rabies virus (RABV). However, the virus was successfully isolated on murine neuroblastoma cell culture [2], and was confirmed as RABV by RT-PCR using specific primers. When the FAT was repeated on other brain specimens, the test was weakly positive.

The complete open reading frame (1,350 nt) of the gene encoding the nucleoprotein (N) was sequenced (GenBank Acc. Number FJ424484) and compared to the sequences available in public databases. Phylogenetic analysis was performed using the neighbour-joining method with 1,000 times bootstrapping, as implemented by the Mega 4 programme [3]. Phylogenetic analysis (Figure 2) identified the isolate as Lyssavirus genotype 1, "classical" rabies virus, according to the classification made by Kissi et al. [4], clustered in the Western European group [5]. As expected, it was closely related to RABV isolated from eastern neighbouring countries (Slovenia, Bosnia and Herzegovina and the former Federal Republic of Yugoslavia) and shared 99% homology with the complete N gene sequence of the strain 86111YOU and 100% homology with a 400 nt fragment of the N gene sequence of the strain 9494SLN, red fox isolates from Bosnia-Herzegovina and Slovenia, respectively.

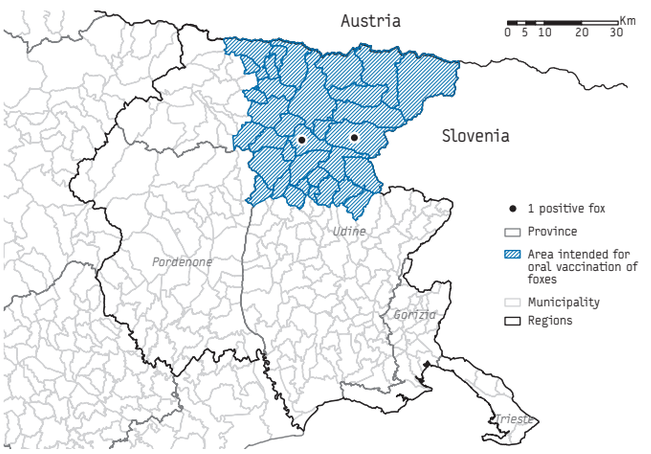
According to the characteristics of the isolate it seems reasonable to believe that the emergence of sylvatic rabies in north-eastern Italy could be linked to infection in the bordering territories of Slovenia, although no cases are currently reported in the area.

### Rabies situation in Italy

The north-eastern territories of the Italian region of Friuli Venezia Giulia have been affected by rabies in the 1970s and 1980s, and more recently in the period from 1991 to 1995 [6]. The municipality of Resia was affected until 1992. At that time, the epidemic of sylvatic rabies was linked to the epidemiological situation of infection in Austria and the nearby territories of former Yugoslavia, now Slovenia. For this reason, the risk of rabies in the northern and eastern border regions of Italy has long been recognised. The rabies surveillance carried out in that region accounted for an annual number of 310, 210, 123, 94 and 85 foxes analysed from 2004 to October 2008, respectively. Vaccination campaigns using oral rabies vaccine baits have been conducted targeting the wild fox population in these areas in 1989 and between 1992 and 2004. The last case of rabies in Italy was diagnosed in a fox in the province of Trieste on the border with Slovenia in December 1995.

**Figure 1**

**Map of Friuli Venezia Giulia region, Italy, showing the two reported cases as well as the area where oral vaccination of foxes is being implemented**



Italy has been classified as rabies-free since 1997. At present, Austria is rabies free, while in Slovenia, rabies cases in foxes are still being reported from the South Eastern regions bordering Croatia [7]. In this area oral vaccination campaigns are systematically conducted in the fox population since the mid-1990s in the framework of a national rabies eradication programme [8].

