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Ongoing outbreak of rubella among young male adults in Poland: increased risk of congenital rubella infections

I Paradowska-Stankiewicz[1], M P Czarkowski[1], T Derrough[2], P Stefanoff (pawel.stefanoff@fhi.no)[3,4]
1. National Institute of Public Health – National Institute of Hygiene, Warsaw, Poland
2. European Centre of Disease Prevention and Control (ECDC), Stockholm, Sweden
3. Norwegian Institute of Public Health, Oslo, Norway
4. European Programme for Intervention Epidemiology Training (EPIET), European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden

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From January to April 2013, Poland reported 21,283 rubella cases (55.2 per 100,000 inhabitants), the highest number since 2007. Some 81% of cases were among 15–29 year-old males. This outbreak reflects the history of immunisation policies – selective vaccination of adolescent girls since 1989, then universal two-dose measles-mumps-rubella vaccination, since 2004. The extent of virus circulation among adults increases the risk of congenital rubella infections and jeopardises the World Health Organization Regional Office for Europe 2015 elimination goal.

Ongoing rubella outbreak

From January to April 2013, physicians in Poland notified 21,283 rubella cases (55.2 per 100,000 inhabitants) [1], a near 10-fold increase compared with the 2,224 cases (5.8 per 100,000 inhabitants) reported during the corresponding period of 2012 (Figure 1).

Physicians use the European Union (EU) case definitions for rubella and congenital rubella syndrome (CRS) [2]. They report each rubella case to the local health department, providing demographic information, International Classification of Diseases (ICD)-10 code [3], case classification and vaccination status. Every two weeks, local health departments report the number of cases notified in their area to the provincial health departments that aggregate data, which they forward to the National Institute of Public Health – National Institute of Hygiene. Every month, local health departments prepare aggregated reports with more detailed data including the number of cases by age group, sex, vaccination status and case classification.

Physicians report CRS cases to the local health departments. Local health departments investigate CRS cases and send individual reports to the National Institute of Public Health – National Institute of Hygiene. There is no routine active case finding search for CRS cases, nor has active CRS surveillance been implemented to date.

We describe here this country-wide rubella outbreak by person, place and time and propose implementation of preventive measures.

We calculated reported rates per 100,000 inhabitants, dividing the number of rubella reported cases by mid-year census estimates [4]. The reported rates of rubella varied substantially between provinces (median: 51.2 per 100,000 inhabitants; range: 7.4–151.1 per 100,000 inhabitants). The majority of cases were reported from three provinces in the south-east of the country (n=8,659; 41%), bordering Ukraine, Belarus and Slovakia, and five provinces in the north-west (n=7,997; 38%), two of which border Germany (Figure 2).

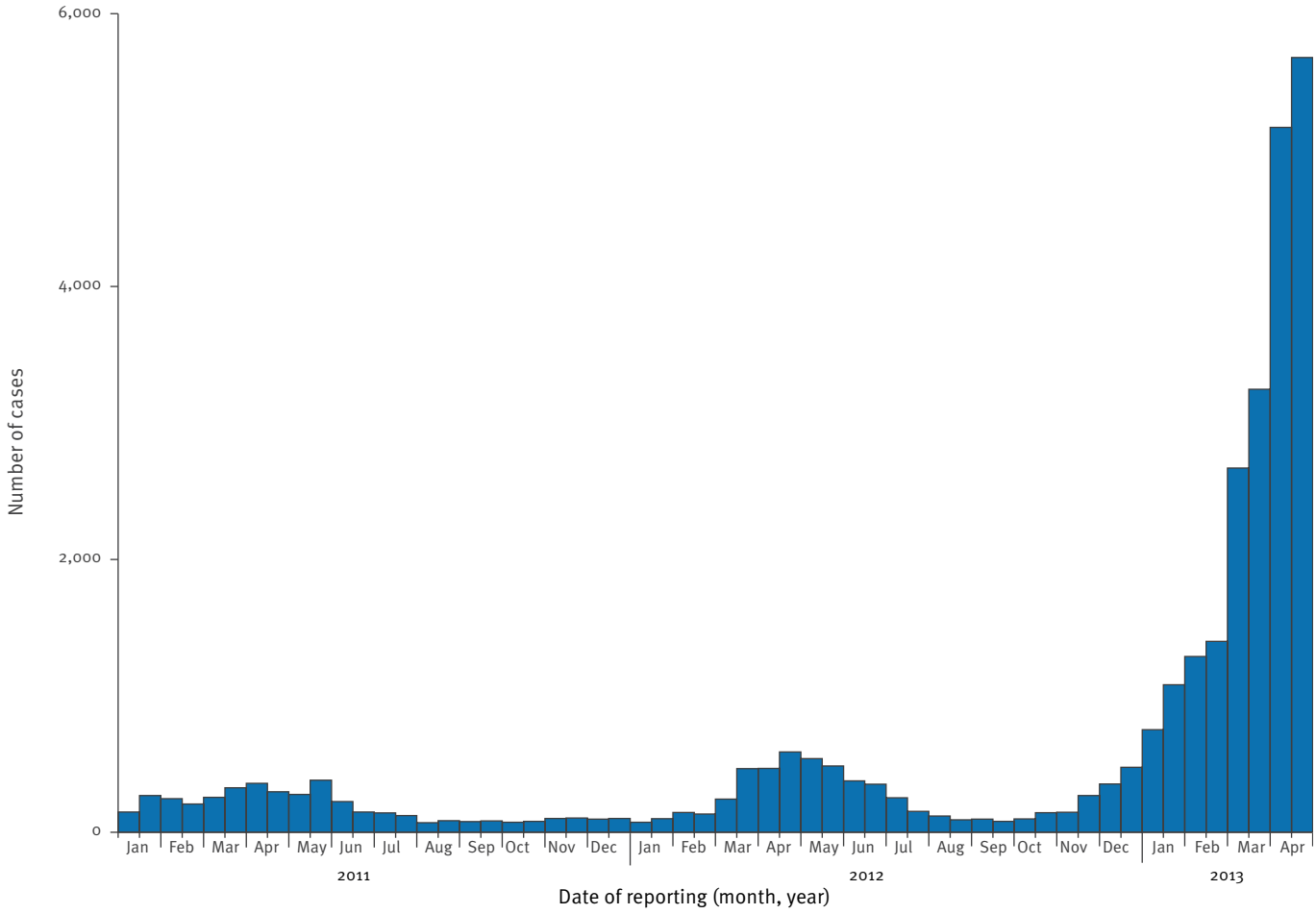
The male to female ratio was 10:1. The most affected groups were persons who were male aged 15–19 years (12,220 cases, reported rate: 1,044.9 per 100,000 inhabitants; 57% of cases), 20–24 years (4,000 cases, reported rate: 286.8 per 100,000 inhabitants; 19% of cases) and 25–29 years (992 cases, reported rate: 61.1 per 100,000 inhabitants; 5% of cases). During 2003 to 2012 and the first four months of 2013, rubella reported rates suggested an increasing trend in 2006–2007 and then in 2012 and the first third of 2013. The increases in 2006–2007 and from 2012 were both more pronounced among males 10 years of age and older, especially in the first four months of 2013 (Figure 3).

Vaccination status was recorded for 15,237 (72%) reported cases. Of these, 1,502 (10%) were vaccinated with one dose of rubella-containing vaccine, and 234 (2%) with two or more doses of rubella-containing vaccine.

Of all reported cases, 29 (0.1%) were confirmed (based on a valid laboratory test), 57 (0.3%) were probable (based on an epidemiological link to a confirmed case), and 21,197 (99.6%) were possible (based on clinical symptoms).

Figure 1

Rubella cases reported in two-week periods, Poland, 1 January 2011–30 April 2013



From January to April 2013, two cases of (CRS) were reported, as compared with four cases reported during 2003 to 2012.

History of rubella immunisation in Poland

In 1989, Poland started to administer monovalent rubella vaccine to 13 year-old girls. In 1992–2006, reported coverage ranged from 94% to 99% [5]. In 2004, measles-mumps-rubella (MMR) vaccine was administered to all children at the age of 13–15 months and 10 years [1]. In 2005–2012, first-dose coverage among 3 year-old children ranged from 91% to 98% [5].

In 2011, a mission of the World Health Organization (WHO) Regional Office for Europe reviewed the rubella situation in Poland, identified an immunity gap among adolescent males and young adults and recommended supplementary immunisation of all adolescents and young adults, as the increased circulation of rubella among young adults increases the risk of congenital rubella syndrome (CRS) [6].

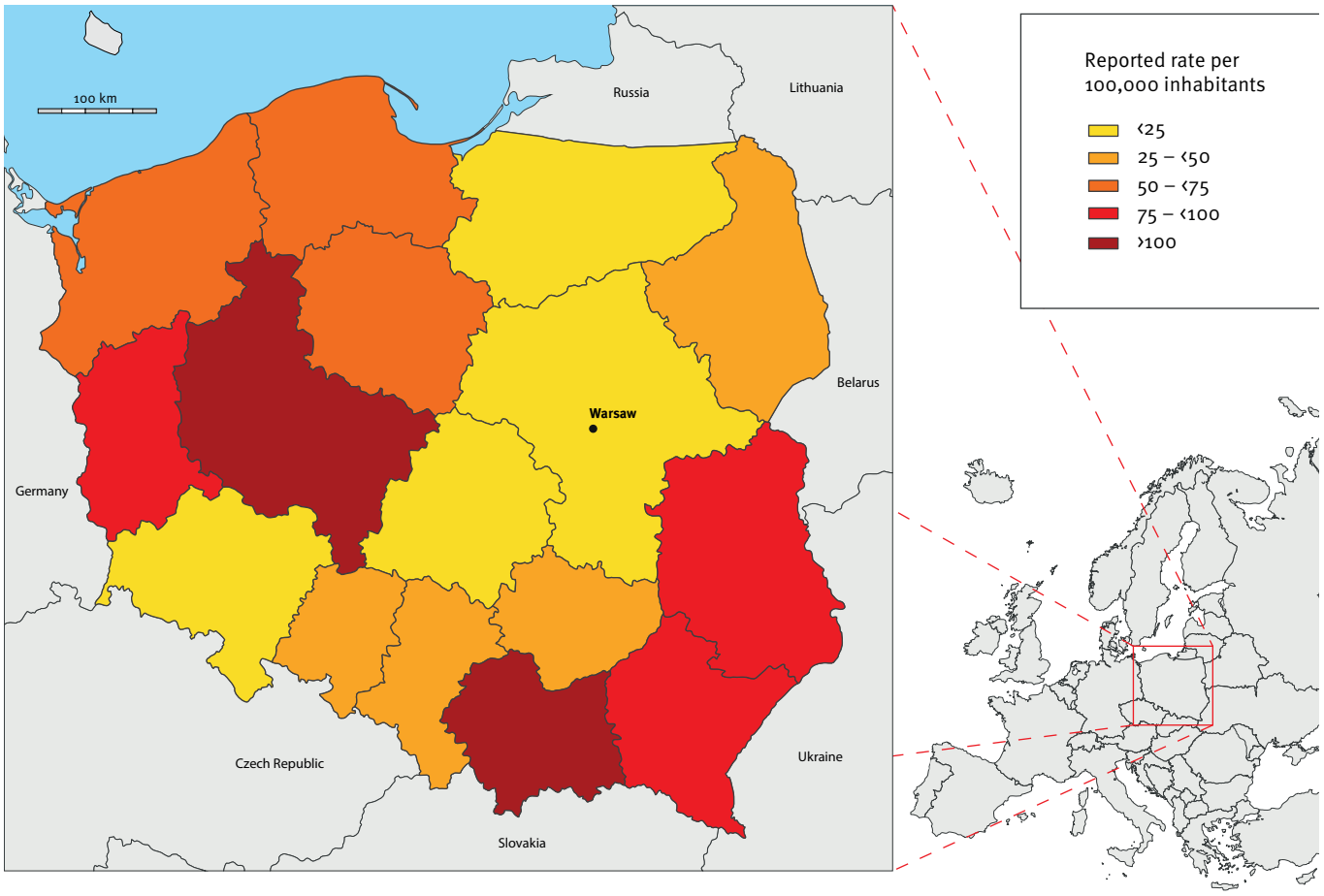
Discussion

The 2013 outbreak of rubella in Poland reinforces the need for public health efforts to meet the WHO Regional Office for Europe target for the elimination of measles and rubella and prevention of CRS by 2015 [7]. From April 2012 to March 2013, rubella cases in Poland comprised 74% of those in the EU/European Economic Area (EEA) countries [8]. Despite the visible impact of the childhood immunisation programme in decreasing rubella activity among the vaccinated age groups, Poland will not achieve rubella elimination without targeted supplementary immunisation activities.