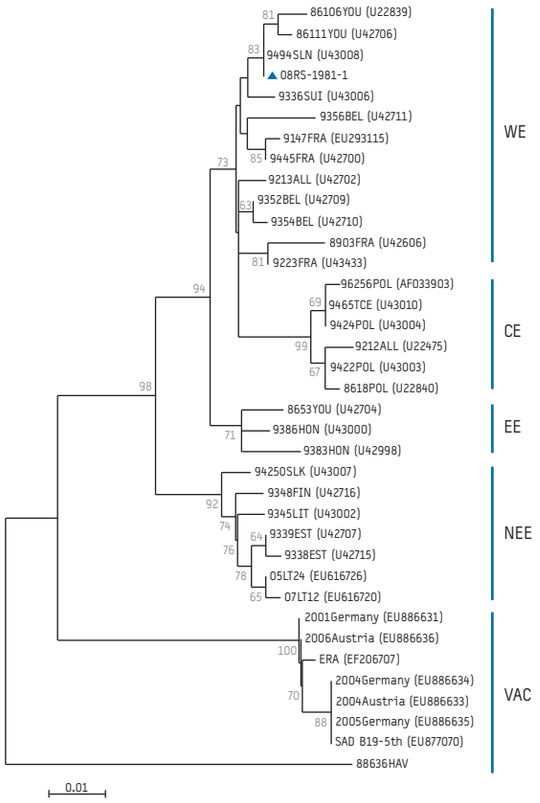
On 27 October 2008, a second fox was found dead and diagnosed with rabies in the municipality of Venzone (Province of Udine) (Figure 1), 12 km west to the one infected earlier the same month. No human exposure has been reported related to this second infected fox.

### Measures taken

Following these outbreaks, the preventative measures implemented in the affected areas of Italy include compulsory rabies vaccination of dogs and domestic herbivores at risk of infection (i.e. cows, horses, sheep and goats kept outdoors), prohibition of hunting with dogs, enhanced surveillance in the wild animal population and implementation of oral vaccination of foxes (Figure 1). Furthermore, an informative campaign on the risk for the local population, as well as visitors and tourists, has been implemented and a protocol for post-exposure prophylaxis and recommendations for pre-exposure immunisation for individuals at high risk (such as hunters, forest workers, game wardens, veterinarians) have been sent to all healthcare facilities and medical associations in the affected area.

**FIGURE 2**

**Phylogenetic tree (neighbour-joining method) of the nucleoprotein gene of a rabies virus isolated from a fox in Italy, October 2008**




The sequence of the Italian isolate is identified with a blue triangle. Sequences of the other genes of this isolate can be found in GenBank.

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# Measures taken to reduce the risk of West Nile virus transmission by transplantation in Italy

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For the first time in Italy, two patients with meningoencephalitis were diagnosed with West Nile virus (WNV) infection in September 2008 [1]. The patients live in the Bologna and Ferrara provinces of Emilia Romagna where WNV infections had previously been noted in horses, crows and magpies [2].

The Italian National Transplant Centre (CNT), which is responsible for the procurement, processing and distribution of organs and tissues in Italy, has now reviewed the risks of transmission of WNV by organ, tissue and cell transplantation and, taking into account the advice and recommendations of the relevant authorities in other countries, issued guidance to the transplant community.

## Background

Although there are no known cases of WNV transmission by tissue transplantation, both blood transfusion and organ transplantation have resulted in transmissions [3-6]. Transmission by transplantation of tissues such as bone, heart valves, skin and corneas is theoretically possible, particularly when the tissues have been minimally processed and/or they contain blood.

European Union Directive 2006/17/EC, Annex 1, defines the criteria to be applied for the selection of tissue and cell donors and Annex 2 defines the testing requirements [7]. Annex 1 states that donors must be excluded from donation if there is evidence of "risk factors for transmissible diseases on the basis of a risk assessment, taking into consideration donor travel and exposure history and local infectious disease prevalence". This requirement is reflected in the CNT's Guidelines for the Procurement, Processing and Distribution of Tissues for Transplantation (19/06/2007), which demand the exclusion of donors who have "a risk of infection associated with travel to endemic areas or exposure to infective agents that cannot be excluded by testing".

The Food and Drug Administration (FDA) in the United States issued draft, non-binding guidance for blood, cell and tissue donations in April 2008 [8]. They recommend that blood specimens from all human cell and tissue donors be tested year-round for WNV by individual donor Nucleic Acid Testing (NAT) using a licensed screening NAT test, and that only donors whose specimens are non-reactive may be considered eligible.