The outbreak reflects the historical immunisation activities in Poland: a combination of selective and universal vaccination led to a shift in the age of rubella infections to cohorts of young adults. This situation increases the risk for CRS, due to high circulation of the rubella virus among adolescents and young adult populations. Despite long-term vaccination of adolescent girls, about 10% of women of childbearing age may still be susceptible to rubella, as documented in a 2004 study [9]. From January to April 2013, two

Figure 2

Reported rates of rubella by province, Poland, 1 January–30 April 2013 (n=21,283)

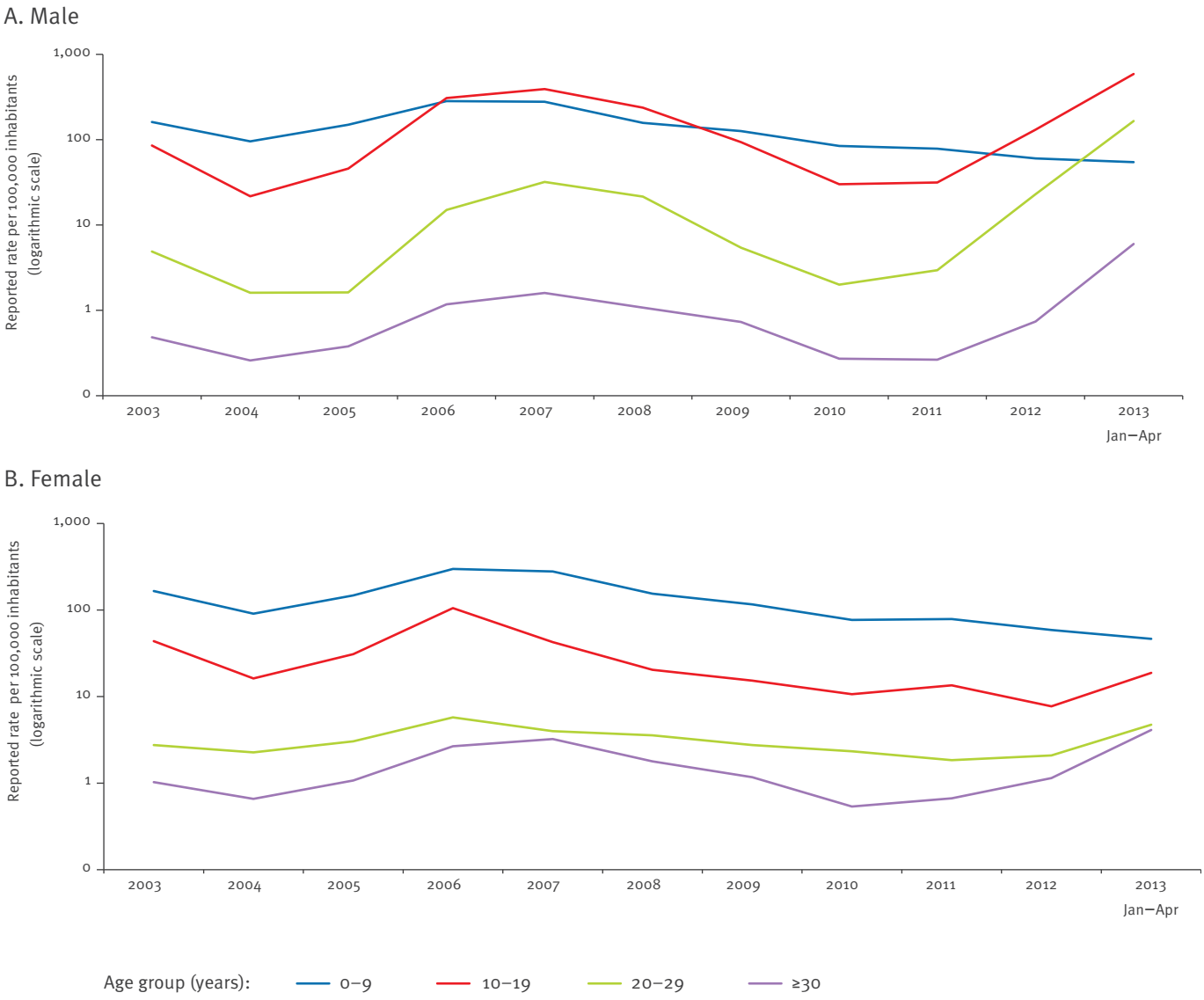


cases of CRS have already been reported, as compared with four cases during 2003 to 2012. Further cases are unfortunately expected and unavoidable, as observed during outbreaks in other EU/EEA countries in recent years. The country most recently affected was Romania. In 2011–2012, Romania reported 20,772 rubella cases, 22 confirmed CRS cases and 11 cases of congenital rubella infection [10,11]. A similar situation was observed in Greece in the 1990s: a large rubella outbreak was described in 1993, with 25 cases of CRS, and another epidemic occurred in 1999, mainly in young adults, with four cases of CRS [12]. These observations in Greece, Romania and now Poland are a consequence of immunisation practices that had been followed, leading to the build-up of susceptible cohorts. Sadly, rubella outbreaks in young adults unavoidably lead to children being born with CRS. This situation is likely to be repeated if women of childbearing age are left unprotected and become infected with the rubella virus. All possible efforts should be undertaken to prevent any cases of CRS in Europe in the future. The availability of rubella-containing vaccines with a long-standing history of good safety and effectiveness profiles, and provision to all those who need it would ensure that the potentially dramatic consequences of rubella infection in pregnancy would become a thing of the past.

Effective control of rubella and prevention of CRS requires high-level political commitment and a long-term strategy for vaccination programmes and implementation of additional control measures, when needed. The WHO Region of the Americas elaborated a regional plan of action and mobilised human and financial resources in support of a rubella and CRS elimination goal for 2010 [13]. In 2009, the last endemic rubella and CRS cases in that region were reported. Since then, rubella cases have been attributed to importations of rubella virus into countries, particularly those that only targeted girls for vaccination. In response to these outbreaks, countries reinforced surveillance activities and vaccination interventions by conducting supplementary immunisation activities among adolescents and adults. The results of a systematic literature review demonstrated that the combined vaccination strategy with a universal approach – two doses of rubella-containing

Figure 3

Trends in age group-specific reported rates of rubella, Poland, 1 January 2003–30 April 2013



vaccine in children 1–6 years of age and one dose in susceptible adult (both men and women) populations through a catch-up campaign – contributed to the elimination of rubella in the Americas, as observed in Costa Rica and in Mexico [14].

Our report has one main limitation. The vast majority of cases were reported based on clinical symptoms and were not laboratory confirmed. Thus, the clinical cases could be potentially due to a concomitant outbreak of another illness causing a rash. Elements that support the hypothesis of a rubella outbreak include: (i) the unvaccinated status of most reported cases and (ii) their age-by-sex distribution. Therefore, rubella remains the most probable explanation for this outbreak.

This outbreak empirically reflects the shift in the age of infections and the accumulation of susceptible cohorts, 10 years after starting universal vaccination. The increased age of infected people leads to an increased risk of CRS. This situation requires immediate public health action to prevent further CRS cases.

Suggested public health action

First, we suggest that rubella elimination and CRS prevention should be made a priority and a plan of action developed. Second, the proportion of laboratory-investigated cases should be increased, to confirm the aetiology of the outbreak. Third, we suggest enhancing the surveillance of congenital rubella infections to monitor the impact of interventions and to estimate the CRS burden. Fourth, following confirmation of the rubella aetiology of this outbreak, emergency catch-up vaccination of young adults should be considered, to stop

further transmission of the virus and prevent further CRS cases. Fifth, we suggest screening for rubella antibodies as part of pre-conception or antenatal care to identify and vaccinate unprotected women [15]. Sixth, a seroprevalence study could be instrumental in documenting the age-specific proportion of susceptible persons after the outbreak.

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Conflict of interest

None declared.

Authors' contributions

Iwona Paradowska-Stankiewicz and Miroslaw Czarkowski were responsible for data collection, verification and summarisation. Pawel Stefanoff and Tarik Derrough drafted the manuscript. All authors revised and approved the final version of the manuscript.

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Detection on four continents of dengue fever cases related to an ongoing outbreak in Luanda, Angola, March to May 2013

E Schwartz[1], E Meltzer (meltzere@zahav.net.il)[1], M Mendelson[2], A Tooke[3], F Steiner[4], P Gautret[5,6], B Friedrich-Jaenicke[4], M Libman[7], H Bin[8], A Wilder-Smith[9], D J Gubler[10], D O Freedman[11], P Parola[5,6]

1. Center for Geographic Medicine and Department of Medicine C the Sheba medical center, Tel Hashomer & Sackler School of Medicine, Tel Aviv, Israel
2. Division of Infectious Diseases & HIV Medicine, Department of Medicine, Groote Schuur Hospital, University of Cape Town, South Africa
3. Department of Medicine, 2 Military Hospital, Wynberg, Cape Town, South Africa
4. Institut für Tropenmedizin und Internationale Gesundheit Charité - Universitätsmedizin Berlin, Germany
5. Assistance Publique Hôpitaux de Marseille, CHU Nord, Pôle Infectieux, Institut Hospitalo-Universitaire Méditerranée Infection, Marseille, France
6. Aix Marseille Université, Unité de Recherche en Maladies Infectieuses et Tropicales Emergentes (URMITE), Faculté de Médecine, Marseille, France
7. J.D. MacLean Centre for Tropical Medicine, McGill University Health Centre Montreal, Quebec Canada
8. The Central Virology Laboratory, Ministry of Health, Tel Hashomer Israel
9. Institute of Public Health, University of Heidelberg, Germany
10. Program on Emerging Infectious Diseases- Duke-NUS Graduate Medical School, Singapore
11. University of Alabama at Birmingham, Birmingham, Alabama, USA

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In April 2013, ten cases of dengue fever in travellers returning from Luanda, Angola, to five countries on four continents, were reported to the globally distributed GeoSentinel Surveillance network. Dengue virus serotype 1 was identified in two cases. The findings indicate that a major dengue outbreak is currently ongoing in Luanda. This report illustrates how cases from an emerging arboviral epidemic focus can spread internationally and highlights the risk of dissemination of a vector-borne disease into receptive areas.