The American Association of Tissue Banks has raised concerns about the necessity for universal testing seeing as there are as yet no reports of transmission by tissue transplantation. They have also raised concerns regarding the reliability of the kits that have been licensed for use with post-mortem samples: They report an unacceptable rate of abnormal initial tests that give a weak positive result but are negative when repeated for the same sample [9].

Apart from these concerns, it is relevant that in the case of the first donor that transmitted WNV by organ transplantation, two serum samples collected at the time of admission did not contain any detectable WNV IgM antibody or nucleic acid. Neither did a serum sample obtained from the patient on the following day, after receipt of transfusions contain detectable levels of WNV nucleic acid. However, serum and plasma samples collected a day later at the time of organ recovery yielded WNV nucleic acid in a quantitative PCR, and WNV could be recovered in culture [4]. In the second case of transmission by organ transplantation, the donor tested negative for WNV RNA, although serum samples were positive for WNV IgG and IgM [5].

These findings underline the problems that can arise when relying on testing and the importance of accurate documentation of the donor's history in the prevention of donor-transmitted infections such as West Nile fever.

It is notable that in the United Kingdom, 18,700 blood donors returning from high-incidence WNV areas during the epidemic season were tested between mid-June 2004 and the end of November 2005 and no positive result was obtained [10]. It can be concluded that the risk of transmission by those who have travelled to affected areas is very low.

## Guidance issued for transplantation in Italy

In the light of the recently reported infections, and taking a precautionary approach, the Italian National Transplant Centre has issued guidance that all potential donors of organs, tissues and cells from the Bologna and Ferrara provinces in the Emilia-Romagna region should be tested to exclude infection. Where there is evidence of infection, organs, tissues and cells will not be used.

In the rest of Italy and in the other Emilia-Romagna provinces, he following rules apply:

- Investigation of the history of potential **tissue donors** will include enquiries regarding a possible overnight stay in the provinces of Bologna and/or Ferrara during the previous 28 days. If a potential donor has visited one of these provinces, they will

not be considered eligible for donation, unless laboratory test results for WNV are negative;

- For **organ donors**, a case by case evaluation is conducted in order to assess the infection risk, which is acknowledged to be very low, taking into account the nature and benefits of transplantation and the health status of the patient on the waiting list.

## Discussion

In the case of organ donation, decisions have to take into account the shortage of organs available for transplant and the great, usually life-saving, benefit that can result from this type of transplantation. The available time is very limited and it may not always be possible for WNV testing to be performed in a particular area of Italy where a donor is identified in time before the transplantation would need to proceed. Under such circumstances, the risk posed by a potential donor who may have spent a night in a WNV-affected area in the previous month and has had no symptoms of infection would be very low and would probably not justify depriving the recipients of the opportunity for transplantation. For this reason, it is necessary to consider each case individually, weighing the risks and potential benefits that face each individual recipient in a balanced and pragmatic way.

In the case of tissue donation, the potential donor pool is much larger and shortages are therefore not a major challenge in the system, particularly in Italy where tissue donation rates are high. In general, tissue transplants result in improved quality of life and are rarely life-saving, so it is important to maintain risk at a very low level. On the other hand, many donated tissues are processed by washing, freezing, freeze-drying and in some cases subjected to gamma irradiation or other types of sterilisation. The tissues that are not highly processed, such as corneas, heart valves and skin, contain very little blood. The risk of an infected tissue transmitting a virus is therefore significantly lower than for blood or organs. Overall, it is considered appropriate to take a precautionary approach to the selection of tissue donors until there is a clearer picture of the extent of the problem.

The guidance described here will be reviewed should further infections be reported, also taking into account changing seasons.

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# Investigations and control measures following a case of inhalation anthrax in East London in a drum maker and drummer, October 2008

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We discuss the investigations and control measures undertaken following the notification of a fatal case of inhalation anthrax in East London. The patient is believed to have acquired the infection from making animal hide drums. Environmental investigations identified one drum and two pieces of animal skins contaminated with anthrax spores.

## Introduction

Anthrax is predominantly a disease of livestock. Naturally acquired infection in humans occurs as a result of contact with infected animals or animal products contaminated with spores of *Bacillus anthracis*. The most common form of the disease in man is cutaneous anthrax. Other forms, including inhalation and gastrointestinal anthrax, are less common.

The incubation period for inhalation anthrax is between two and 43 days, but may be up to 60 days or as short as one day [1]. An initial prodromal stage may include symptoms such as fever, malaise, fatigue and anorexia followed by sudden increase in fever, severe respiratory distress, excessive sweating and shock. The case fatality can be as high as 92% in sporadic cases where the diagnosis is usually only made during the fulminant stages [2].

*B. anthracis* spores are considered as one a potential biological weapon [3] and deliberate release of anthrax spores has occurred in the United States (US) in 2001 [4].

Anthrax is now a very rare disease in the United Kingdom (UK). Between 1981 and 2006, 18 possible cases of cutaneous anthrax were notified in England and Wales, with *B. anthracis* isolated in only one case and serological confirmation in another two. The last case of pulmonary anthrax in England and Wales was reported in 1974, and the last case before that was in 1965 [5].

A death from anthrax occurred in Scotland in 2006; this was a case of disseminated anthrax following exposure to imported animal hides in a drummer and drum maker [6]. One case of naturally acquired inhalation anthrax was also reported in the US in 2006 in a drum maker who used imported animal skins [7].

The case reported below is the first case of inhalation anthrax reported in England and Wales in more than 30 years [8].

## The case

On 21 October 2008, the patient presented to a London hospital with a two-day history of fever, night sweats and rigors. He rapidly deteriorated during early hours of 23 October and was transferred to the intensive care unit with respiratory failure. Multiple organ failure developed the following day. The admission chest X-ray showed some basal shadowing and a widened mediastinum.

On 22 October, admission blood cultures had become positive and Gram-positive rods were seen on microscopy. On 23 October, the organism produced pure growth on media plates. A preliminary diagnosis of *B. anthracis* was made on 24 October.

The results were reported to North East and North Central London Health Protection Unit on Friday 24 October, and an incident was declared immediately. The sample was couriered to the Health Protection Agency's (HPA) laboratory for Novel and Dangerous Pathogens (NADP) in Porton Down, where the identity of the organism was confirmed on the same day as *B. anthracis*. Further molecular and microbiological investigations on the next day confirmed the identification of the organism and drug sensitivities.

The patient commenced on oral and intravenous antibiotics immediately after admission. Antibiotic treatment was changed to rifampicin, ciprofloxacin and clindamycin following diagnosis of anthrax on 24 October. Following consultation with the US Centers for Disease Control and Prevention (CDC) in Atlanta, anthrax immunoglobulin (Cangene Corporation), was flown in from the US and administered on 27 October. The patient remained in

critical condition requiring multi-organ support until he died on 2 November,

A *post mortem* examination was carried out on 5 November to confirm the diagnosis and to clarify the circumstances of death. The *post mortem* report is awaited after the inquest in March 2009 but the preliminary results confirm the primary cause of death as pulmonary anthrax.

Although cremation is the preferred disposition method [9] the body was buried in a sealed coffin according to the family's wishes.

## Epidemiological investigation

When the diagnosis had been made, the patient was in a critical condition and unable to communicate. Therefore all information about his activities was provided by his family and friends. The family was interviewed in depth in order to identify the potential source of infection. However, they were not able to provide all the required information about his activities during the incubation period. Some of his friends, colleagues and clients were also interviewed.

The patient made and played animal hide drums. All drum making activities in the two months preceding the onset of disease took place in a studio flat in East London, while the family lived at a different address.

A supplier of animal skins, who had been reported to have supplied skins to the patient, was also interviewed and reported importing hides from Gambia; however, it was also reported that the patient made and repaired drums for clients who brought him animal skins from various sources. Based on the available information and evidence from previous cases [6,7,10-12] a working hypothesis was formed that he possibly acquired the infection while making the drums in his studio flat.