GeoSentinel provides a sentinel sample of returning travellers at 56 clinics in 24 countries on six continents [1]. During April 2013, GeoSentinel sites in Canada, France, Germany, Israel and South Africa reported a total of 10 cases of dengue in business travellers returning from Angola, with Luanda as the only likely place of exposure. Meanwhile, on 15 April, the Instituto de Higiene e Medicina Tropical in Lisbon, Portugal reported 19 cases of dengue acquired in Luanda since late March 2013, in four of whom dengue virus (DENV)-1 was detected by polymerase chain reaction (PCR) [2]. The nearly simultaneous reports of dengue cases related to travel to Luanda from five GeoSentinel sites on four continents, as well as Portugal, suggest that a large scale outbreak of dengue may in fact be unfolding in Angola.

Background

In Angola, DENV activity has been reported sporadically. Early surveys in the 1960s revealed no evidence of DENV activity [3], while outbreaks of clinically suspected dengue in the 1970s were proven to be caused by chikungunya [4]. In the 1980s an outbreak of dengue was reported from Luanda, with subsequent reports of travel-related dengue acquired in Angola, by travellers from the Netherlands [5] and Brazil [6]. For a Brazilian travel-related case, the serotype identified was DENV-2. Since then, there has been little information on the risk of dengue in Angola. This may represent an absence of disease activity, or a lack of awareness, diagnostic resources and active surveillance.

Travellers may serve as sentinels to local epidemic risks, and this role is especially important in areas with scarce public health reporting and resources. Thus, cases of dengue among European travellers returning from the Comoros islands in east Africa [7] and Benin in west Africa [8] have called attention to local DENV transmission. In a recent review, 12 of 27 countries in Africa where travellers/expatriates had acquired dengue, had not reported local DENV transmission [9].

Here, we report on an apparent outbreak of dengue in Luanda, Angola diagnosed among travellers presenting to travel clinics on four continents.

Table

Travel-related dengue infections acquired in Luanda, Angola, reported from GeoSentinel sites, March–May 2013 (n=10)

Country of origin of the case	Fever onset date	Time from fever onset to test (days)	NS1	Serology-IgM	Qt-PCR
Germany	30 March	4	Positive	Positive	ND
Canada	3 April	10	Negative	Positive	ND
France	5 April	12	Negative	Positive	Negative
Germany	7 April	14	Negative	Positive	ND
South Africa	10 April	7	ND	Positive	Negative
Israel	11 April	14	ND	Positive	ND
Israel	17 April	7	Positive	Positive	ND
Israel	18 April	4	Positive	Positive	DENV-1
Israel	25 April	5	Positive	Positive	DENV-1
Israel	2 May	6	Positive	Positive	ND

DENV: dengue virus; NS1: non-structural protein 1; ND: not done; Qt PCR: quantitative polymerase chain reaction.

Case descriptions

Overall the male/female ratio of cases reported to GeoSentinel was 9:1 and the traveller's age was 41.3±10.7 (mean ±SD) years.

All cases presented with an acute febrile illness and symptoms suggestive of classic dengue, including headache and joint pain. In three of the 10 cases a rash was reported. Laboratory studies during the febrile period revealed leucopenia (range: 1.2–2.9 x 10^9/L, norm: 4.0–10.0 x 10^9/L) and thrombocytopenia (range: 13–124 x 10^9/L, norm: 140–440 x 10^9/L) in all the cases. None of the cases had features of severe dengue and all recovered without complications.

Dengue diagnosis was confirmed by one or more of three methods; non-structural protein 1 (NS1) antigen, DENV IgM enzyme-linked immunosorbent assay (ELISA) serology or DENV viraemia by quantitative (Qt)-PCR (Table). DENV IgM was detected in all 10 cases, whereas five cases also tested positive for NS1 antigen. For all these latter cases except one from Germany (Table), NS1 antigen and DENV IgM were detected in a single sample. For the German case, a blood sample drawn at four days post symptom onset was NS1 antigen positive but seroconversion was verified in subsequent samples. In two viraemic Israeli patients, Qt-PCR revealed the virus to be DENV-1 similar to the imported cases seen in Portugal [2].

Discussion

Dengue has long been known to exist in Africa, but its epidemiology is poorly documented. Recent prediction models of dengue suggest that the true burden of dengue in Africa may approach that of South America [10]. Moreover, limited serological surveys in locations such as Burkina Faso [11] have suggested that the disease is far more prevalent than previously recognised. In the last four years, large dengue epidemics were reported on Macaronesian islands of Cape Verde (DENV-3) [12] and Madeira (DENV-1) [13] off the northwest African coast. Common models of dengue epidemiology suggest that clinically diagnosed cases of classic dengue represent the tip of an iceberg, with actual case numbers being much higher [14].

On 1 April 2013 local health authorities in Luanda reported six cases of dengue fever acquired in the city [15]. The true extent of the dengue outbreak in Luanda is likely to be much higher than currently acknowledged. Anecdotally, returning Israeli travellers with dengue have maintained that multiple additional cases of similar febrile illness were extant in the expatriate community in Luanda.

The origin of the present DENV-1 strain responsible for the current Luanda outbreak is as yet undetermined, but the possibility of an imported strain is of concern. Of the 190,000 ill returned travellers in the GeoSentinel database since 1997, no previous cases of dengue acquired in Angola have been reported. Strains of DENV appear to be circulating between east Africa and the Indian subcontinent [16] and recent DENV-1 isolates from Madeira appear to be closely related to strains circulating in Central or South America [17,18]. Thus, it is well established that dissemination of dengue from DENV endemic countries in America and Asia occurs both in east Africa and off the northwest African coast. In this regard, it is important to note that according to the World Tourism Organization (WTO) data, major source countries of travellers to Angola included DENV

endemic China and Brazil, with 69,900 and 29,700 travellers respectively during 2011 [19].

Another source of concern is the possibility of the spread of dengue to susceptible countries by returning, viraemic travellers. *Aedes albopictus*, one of the DENV vectors is currently endemic throughout most of the Mediterranean basin, and has recently been documented as far north as the Netherlands [20]. In Israel for example, the presence of *Aedes albopictus* in dense population centres, creates prime conditions for a dengue outbreak [21].

At present, health practitioners should be aware of the possibility of dengue in febrile travellers returning from Angola. Such travellers would be best served by clinicians with access to rapid diagnostic tests, and should be advised to implement measures to avoid mosquito bites, for the likely duration of viraemia.

This report serves to illustrate the possible speed of global dissemination of cases from an emerging arboviral epidemic focus, and the potential for introduction of novel viruses or novel strains into receptive countries.

Authors' contributions

Eli Schwartz – study conception; Eyal Meltzer – Drafting of the article; Marc Mendelson – Critical review and editing of the manuscript; Alan Tooke – Collection of data; Florian Steiner – Critical review and editing of the manuscript, collection of data; Philippe Gautret – Critical review and editing of the manuscript, collection of data; Barbara Friedrich-Jaenicke – Collection of data; Michael Libman – Critical review and editing of the manuscript, epidemiological data; Hanna Bin – Laboratory analysis; Annelies Wilder-Smith – Critical review and editing of the manuscript; Duane J Gubler – Laboratory analysis, critical review and editing of the manuscript; David O. Freedman – draft editing, and patient classification; Philippe Parola – Critical review and editing of the manuscript. David O. Freedman and Philippe Parola, also linked the cases together using the GeoSentinel system.

Conflict of interest

None declared.

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Hepatitis A outbreak in Bijeljina, Bosnia and Herzegovina, August 2012 - April 2013

Z Dakic (z.dakic@live.com)[1], S Musa[2]
1. Health Center Bijeljina, Bijeljina, Bosnia and Herzegovina
2. Institute for Public Health Federation of Bosnia and Herzegovina, Sarajevo, Bosnia and Herzegovina

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From August 2012-April 2013, an outbreak of hepatitis A with 28 laboratory-confirmed cases occurred in Bijeljina, Bosnia and Herzegovina. The index case was in a seven year old child from the local Roma community. Cases were 7-70 years old, 7-15 year-olds (9 cases) were the most affected age group. The event highlights the susceptibility of the population due to reduced hepatitis A virus circulation with consecutive lower immunity in the population in the past years.

In January 2013, the local health authorities in Bijeljina, the fifth largest city in Bosnia and Herzegovina, informed the national authorities about an ongoing outbreak of hepatitis A. A first case had been notified in August 2012 and by mid-January the case count had raised to 20. Here we describe the outbreak investigation and control measures taken by the local health authorities.

Figure 1
Location of Bijeljina, Bosnia and Herzegovina

Background

Bijeljina is a town and municipality with about 120,000 inhabitants, located in the north-east of Bosnia and Herzegovina (Figure1). It is the fifth largest city in Bosnia and Herzegovina and second largest in the Republic of Srpska. Republic of Srpska is one of the two main - political entities of Bosnia and Herzegovina. Bijeljina municipality shares borders with Croatia and Serbia. It is a significant agricultural, trade and transit area in Bosnia and Herzegovina, with a high population density.

In the past 21 years, the epidemiological situation of reported intestinal infectious diseases in Bijeljina municipality was stable. Only sporadic cases of salmonellosis and few family outbreaks of trichinellosis were notified (data not shown).

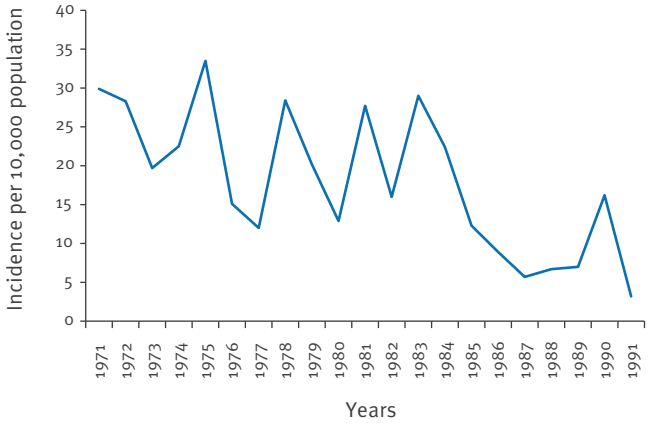
In the Republic of Srpska, hepatitis A is a notifiable disease in accordance with the law on protection of the population against infectious diseases [1]. An infectious disease reporting is regulated by respective rules which do not define the criteria for reporting in detail [2].

Despite numerous challenges such as the Bosnian war from 1992 to 1995, migration of inhabitants elsewhere, poor socio-economic conditions and the unprecedented floods of the Drina river at the end of 2010, no hepatitis A cases were recorded before the current outbreak, in Bijeljina in the past 21 years. There is a possibility however, that cases of hepatitis A had occurred that were neither detected nor registered due to severe disruption of the surveillance of infectious diseases, especially during wartime. Before that period, from 1971 to 1991, a total of 3,399 cases of hepatitis A with an average of 154 cases per year, were registered (Figure 2).

In the last 15 years, the overall incidence of hepatitis A in Bosnia and Herzegovina decreased (Figure 3). In the same period, declining hepatitis A incidence trends were also observed in many other European countries [3].

Figure 2

Incidence of notified hepatitis A cases per 10,000 population in Bijeljina, Bosnia and Herzegovina, 1971-1991 (n= 3,399)



Outbreak investigation

On 17 January 2013, the epidemiological service of the Health Center Bijeljina notified the the Republic of Srpska Institute of Public Health about a hepatitis A outbreak in Bijeljina, due to an increased number of hepatitis A cases since August 2012.

Case definition

The case definition for the investigation of the hepatitis A outbreak corresponds to the European Union case definition [4]. Confirmed cases are only those with clinical symptoms and laboratory confirmation (IgM antibody to hepatitis A virus (anti-HAV IgM)) reported from 1 August 2012 in Bijeljina.

All available relevant clinical and epidemiological data on cases and their contacts were collected using a paper-based questionnaire by the staff of the epidemiological service of the Health Center Bijeljina.

From 15 August 2012 to 2 April 2013 a total of 28 confirmed cases were reported (Figure 4).

The index case was a seven year-old child from the Roma population that resides in the area close to the canal Dasnica, to where untreated domestic sewage and wastewater of the entire city is disposed. The other cases were from the general population, mostly pupils (9 cases) and unemployed persons (13 cases). The youngest case was seven years and the oldest 70 years old (median 34 years). With nine of the 28 cases, the most affected age group was that of 7to 14 year-olds. Age and sex distribution of cases are depicted in Figure 5.

Clinical findings

All 28 cases were hospitalised; 23 were treated at the general hospital in Bijeljina and five were transported to and hospitalised at the Clinic for Infectious Diseases in Banja Luka because of limited availability of hospital beds in Bijeljina. All patients had several of the following clinical findings: jaundice, fever, weakness, fatigue, abdominal pain, vomiting, diarrhoea, light colored stools and dark colored urine. The clinical course was favourable for all and there were no complications.

Laboratory reports

From all 28 patients serum samples were taken and analysed for the following hepatitis markers: hepatitis B surface antigen (HBsAg), antibodies to hepatitis E virus (anti-HEV IgM), antibodies to hepatitis C virus (anti-HCV IgM and IgG) and anti-HAV IgM.

After an initial analysis at the general hospital in Bijeljina, all samples which were not reactive for HBsAg, anti-HEV and anti-HCV, were further tested for hepatitis A at the Clinical Center of Banja Luka.

Specimens with signal to cutoff (S/CO) values ≥ 1.21 were considered reactive for IgM anti-HAV (analysed by Abbott ARCHITECT® apparatus). All reported cases were reactive. The observed values ranged from 7.08 to 19.64 (median 13.1).

Figure 3

Incidence of notified hepatitis A cases per 100,000 population, in Federation of Bosnia and Herzegovina and Republic of Srpska, Bosnia and Herzegovina, 1996-2012

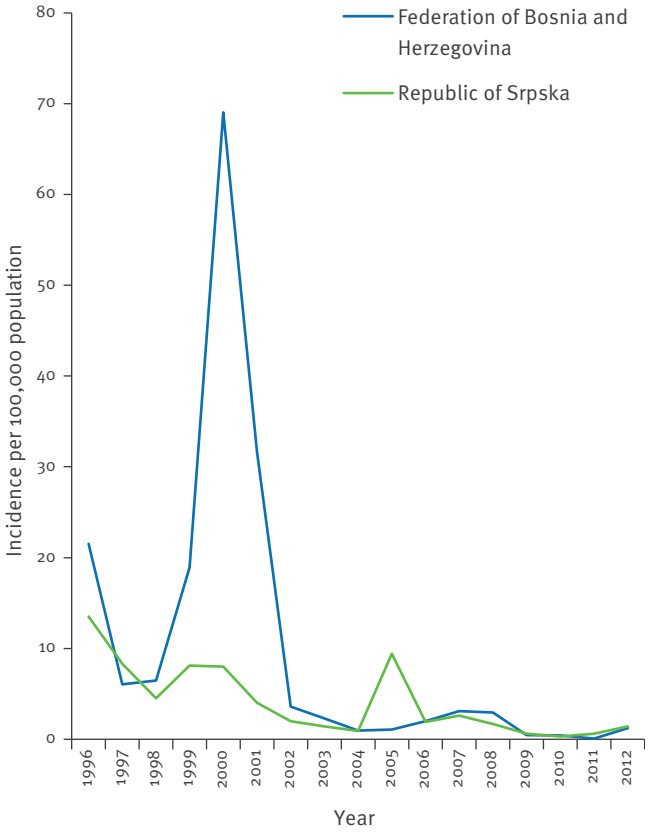
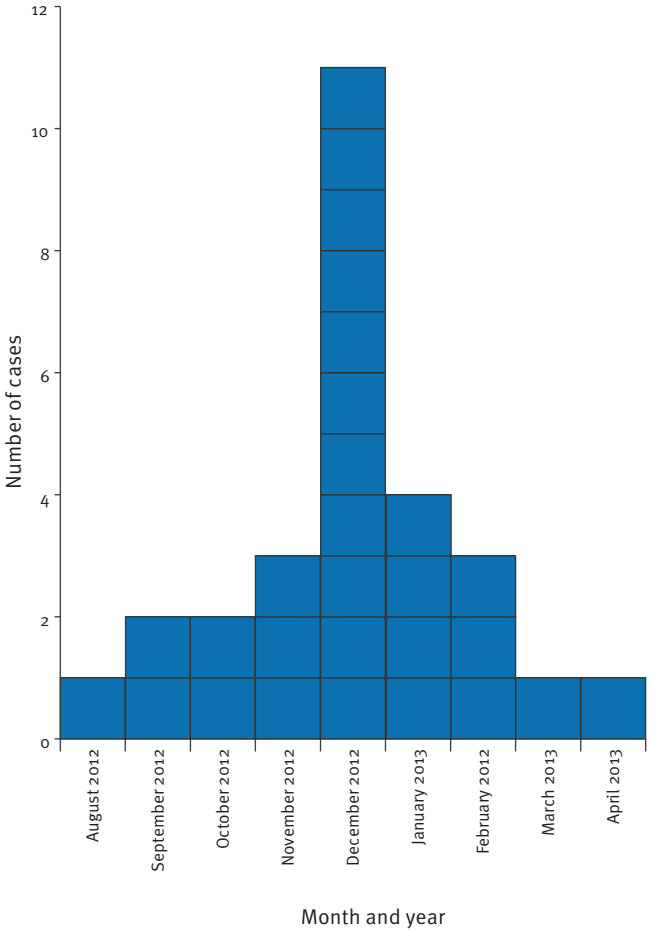


Figure 4

Notified cases of hepatitis A, in Bijeljina, Bosnia and Herzegovina, 15 August 2012 - 2 April 2013 (n=28)



Control measures

Specific guidelines for case management of hepatitis A do not exist in Republic of Srpska. We inspected schools and public buildings in Bijeljina. All cases and contacts were provided with general information about the nature of the disease, ways of transmission and how to prevent hepatitis A. The local health authorities disinfected houses (with chlorine granulates dissolved in water) and the immediate environment of patients as well as premises of kindergartens and pre-schools and local boarding school facilities and other collective accommodation buildings in Bijeljina.

Monitoring of drinking water quality from the waterworks and affected households was intensified and showed that water was safe for drinking.

Vaccines and immunoglobulin against hepatitis A are not available in Republic of Srpska.

Discussion

The surveillance system in Bosnia and Herzegovina dates back to the time of the former Socialist Republic of Yugoslavia and is a passive reporting system of infectious diseases. Reporting is done through paper forms and depends on cooperation of doctors who report diseases to the relevant epidemiological service in the public health authorities. Diagnosis is usually only clinical; microbiological confirmation of diseases is still quite limited.

In the current hepatitis A outbreak the first case and five more cases occurring in September, October and November 2012, were reported in the Roma population living in the southern part of Bijeljina, in an area with significant infrastructure problems e.g. unregulated water supply system and sewage. During the past 20 years this area has been exposed to a large migration of the Roma population many of whom left the town.

We assume that the infection transmission in the current outbreak which started in the Roma community, reached the general community through transmission in schools. The age distribution of cases with the most affected age group being that of children between 7 to 14 years old, compared with cases in outbreaks in Czech Republic [5], Latvia [6], Estonia [7] and Slovakia [8], is most similar to that in Estonia. These outbreaks confirm the susceptibility of the population due to a reduced HAV circulation with consecutive lower immunity in the population in the past years.

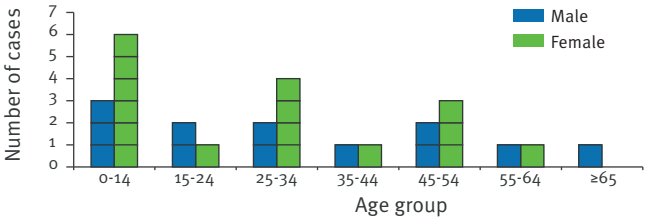
Based on epidemiological data and results of analysis of drinking water, most probable routes of transmission are (mainly) indirect faecal-oral transmission contact via common use items: door handles, sanitary devices in school and public toilets etc.

The last outbreak case was reported on 2 April 2013. The average incubation period of hepatitis A is 28–30 days (range 15–50 days) [8]. After the double maximum incubation period from last the notified hepatitis A case, we will announce the end of this outbreak most probably at the beginning of July this year if no further cases occur.

A lesson learnt from this event is that in an increasingly susceptible population, unresolved problems

Figure 5

Age and sex distribution of notified hepatitis A cases, in Bijeljina, Bosnia and Herzegovina, 15 August 2012 - 2 April 2013 (n=28)



in sanitary infrastructures can increase the risk of an outbreak of hepatitis A. Public hygiene was evaluated by inspection authorities, as soon as the outbreak had been declared by the local public health authorities. There is an obvious need for larger involvement of institutions responsible for public health and for the educational sector to raise the awareness of the population about the need to improve hygiene through the mass media and educational campaigns.

Also, cross-border collaboration is necessary because of the vicinity to other countries and the mobility of the local population. Hepatitis A is resurging in Europe at the moment with a number of food-related multi-country outbreak(s) [10]and travel-related cases which show the challenge of increased susceptibility in the population [11].

Finally, in the present case, the surveillance system was able to pick up the outbreak and this can be considered as a positive signal for the future.

Author contributions

ZD and SM wrote the manuscript were responsible for its conception and design, as well as for data analysis and interpretation.

Conflict of interest

None declared.

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Evidence of false-positive results in a commercially available rotavirus assay in the vaccine era, Australia, 2011 to 2012

S Ye[1,2], S Roczo-Farkas[3], D M Whiley[1,2], S B Lambert (sblambert@uq.edu.au)[1,4], J Robson[5], C Heney[2], G Nimmo[2], K Grimwood[1], T P Sloots[1,2], C D Kirkwood[3,6]

1. Queensland Paediatric Infectious Diseases Laboratory, Queensland Children's Medical Research Institute, The University of Queensland, Brisbane, Queensland, Australia
2. Microbiology Division, Pathology Queensland Central Laboratory, Herston, Queensland, Australia
3. Enteric Virus Research Group, Murdoch Childrens Research Institute, Parkville, Victoria, Australia
4. Queensland Health Immunisation Program, Queensland Health, Brisbane, Australia
5. Sullivan Nicolaides Pathology, Brisbane, Australia
6. Department of Microbiology, La Trobe University, Bundoora, Victoria, Australia

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Concerns were raised about specificity of the VIKIA Rota-Adeno immunochromatographic kit. Only 28-37% of samples positive with the VIKIA kit could be confirmed using two real-time RT-PCR assays and three ELISA kits. On re-analysis of a subset of the positive samples, 86% remained positive with the VIKIA kit, however, 90% remained negative in the other assays. In a highly vaccinated population we found a high number of false-positive rotavirus tests with a widely-used commercial kit.