## Environmental investigation

The studio flat was investigated and environmental samples were collected by staff from the NADP laboratory. Samples included five drums, animal skins left in the property, drum making equipment, surfaces, and air samples. The remaining skins from the same batch supplied to the patient by the main supplier of animal skins were also tested, as were a further six drums kept at the family's home.

Of the samples taken from the studio flat one drum and two pieces of leftover animal skins proved to be contaminated with *B. anthracis*. All other samples from the studio flat were negative. Neither the animal hides belonging to the main supplier, nor the drums kept at the case's family home showed any evidence of contamination with anthrax spores.

## Control measures

### Prophylaxis

The HPA started a risk assessment as soon as the incident was declared to identify individuals who might have been present when the case was making drums in the 60 days before onset of symptoms. The patient's immediate family, the main supplier of the skins, and a person who assisted him with drum making were offered prophylaxis with ciprofloxacin. A staff member at the hospital was also concerned about potential exposure to aerosolised spores and started prophylaxis on 24 October. No one else had been identified as being at risk.

All contacts started the recommended course of prophylaxis with ciprofloxacin (500 mg oral, twice daily for 60 days) [13] on 24 October or as soon as they were identified. Due to reported minor side effects including gastrointestinal upset, the treatment was switched to doxycycline (100 mg oral, twice daily) in one contact and to amoxicillin (500 mg oral, three times daily) in another. The latter stopped taking antibiotics after three weeks. All other contacts were still taking antibiotics at the time of publication of this report (more than seven weeks after start of prophylaxis).

### Decontamination

None of the surfaces at the studio flat were positive for anthrax. It was therefore decided that extensive decontamination of the flat would not be necessary. However, the contaminated drum was removed and the area surrounding it was decontaminated using 10,000 ppm hypochlorite solution [14]. It was agreed that the skin from this drum should be removed and incinerated but that the base of the drum could be returned to the family following decontamination. Other animal skins found at the property were also incinerated.

### Advice to drummers

During interviews with the case's friends and colleagues they were informed about the possible risks of handling untreated animal skins and were advised to avoid importing skins from uncertified sources. Based on evidence from previous cases they were advised that the risk from playing drums was minimal and the main risk would be during the process of making drums, particularly shaving hair from animal skins as this could result in aerosolised anthrax spores being inhaled [7]. The HPA is now working on information leaflets on anthrax to be distributed to drummers through existing drumming networks and websites. Advice for drum makers on manipulation of animal hides is available on HPA website [15].

## Discussion

Inhalation anthrax continues to be a rare event but this case illustrates the need for rapid diagnosis and treatment. In the anthrax letters in the United States in 2001 the mortality of inhalation of anthrax was reduced from 92% to 45%. This reduction in case fatality rate was associated with factors including rapid instigation of treatment. [12].

Following the identification of one contaminated drum and two animal skins at the patient's workshop we are continuing our investigations about his activities in relation to these objects in more detail. However, we are aware that retrospective information gathering about his exposure may not yield reliable and conclusive information due to several factors including recall bias, vested interest and fear of legal challenges regarding imported animal skins by his clients and work associates.

Inhalation anthrax is very rare. The above case is the first reported in England and Wales in more than 30 years and the second case in the UK after a patient died in Scotland in 2006 [6]. Both of these UK cases, as well as a case reported from US in 2006 [7], were drum makers. The microbiological evidence and epidemiological investigations into the Scottish case concluded that the infection was linked to drumming activities, although the exact nature of the exposure is unknown. It is believed that the most likely source

of infection in the case in London was the imported hides he used to make the drums.

Despite the popularity of African drums and drumming in many countries, there are few documented cases of anthrax associated with these activities. According to an internet search for English and French reports, the case reported here is the sixth case in the literature. In four cases there was a known exposure via manipulating skins in drum making, and for two the exposure was thought to be handling or playing the drums [6,7,10-12].

To prevent future similar cases it is important to raise public awareness, in particular amongst drum makers and drummers about potentially contaminated animal skins, the risks of particular sources of these animal products, and the early signs of anthrax so that they can seek professional advice in a timely manner. This should be available through websites such as the HPA site, and supplied to the drumming community for dissemination.

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