We recently became concerned about the specificity of the VIKIA Rota-Adeno assay (bioMérieux, France) following an unexplained increase in positive results and feedback from clinicians.

Accurate detection of rotavirus is essential for prevention and control of rotavirus outbreaks and disease monitoring. There are two common methods used for routine diagnosis: immunochromatographic (ICT) assays and enzyme-linked immunosorbent assays (ELISA). ICT assays are relatively inexpensive, easy to use, rapid (results within 20 min) and with reportedly good sensitivity (96.6%) and specificity (92.9%) [1]. Many diagnostic laboratories in Australia use the VIKIA Rota-Adeno assay for detection of rotavirus in faecal specimens.

We therefore re-examined samples initially testing positive in the VIKIA Rota-Adeno ICT with other commercially available ELISA rotavirus assays and, for a subset of specimens, by RT-PCR.

Methods

Ethics approval for this study was provided by the Children's Health Queensland Human Research Ethics Committee.

Clinical specimens

We obtained a convenience sample set of 133 faecal specimens submitted for diagnostic rotavirus testing and collected between July 2011 and August 2012 from patients with symptoms of acute gastroenteritis. Specimens were from two laboratories in Queensland (n=113: Pathology Queensland, a publically funded laboratory, and Sullivan Nicolaides Pathology, a private laboratory) and from a private laboratory network in Victoria (n=20: Melbourne Pathology). The latter were submitted to the National Rotavirus Reference Centre (NRRC) in Melbourne, Victoria, for genotyping. All samples had been tested initially for rotavirus using the VIKIA ICT method according to the manufacturer's instructions (Queensland: 81 positive, 32 negative; Victoria: 20 positive).

Real-time RT-PCR, Queensland samples only
All 113 Queensland specimens were tested initially in Queensland employing two real-time RT-PCR assays, using primers and TaqMan probe sequences described previously:
NVP3-PCR (NVP3-F1 ACCATCTACACATGACCCTC, NVP3-F2 ACCATCTTCACGTAACCCTC, NVP3-R GGTCACATAACGCCC, NVP3 probe ATGAGCACAATAGTTAAAAGCTAACACTGTCAA) [2,3], JVK-PCR (JVK-F CAGTGGTTGATGCTCAAGATGGA, JVK-R TCATTGTAATCATATTGAATACCCA, JVK probe ACAACTGCAGCTTCAAAAGAAGWGT) [4].
RNA extraction was performed by homogenising ca. 25 µl of stool specimen with 225 µl of phosphate buffered saline to provide a concentration of ca. 10%. Then 200 µl of this suspension were extracted into a volume of 50 µl using the Roche High Pure Nucleic Acid extraction kit as per kit instructions (Roche Diagnostics, Australia). As described previously, specimens were

Table 1

Comparison of original VIKIA test results and repeat VIKIA test results for specimens with sufficient volume for re-testing, Queensland, July 2011–August 2012 (n=61)

		Repeat VIKIA test		
		Positive	Negative	Total
Original VIKIA test	Positive	30	5	35
	Negative	1	25	26
	Total	31	30	61

spiked before extraction with 5 µl of equine herpes virus as an extraction and inhibition control [5].

All real-time RT-PCR reactions were performed using a Qiagen one-step RT-PCR kit. Each reaction mix contained in a total volume of 25.0 µl in RNase-free water: 0.4 µM of forward and reverse primers, 0.16 µM of Taqman probe, 1.0 µl of Qiagen one-step RT-PCR dNTP mix, 5.0 µl of Qiagen one-step RT-PCR buffer (5x), 1.0 µl of RT-enzyme and 2.0 µl of RNA extract or control. Cycling was performed on a Rotor-Gene instrument (Qiagen, Australia) or Applied Biosystems 7500 real-time PCR system (Life Technologies, United States) with the following cycling conditions: initial hold steps at 50 °C for 20 min and 95 °C for 15 min, followed by 45 cycles at 95 °C for 15 sec and 60 °C for 30 sec, with fluorescence signal read on green at 60 °C.

ELISA testing

There were 103 samples available at the NRRC for further testing: 83 from Queensland (51 VIKIA-positive, 32 VIKIA-negative specimens) and 20 specimens from Victoria. Thirty VIKIA-positive specimens from Queensland were not sent to the NRRC due to insufficient sample volume. Available specimens were retested using three commercial rotavirus ELISA assays: ProSpecT (Oxoid, United Kingdom), Premier Rotaclone (Bioline, United Kingdom) and Ridascreen (R-Biopharm AG, Germany). All three methods were performed as per the manufacturer's instructions.

VIKIA retesting

To confirm initial VIKIA assay results, Queensland specimens with sufficient remaining sample (positive: n=35; negative: n=26) after PCR and ELISA testing, were retested using the VIKIA assay.

In-house VP6 RT-PCR

At NRRC, any samples that gave a discordant result for the ELISA methods or appeared to be falsely positive in the VIKIA assay (n=55), were further tested using a rotavirus VP6-specific RT-PCR with primers ROT3 AAAGATGCTAGGGACAAAATTG and ROT5 TTCAGATTGTGGAGCTATTCCA [6,7].

Results

Samples from Queensland retested in a second VIKIA assay

Of the 81 VIKIA-positive and 32 VIKIA-negative Queensland samples, there was sufficient remaining specimen for VIKIA retesting on 35 and 26 specimens, respectively. Thirty of 35 initially VIKIA-positive and one of 26 initially VIKIA-negative Queensland specimens were positive on retesting (Table 1).

Twenty-seven of the 30 VIKIA twice-positive samples were negative in every other assay applied (Table 2). Of the 10 VIKIA retest-positive specimens with sufficient sample volume available for testing at the NRRC, seven were negative by all three ELISA assays (Table

Table 2

Test results for specimens with sufficient volume for VIKIA re-testing, in PCR and ELISA assays, Queensland, July 2011–August 2012 (n=61)

		Queensland PCR (NVP3 and JVK)			Melbourne ELISA assays (ProSpect, Rotaclone, Ridascreen)			Victorian PCR			
		Positive	Negative	Total	Positive	Negative	Total	Positive	Negative	NP	Total
Repeat VIKIA test	Positive	2	29	31	3[a]	7	10	0	7	3	10
	Negative	1[b]	29	30	0	25	25	0	0	25	25
	Total	3	58	61	3	32	35	0	7	28	35

ELISA: enzyme-linked immunosorbent assay; Negative: negative in all assays; NP: specimen not tested in this assay; PCR: polymerase chain reaction; positive: positive in any assay.

[a] Two specimens positive in all three assays, one specimen positive in Rotaclone only.

[b] One sample positive in single RT-PCR assay, NVP3, only (cycle threshold: ca. 37 cycles).

Table 3

Restesting with different diagnostic assays of faecal specimens positive in the VIKIA assay, Queensland and Victoria, July 2011-August 2012 (n=133)

VIKIA initial[a]	Queensland PCR assays		ELISA assays			VIKIA retest[b]	Melbourne PCR assay	Number
	NVP3-PCR[c]	JVK-PCR[c]	ProSpecT[d]	Rotaclone[d]	Ridascreen[d]		VP6-PCR[e]	
Queensland specimens from Pathology Queensland and Sullivan Nicolaides Pathology, original diagnostic test: VIKIA								113
Queensland specimens with insufficient material for sending to the NRRC								30
POS	POS	POS	NP	NP	NP	NP	NP	2
POS	NEG	NEG	NP	NP	NP	POS	NP	21
POS	NEG	NEG	NP	NP	NP	NEG	NP	5
POS	NEG	NEG	NP	NP	NP	NP	NP	2
Queensland specimens with sufficient material for sending to the NRRC								83
POS	POS	POS	POS	POS	POS	POS	NP	2
POS	POS	POS	POS	POS	POS	NP	NP	14
POS	POS	POS	POS	POS	NEG	NP	POS	1
POS	POS	POS	NEG	POS	POS	NP	NEG	1
POS	NEG	POS	NEG	NEG	NEG	NP	NEG	1
POS	NEG	POS	NEG	NEG	POS	NP	NEG	1
POS	POS	NEG	NEG	NEG	NEG	NP	POS	1
POS	NEG	NEG	NEG	POS	NEG	POS	NEG	1
POS	NEG	NEG	NEG	NEG	NEG	POS	NEG	6
POS	NEG	NEG	NEG	NEG	NEG	NP	NEG	23
NEG	POS	NEG	NEG	NEG	NEG	NEG	NP	1
NEG	NEG	NEG	NEG	NEG	NEG	POS	NP	1
NEG	NEG	NEG	NEG	NEG	NEG	NP	NP	6
NEG	NEG	NEG	NEG	NEG	NEG	NEG	NP	24
Specimens from Victoria provided to the NRRC for genotyping, original diagnostic test: VIKIA								20
POS	NP	NP	NEG	NEG	NEG	NP	NEG	13
POS	NP	NP	NEG	POS	NEG	NP	NEG	1
POS	NP	NP	POS	POS	POS	NP	POS	6

ELISA: enzyme-linked immunosorbent assay; NEG: specimens negative in this assay; NP: test not performed on this specimen; NRRC: National Rotavirus Reference Centre; PCR: polymerase chain reaction; POS: specimens positive in this assay.

[a] Initial diagnostic test.

[b] VIKIA retest performed on specimens with remaining adequate volume after PCR/ELISA testing.

[c] Queensland PCR, not performed on Victorian samples.

[d] ELISA tests performed at the National Rotavirus Reference Centre, Melbourne.

[e] PCR performed at the National Rotavirus Reference Centre, Melbourne, on specimens discordant for any of the ELISA tests.

2). The initially negative, but retest-positive specimen was negative by the NVP3 and JVK PCR assays, and all three ELISA tests.

Other specimens from Queensland and Victoria available for testing in other assays

There were further specimens from Queensland (n=52; 46 positive, six negative) and Victoria (n=20; all positive) which were not retested using the VIKIA assay, but for which PCR and ELISA results were available.

Of 20 VIKIA-positive specimens from Victoria, 13 were negative in all three ELISA assays and the VP6 PCR assay (Table 3). The six negative specimens from Queensland were negative in both NVP3 and JVK PCR assays, and all three ELISA assays. Four of the 46 positive specimens did not have sufficient specimen volume remaining for ELISA testing; two of these positive in both the NVP3 and JVK assays, and two negative in both (Table 3). Of the remaining 42 specimens, 14 were positive in both Queensland PCR assays and all three ELISA assays, and 23 were negative in each of these

assays as well as the VP6 PCR assay. The remaining five specimens provided mixed results.

Discussion

The results of our study highlight the need to review the validity of diagnostic assays when disease incidence changes unexpectedly. Australia implemented a nationwide rotavirus vaccination programme in July 2007, and since that time notifications of laboratory-confirmed rotavirus infections and hospitalisations have fallen quickly in targeted and older age-groups [8-10]. Anecdotal feedback from clinicians and an unexplained increase in disease notifications prompted this investigation, which has identified a problem with false positivity in an ICT assay used widely in Australia and elsewhere.

Even though ours is a convenience sample, the results point towards inability to confirm by a variety of PCR and ELISA methods a substantial proportion of specimens twice positive using the VIKIA kit. These findings were reinforced by specimens from Queensland and Victoria which were tested only once using the VIKIA assay. As a sensitivity analysis, if we assume the remaining 46 initially VIKIA positive specimens with insufficient volume for retesting had all retested negative, there would still remain 27 of 81 Queensland specimens that were twice positive by the VIKIA assay, but were negative in two PCR assays (n=21) or three PCR assays and three ELISA assays (n=6) (Table 3).

Given the consistency of other methods it is unlikely that the ICT assay is detecting true positive results. Notably, our data suggest that between one and two thirds of VIKIA-positive samples may be actually false-positive results. Furthermore, of initially positive samples from Queensland with sufficient volume for repeat testing using the VIKIA kit, 86% remained positive on retest, with only three of these 30 specimens positive in one or more other assay, by PCR or ELISA. Given the consistency of the VIKIA retest values and our PCR and ELISA assay findings, conducted at different times in different locations, with all assays performed according to the manufacturer's instructions, we do not believe specimen degradation or test conditions are a logical or sustainable explanation for the apparent specificity issue. There were six Queensland specimens for which the VIKIA retest value differed from the original result, with five of these initially positive and negative in the repeat test. Possible reasons for these discrepancies include sample stability, human error in result interpretation, and specimens with low virus load.

The VIKIA ICT kit insert states that the method has 100% sensitivity and 100% specificity for rotavirus detection, based on testing of 103 positive and 290 negative stools [11]. In a prospective study of 57 samples from children younger than 36 months in Lyon in childcare centres during 2004-05, the reported sensitivity and specificity of this kit, compared to a PCR-based method, was 96.6% and 96.4% respectively (PPV: 96.5%, NPV: 92.9%) [1]. Similar high specificity (100%) was reported by Bon et al. in 2006 [12]. Given this, it is difficult to know if our findings are due to recent changes in the assay or to specificity problems exposed by reduced disease incidence in a high vaccine coverage setting. We are therefore investigating further the specificity of this and other assays in a prospective study.

Conclusion

We have shown a suboptimal test specificity using a commercially available rotavirus ICT assay. Assay-specific issues should be considered in the event of unexplained increases of rotavirus disease in the vaccine era.

Conflict of interest

None declared.

Authors' contributions

All authors collaborated on the design and conduct of the study. JR, CH, GN provided specimens. SY, SR-F performed PCR and ELISA testing on specimens. SY, DMW, SBL, KG analysed the data. SY wrote the first draft of the paper. All authors contributed to critical revision of the manuscript and have seen and approved the final version of the manuscript.

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A comparison of rapid point-of-care tests for the detection of avian influenza A(H7N9) virus, 2013

C Baas[1,2], I G Barr[1,2], R A Fouchier[3], A Kelso[1], A C Hurt (aeron.hurt@influenzacentre.org)[1,2]
1. World Health Organization Collaborating Centre for Reference and Research on Influenza, Victorian Infectious Diseases Reference Laboratory (VIDRL), Melbourne, Victoria, Australia
2. Monash University, School of Applied Sciences, Churchill, Victoria, Australia
3. Department of Viroscience, Erasmus MC, Rotterdam, the Netherlands

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Six antigen detection-based rapid influenza point-of-care tests were compared for their ability to detect avian influenza A(H7N9) virus. The sensitivity of at least four tests, standardised by viral infectivity ($TCID_{50}$) or RNA copy number, was lower for the influenza A(H7N9) virus than for seasonal A(H3N2), A(H1N1) pdm09 or other recent avian A(H7) viruses. Comparing detection limits of A(H7N9) virus with Ct values of A(H7N9) clinical specimens suggests the tests would not have detected most clinical specimens.

Human infections with influenza viruses derived directly from wild birds or poultry are relatively rare, although since 2003, over 600 human infections with influenza A(H5N1) viruses have been detected, many of which were fatal [1]. During the same period, a small number of influenza A(H7) virus infections worldwide have also occurred in humans upon contact with infected poultry, generally resulting in mild symptoms such as conjunctivitis with occasional respiratory involvement and one death [2-4]. In contrast, China announced in March 2013 human infections with a novel reassortant avian influenza A(H7N9) virus which caused severe pneumonia resulting in a number of deaths [5]. Cases have occurred predominantly in men over 60 years of age living in urban areas, and most cases had a history of recent contact with poultry or poultry products [5]. By 16 May 2013, 131 human cases of influenza A(H7N9) virus infection, in 10 provinces and municipalities in eastern China, had been reported to the World Health Organization (WHO), of which 32 had resulted in death [6]. To date there have not been any reports of sustained human-to-human transmission of the influenza A(H7N9) virus, but the rapid emergence of the virus has led to significant concerns that it could in the future acquire human transmissibility and spread globally, causing the next influenza pandemic.

Rapid testing and diagnosis of possible human influenza A(H7N9) virus infections is an important diagnostic and public health task. An accurate diagnosis will allow the timely administration of antiviral therapy [7,8] and may also enable the quarantining of infected cases to prevent further spread of the virus. Real-time PCR is now considered the gold standard laboratory-based assay for the detection of influenza virus infections due to its high sensitivity and specificity [6] and, although such assays have already been developed for the detection of influenza A(H7N9) virus [6], they require a high level of laboratory expertise and may not be available in all places where cases occur.

Point-of-care tests (POCTs) based on antigen detection, however, are simple to use and are designed for use in a medical clinic or outpatient setting, enabling the rapid testing of patient specimens within 15 minutes [9]. POCTs have mostly been licensed for detection of seasonal human influenza viruses, for which they generally have good specificity but low sensitivity [10]. Recently however, some POCTs have been specifically developed to utilise automated readers which have resulted in improved sensitivity. For public health purposes, it is important to determine whether the new or existing POCTs can detect the novel influenza A(H7N9) virus, particularly as previous studies have found that some POCTs had poorer sensitivity in detecting avian influenza strains compared to circulating human seasonal influenza strains [9]. If POCTs could reliably detect influenza A(H7N9) virus at clinically relevant levels, they would be a useful adjunct to real-time PCR in the detection of possible human cases, especially where technical resources are limited.

We evaluated six widely available POCTs that are based on detection of the nucleoprotein antigen (Table 1) for their ability to detect the avian influenza A(H7N9) virus A/Anhui/01/2013 [5], compared with three other low pathogenic avian influenza A(H7) viruses (A/Northern Shoveller/Egypt-EMC/1/2012, A/Mallard/Netherlands/4/2010 and A/Mallard/Lithuania-EMC/2/2010), two human seasonal influenza A(H3N2) (A/Sydney/506/2013 and A/Victoria/361/2011) and two influenza A(H1N1)pdm09 viruses (A/Auckland/1/2009 and A/Brisbane/292/2010).

Table 1

Details of influenza point-of-care tests evaluated in this study

Point-of-care test	Manufacturer	Specimen type approved	Proportion of virus sample following addition of diluent[a]	Format	Time (minutes)	Analysis of result
SD Bioline Influenza Ag/A/B/ A(H1N1)Pandemic	Standard Diagnostics, Korea	NPS, NS, NA, NPA	50% (S:100 µl + D:100 µl)	Test strip	10–15	Eye
Binax Now Influenza A & B Card	Alere, Unites States	NW, NA, NPS, NS	100% (S:100 µl)	Card	15	Eye
Clearview Exact Influenza A & B	Inverness Medical, Australia	NS	29% (S: 50 µl[b] + D:120 µl)	Test strip	15	Eye
BD Veritor System for rapid detection of Flu A+B	Becton, Dickinson, Unites States	NS, NPS	11% (S: 50 µl[b] + D: 400 µl)	Cartridge	10	Automated reader
BD Directigen EZ Flu A+B	Becton, Dickinson, Unites States	NW, NA, NPS, TS	83% (S: 300 µl + D: 60 µl)	Cartridge	15	Eye
Sofia Influenza A+B FIA	Quidel, Unites States	NS, NPS, NPA, NW	46% (S: 260 µl + D: 300)	Cartridge	15	Automated reader

D: diluent; NA, nasal aspirate; NPA, nasopharyngeal aspirate; NPS, nasopharyngeal swab; NS, nasal swab; NW, nasal wash; S: specimen.

[a] Dilution of specimen in kit diluent is presented as a percentage, where volumes of specimen (S) and diluent (D) are shown in parentheses

[b] Because the kit is not approved for testing of wash or aspirate samples, the specimen was absorbed by the swab provided after at least a 15 second immersion in the virus sample. The volume taken up by the swab was found to be approximately 50 µl.

Methods

All viruses were cultured in Madin-Darby Canine Kidney (MDCK) cells at a low multiplicity of infection for at least one passage before testing. All viruses were harvested at near full cytopathic effect (CPE), supernatant was centrifuged at low speed to remove cell debris, and viruses were frozen at -70°C prior to testing. A mean tissue culture infectious dose 50 ($TCID_{50}$) per mL was determined for each virus, based on at least three independent assays. Viruses were standardised to an infectivity titre of $1x10^6$ $TCID_{50}$/mL and then diluted in phosphate-buffered saline (PBS) in half-log_{10} dilutions. Real-time RT-PCR analysis was conducted on each virus dilution to determine a cycle threshold (Ct) value and RNA copy number, using an Applied Biosystems 7500 Fast cycler and the real-time RT-PCR primer and probe set recommended by the United States Centers for Disease Prevention and Control (US CDC) for the detection of influenza A matrix genes (version 4 April 2006). RNA copy number was calculated using a standard curve of RNA standards (10-fold dilutions) of known copy number prepared from a pGEMT-A/ California/7/2009 matrix plasmid using the Riboprobe In Vitro Transcription System (Promega, United States).

Each virus dilution was then tested in each POCT according to the manufacturer's instructions and a limit of detection (LOD), based on either the $TCID_{50}$/ mL or the RNA copy number/µL, was determined. Standardising viruses by viral infectivity ($TCID_{50}$/mL) is the most widely used method for the evaluation of POCTs, however it does not account for defective viral particles which may react in these antigen-detection assays. Therefore comparison of the LOD based on both $TCID_{50}$/mL and RNA copy number/µL (which accounts for both infective and defective viruses) can be informative. Half-log_{10} dilutions of influenza A/Anhui/01/2013 virus were prepared in duplicate and both sets tested with the six POCTs. The number of available test kits was not sufficient to conduct duplicate testing of the other seven viruses. The duplicate sets of influenza A/ Anhui/01/2013 virus concentrations gave highly comparable LOD data, therefore data for only the first set is presented. Four of the kits were read by eye, while two POCTs (Veritor and Sofia) utilised a mechanical reader (Table 1).

Results

Based on the $TCID_{50}$/mL, the LOD of five of the six POCTs for the A/Anhui/01/2013 influenza A(H7N9) virus ranged from $1x10^5$ to $1x10^{5.5}$ $TCID_{50}$/mL, with the Sofia and Directigen EZ detecting virus at the lower limit. The Clearview POCT was unable to detect the influenza A(H7N9) virus at any of the concentrations tested ($1x10^6$ $TCID_{50}$/mL or lower) (Table 2). In comparison, the LOD of the POCTs for the other influenza A(H7) viruses tested was generally better than that seen with the A/ Anhui/01/2013 virus, with some tests detecting virus levels as low as $1x10^2$ $TCID_{50}$/mL. Seasonal influenza A viruses were also more easily detected by most POCTs

Table 2

TCID$_{50}$ limit of detection of the influenza point-of-care tests evaluated in this study

Influenza virus origin/ subtype	Designation	Limit of detection (log_{10} TCID$_{50}$/mL)					
		SD Bioline	Binax Now	Clearview	Veritor	Directigen EZ	Sofia
Human A(H7N9)	A/Anhui/01/2013	5.5	5.5	>6[a]	5.5	5	5
Avian A(H7)	A/Northern Shoveller Egypt-EMC/1/2012	5	4	>6[a]	4	4	3
	A/Mallard/Netherlands/4/2010	5	4	>6[a]	4	4	2.5
	A/Mallard/Lithuania-EMC/2/2010	4	3	4.5	2.5	2.5	2
Human A(H1N1)pdm09	A/Auckland/1/2009	4.5	3.5	5	3	3	2.5
	A/Brisbane/292/2010	4	3	4.5	3	2.5	2
Human A(H3N2)	A/Sydney/506/2013	5	4	5	4	4	3
	A/Victoria/361/2011	4	3.5	4.5	3	3	2.5

TCID$_{50}$: tissue culture infectious dose 50.

[a] >6, the virus was not detected at any of the concentrations tested.

Table 3

RNA copy number and Ct value limit of detection of the influenza point-of-care tests evaluated in this study

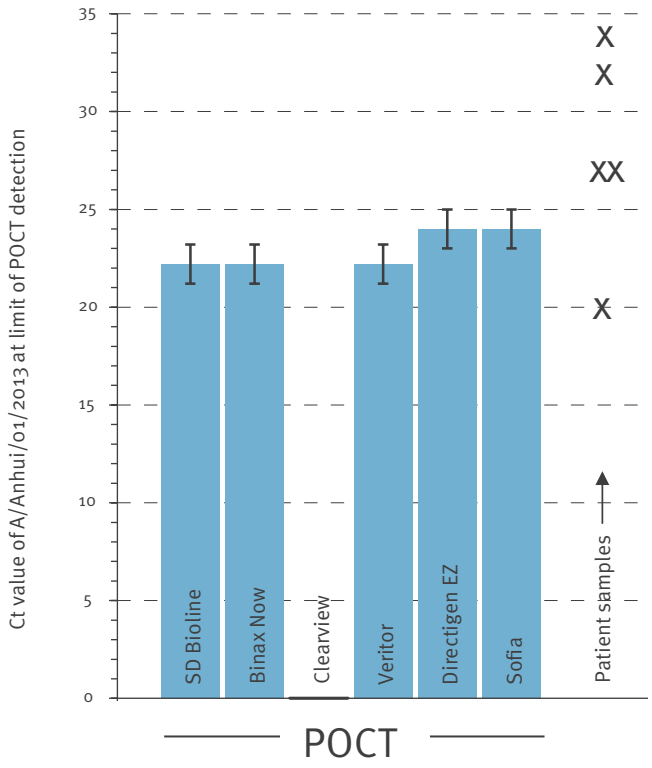
Influenza virus origin/subtype	Designation	Limit of detection (RNA copies/µL [Ct value])						RNA copies/ µL [Ct value] of 1x10$^{4.5}$ TCID$_{50}$/mL concentrations
		SD Bioline	Binax Now	Clearview	Veritor	Directigen EZ	Sofia	
Human A(H7N9)	A/Anhui/01/2013	5.0x10^5 [22.2]	5.0x10^5 [22.2]	>1.5x10^6 [a] [<20.4]	5.0x10^5 [22.2]	1.6x10^5 [24.0]	1.6x10^5 [24.0]	3.6x10^4 [26.2]
Avian A(H7)	A/Northern Shoveller/ Egypt-EMC/1/2012	2.3x10^6 [19.7]	2.3x10^5 [23.3]	>2.9x10^7 [a] [<15.8]	2.3x10^5 [23.3]	2.3x10^5 [23.3]	2.1x10^4 [27.2]	4.1x10^5 [22.5]
	A/Mallard/ Netherlands/4/2010	6.3x10^5 [21.6]	5.6x10^4 [25.7]	>1.1x10^7 [a] [<17.2]	5.6x10^4 [25.7]	5.6x10^4 [25.7]	6.0x10^2 [31.8]	1.3x10^5 [24.2]
	A/Mallard/ Lithuania-EMC/2/2010	7.4x10^5 [21.7]	6.6x10^4 [25.5]	1.5x10^6 [20.5]	1.5x10^4 [27.6]	1.5x10^4 [27.6]	8.8x10^3 [28.6]	1.5x10^6 [20.5]
Human A(H1N1) pdm09	A/Auckland/1/2009	1.2x10^6 [20.8]	8.9x10^4 [25.0]	4.5x10^6 [18.8]	5.6x10^4 [25.3]	5.6x10^4 [25.3]	4.6x10^3 [28.2]	1.2x10^6 [20.8]
	A/Brisbane/292/2010	2.7x10^6 [19.5]	3.2x10^5 [23.0]	4.5x10^6 [19.0]	3.2x10^5 [23.0]	5.7x10^4 [25.5]	1.3x10^4 [26.5]	4.5x10^6 [19.0]
Human A(H3N2)	A/Sydney/506/2013	2.6x10^6 [19.7]	2.2x10^5 [23.5]	2.6x10^6 [19.7]	2.2x10^5 [23.5]	2.2x10^5 [23.5]	6.3x10^3 [26.8]	4.9x10^5 [22.2]
	A/Victoria/361/2011	5.9x10^5 [21.9]	1.1x10^5 [24.3]	1.1x10^6 [21.0]	5.7x10^4 [26.0]	5.7x10^4 [26.0]	7.9x10^3 [27.8]	1.1x10^6 [21.0]

Ct: cycle threshold; TCID$_{50}$: tissue culture infectious dose 50.

[a] The virus was not detected at any of the concentrations tested.

Figure

Mean Ct limit of detection for influenza A/Anhui/01/2013 in point-of-care tests compared with Ct values reported for four influenza A(H7N9) cases confirmed by RT-PCR



Ct: cycle threshold; $TCID_{50}$: tissue culture infectious dose 50; POCT: point-of-care test.

The Ct values of the A/Anhui/01/2013 concentrations containing $1x10^6$, $1x10^{5.5}$, $1x10^5$, $1x10^{4.5}$ $TCID_{50}$/mL were 20.4, 22.2, 24.0 and 26.2 respectively, while the RNA copy number was $1.5x10^6$/µL, $5.0x10^5$/µL, $1.6x10^5$/µL, and $3.6x10^4$/µL respectively. Error bars indicate the standard deviation based on triplicate real-time RT-PCR analysis of the virus concentration at the POCT limit of detection. Influenza A matrix gene Ct values for the cases were 27, 32-34, 20 and 27 for the four patients, respectively, and were taken from the published article by Chen et al. [11]. RT-PCR efficiencies and therefore Ct values may differ slightly between the assay used here and that used by Chen et al. Because the Clearview POCT did not detect the influenza A/Anhui/01/2013 virus at any concentration tested, a Ct value of the limit of detection could not be determined for this kit.

than the influenza A(H7N9) virus, with the Sofia kit performing best: LOD ranging from $1x10^2$ to $1x10^3$ $TCID_{50}$/mL for the human influenza A(H3N2) and A(H1N1) pdm09 viruses.

Comparison of POCT LODs based on RNA copy number/µL showed similar results to those based on $TCID_{50}$/mL for four of the kits (Binax Now, Clearview, Veritor and Sofia). These POCTs were less sensitive for the detection of the influenza A(H7N9) virus compared to the seasonal or other influenza A(H7) viruses (Table 3). However, for the SD Bioline and the Directigen EZ tests, comparison of the LODs based on RNA copy number/µL showed that influenza A(H7N9) was detected at a similar sensitivity to the other viruses (Table 3).

LODs based on RNA copy number/µL or Ct also allowed an estimate of the expected performance of the POCTs in detecting influenza A(H7N9) virus in clinical samples (Figure). Comparison of the published Ct values of clinical samples from patients with confirmed influenza A(H7N9) infection [11] suggested that five of the six POCTS would have detected only one of the four influenza A(H7N9)-positive clinical specimens, with the other three specimens being outside the LOD of these assays (Figure).

Discussion

For all viruses tested, the Sofia POCT, which uses an automated reader, had the highest sensitivity. The BD Veritor test, which also uses an automated reader, had comparable sensitivity to the BD Directigen EZ and the Binax Now tests, both of which are read by eye. The Clearview and SD Bioline POCTs demonstrated the poorest sensitivity.

It is important to note that both the Clearview and the BD Veritor tests are only approved for analysis of swab specimens, therefore the test method used here may not have been appropriate. Similarly, all POCT assays may perform better using a particular specimen type, which was not tested here. The collection of the virus sample used for the Clearview and the BD Veritor POCTs (dipping the swab into liquid and waiting at least 15 seconds for absorption) resulted in a sample volume of approximately 50 µL which, when combined with the recommended diluent volume, resulted in the lowest concentrations of virus used in this evaluation (Table 1).

Other limitations of this study include the use of only a single influenza A(H7N9) isolate A/Anhui/01/2013 (although this virus is genetically closely related to other human influenza A(H7N9) viruses for which sequences have been reported) and the fact that clinical specimens were not available for analysis. It is also important to note that these POCTs have not been primarily designed or licensed to detect influenza A(H7N9) viruses or other avian-derived viruses.

Nevertheless, this study does demonstrate that the sensitivity of at least four of the six evaluated POCTs is lower for the novel influenza A(H7N9) virus than for seasonal influenza viruses and the other avian influenza A(H7) viruses tested. Comparison with published Ct values for clinical specimens from influenza A(H7N9) patients suggested that these POCTs may not detect the majority of influenza A(H7N9) cases, particularly if samples are taken late in the course of disease. Therefore RT-PCR remains the diagnostic test of choice for the testing of suspected influenza A(H7N9) influenza cases.

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Conflict of interest

None declared

Authors' contributions

Designed the study: CB, IB, AH. Analysed and interpreted the data: CB, RF, AK, IB and AH. Drafted the article: CB and AH. Revised the article: CB, RF, AK, IB and AH.

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Temporal trends of extended-spectrum cephalosporin-resistant *Escherichia coli* and *Klebsiella pneumoniae* isolates in in- and outpatients in Switzerland, 2004 to 2011

A Kronenberg (andreas.kronenberg@ifik.unibe.ch)[1,2], M Hilty[1,2], A Endimiani[1], K Mühlemann[1,2]
1. Institute for Infectious Diseases, University of Bern, Bern, Switzerland
2. Department of Infectious Diseases, University Hospital Bern, Bern, Switzerland

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Increasing trends for invasive infections with extended-spectrum cephalosporin-resistant (ESC-R) *Enterobacteriaceae* have been described in many countries worldwide. However, data on the rates of ESC-R isolates in non-invasive infections and in the outpatient setting are scarce. We used a laboratory-based nationwide surveillance system to compare temporal trends of ESC-R rates in *Escherichia coli* and *Klebsiella pneumoniae* for in- and outpatients in Switzerland. Our data showed a significant increase in ESC-R rates from 1% to 5.8% in *E. coli* (p<0.001) and from 1.1% to 4.4% in *K. pneumoniae* (p=0.002) during an eight-year period (2004–2011). For *E. coli*, the increase was significantly higher in inpatients (from 1.2% to 6.6%), in patients residing in eastern Switzerland (from 1.0% to 6.2%), in patients older than 45 years (from 1.2% to 6.7%), and in male patients (from 1.2% to 8.1%). While the increase in inpatients was linear (p<0.001) for *E. coli*, the increase of ESC R *K. pneumoniae* isolates was the result of multiple outbreaks in several institutions. Notably, an increasing proportion of ESC-R *E. coli* was co-resistant to both trimethoprim-sulfamethoxazole and quinolones (42% in 2004 to 49.1% in 2011, p=0.009), further limiting the available oral therapeutic options.

Introduction

Extended-spectrum cephalosporins (ESCs), such as those of third-generation (3GC, e.g. ceftriaxone, cefotaxime, ceftazidime) and fourth-generation (4GC, e.g. cefepime), are frequently used antibiotics for the treatment of severe infections, because of their ample spectrum, strong bactericidal activity, and low toxicity. However, during the past three decades, an increasing number of ESC-resistant (ESC-R) Gram-negative pathogens have been reported worldwide [1].

Several mechanisms such as production of extended-spectrum beta-lactamases (ESBLs), chromosomal (cAmpCs) or plasmid-mediated AmpCs (pAmpCs) or carbapenemases may lead to phenotypic resistance to ESCs. However, since their first description in 1983, ESBLs have been recognised as the most prevalent mechanism responsible for resistance to ESCs in *Enterobacteriaceae*. While ESBLs during the 1990s were mainly described in *Klebsiella pneumoniae* isolates causing nosocomial outbreaks, ESBL-producing *E. coli* causing community-acquired infections (especially urinary tract infections (UTIs)) nowadays also represent a serious challenge for the therapeutic armamentarium [2]. According to the Infectious Diseases Society of America (IDSA), ESBL-producing *Klebsiella* spp. and *E. coli* belong to the six most important multidrug-resistant (MDR) pathogens for which new therapies are urgently needed [3].

The prevalence of ESC-R *Enterobacteriaceae* varies worldwide. In 2009, 39% of *E.coli* isolated from intensive care patients in the Asian/Pacific area were ESBL producers, while rates were lower in Latin America (25%), Europe (16.3%) and North America (8.7%) [4]. As described by the European Antimicrobial Resistance Surveillance Network (EARS-Net), the prevalence rate of ESC-R *Enterobacteriaceae* causing invasive infections increased significantly from 2007 to 2010 in half of the reporting countries, but to different levels. For instance, while the rates in 2010 were below 5% in Scandinavia and Iceland, rates above 10% were found in 10 European countries (Bulgaria, Cyprus, Czech Republic, Greece, Hungary, Italy, Latvia, Malta, Romania and Spain) [5]. However, most surveillance programmes have taken into consideration only isolates responsible for invasive infections in hospitalised patients [6], and only a few studies at national and local level have described rates of ESBL producers in community-acquired UTIs or bloodstream infections (BSIs) [7-10]. As a result, data regarding the temporal

trends of antibiotic resistance in the community are still needed.

In this study, we describe in detail the temporal trends of ESC-R *E. coli* and *K. pneumoniae* isolates in hospital and community settings in Switzerland during an eight-year epidemiological study performed using the data of the Swiss Antibiotic Resistance Surveillance database ANRESIS [11].

Methods

Data collection

Antibiotic resistance data for *E. coli* and *K. pneumoniae* isolates were analysed using the ANRESIS database [11]. The ANRESIS programme collects all routine antibiotic resistance data from currently 22 clinical microbiology laboratories located in Switzerland. The ANRESIS laboratories are homogeneously distributed across the country. They include university laboratories, representing isolates mainly from tertiary-care hospitals, as well as cantonal and private laboratories, representing data from smaller hospitals and outpatient clinics. They send antimicrobial susceptibility test results (AST) of all routinely performed analyses, including isolates from none-sterile sites.

From 2004 to 2010, all participating laboratories interpreted their ASTs according to the Clinical Laboratory Standards Institute (CLSI) criteria in use at that time [12]. During 2011, five of the 11 laboratories included in this study changed their breakpoints according to the European Committee of Antimicrobial Susceptibility Testing (EUCAST) [13]. All institutions participated in at least one quality control programme from the United Kingdom National External Quality Assessment Service (UK NEQAS), and/or the National External Quality Program of the Institute of Medical Microbiology, Zurich [14]. AST results from each laboratory were sent on a regular basis, weekly or monthly, to the central database located at our institution (Institute for Infectious Diseases of the University of Bern).

For the present analysis, we used only data from the 11 laboratories sending data during the whole study period. These laboratories were representative for Switzerland. Moreover, hospitals performing fewer than 200 microbiological samples per year were excluded. Only clinical samples were analysed. When there were multiple isolates with an identical resistance pattern from the same patient and calendar year, only the first was included into the analysis. According to the hospital statistics of Switzerland for the year 2007 [16] and the statistics of Swiss physicians 2009 [17], the restricted data used for this analysis represent 30% of acute-care hospital beds in Switzerland and 11.2% of all Swiss family physicians. The number of BSIs was extrapolated using the 30% coverage of acute-care hospitals in this study.

Interpretation of the results

Resistant isolates were defined as those that were resistant or had intermediate susceptibility against the antibiotic tested. *E. coli* and *K. pneumoniae* isolates resistant to at least one 3GC and/or 4GC antibiotic were defined as ESC-R. The selection of 3GC and/or 4GC antibiotics tested differed between laboratories. If one antibiotic of an antibiotic class (e.g. quinolones or carbapenems) was resistant, the antibiotic class was classified as resistant. Results were stratified according to geographical region, in- versus outpatients (where outpatient was defined as a person attending a medical practice or outpatient clinic), age and sex of the patient, and whether the isolate was from UTI or BSI. For inpatients, nosocomial infection data (i.e. sample(s) positive for ESC-R isolates at least three days after hospitalisation) were analysed separately.

To examine seasonal trends, the period from April to September was defined as 'summer season', whereas that from October to March was defined as 'winter season'. To compare prevalence rates of ESC-R *E. coli* and confirmed ESBL-positive *E. coli*, we used data from our own institution for the years 2009 and 2010. In this case, detection of ESBL producers was performed by double-disk synergy test (i.e. ceftriaxone, ceftazidime, cefpodoxime discs combined with an amoxicillin-clavulanate disc, distance centre-to-centre 20 mm).

Statistical analysis

To calculate 95% confidence intervals (CIs) of proportions, we used the modified Wald method [18]. Proportions were compared using two-tailed chi-square test or Fisher's exact test using Epi info Version 3.4.3 (Centers for Disease Control and Prevention, Atlanta, United States). We analysed the time trends with linear regression using GraphPad Prism Version 5.04.

Results

The overall study included 160,010 *E. coli* and 21,290 *K. pneumoniae* isolates. ESBL confirmation was done for 225 ESC-R *E. coli* and 48 ESC-R K. pneumonia isolated during the years 2009 and 2010 at the University Hospital of Bern. Of these, 210 (93.3%) *E. coli* and 46 (95.8%) *K. pneumoniae* isolates were confirmed as ESBL producers by the double disc synergy test.

During the eight-year period, a total of 63,743 *E. coli* (39.8%) and 11,083 (52.1%) *K. pneumoniae* were isolated from hospitalised patients from 34 different hospitals, and 96,267 *E. coli* (60.2%) and 10,207 *K. pneumoniae* (47.9%) isolates were collected from outpatients. Of the outpatient samples, 45,395 (47.2%) of *E. coli* isolates and 4,746 (46.5%) of *K. pneumoniae* isolates were collected in outpatient clinics, the rest were collected by general physicians.

Escherichia coli

Between 2004 and 2011, the prevalence of ESC-R *E. coli* increased significantly from 1.0% to 5.8% ($p<0.001$).

Table

Number and proportion of *Escherichia coli* and *Klebsiella pneumoniae* isolates with extended-spectrum cephalosporin resistance, Switzerland, 2004 and 2011

	Escherichia coli			*Klebsiella pneumoniae*		
	2004	2011	P[a]	2004	2011	P[a]
	n/all (%)	n/all (%)		n/all (%)	n/all (%)	
Switzerland all	157/15,469 (1.0)	1,425/24,631 (5.8)	<0.001	23/2,070 (1.1)	153/3,490 (4.4)	0.002
In- outpatient	P[b]=0.027			P[b]=0.189		
Inpatients	81/7,012 (1.2)	582/8,846 (6.6)	<0.001	16/1,163 (1.4)	87/1,659 (5.2)	0.007
Outpatients	76/8,457 (0.9)	843/15,785 (5.3)	<0.001	7/907 (0.8)	66/1,831 (3.6)	<0.001
Hospital size	P[b]=0.457			P[b]=0.733		
<200 beds (n=4)	28/2,827 (1.0)	251/3,883 (6.5)	<0.001	5/389 (1.3)	30/657 (4.6)	0.030
200–500 beds (n=12)	27/2,730 (1.0)	202/3,310(6.1)	<0.001	6/464 (1.3)	37/645 (5.7)	0.020
>500 beds (n=18)	26/1,455 (1.8)	129/1,653 (7.8)	<0.001	5/310 (1.6)	20/357 (5.6)	0.005
Departments	P[b]=0.546			P[b]=0.451		
Intensive care unit	5/398 (1.3)	35/500 /7.0)	<0.001	1/134 (0.8)	10/190 (5.3)	0.261
Others	76/6,614 (1.2)	547/8,346 (6.6)	<0.001	15/1,029 (1.5)	77/1,469 (5.2)	0.006
Outpatients	P[b]=0.399			P[b]=0.117		
Outpatient clinics	33/3,743 (0.9)	360/7,255 (5.0)	<0.001	3/417 (0.7)	35/824 (4.3)	0.002
General physicians	43/4,714 (0.9)	483/8,530 (5.7)	<0.001	4/490 (0.8)	31/1,007 (3.1)	0.001
Regions[c]	P[b]=0.037			P[b]=0.417		
Eastern Switzerland	94/9,161 (1.0)	896/14,402 (6.2)	<0.001	18/1,364 (1.3)	89/2,237 (4.0)	0.004
South-western Switzerland	63/6,308 (1.0)	529/10,229 (5.2)	<0.001	5/706 (0.7)	64/1,253 (5.1)	0.007
Sample[d]	P[b]=0.942			p[b]=0.869		
Blood	12/795 (1.5)	68/1,095 (6.2)	<0.001	2/145 (1.4)	8/226 (3.5)	0.047
Urine	119/12,815 (0.9)	1,112/20,815 (5.3)	<0.001	10/1,386 (0.7)	104/2,523 (4.1)	<0.001
Respiratory	3/373 (0.8)	57/547 (10.4)	<0.001	2/307 (0.7)	18/449 (4.0)	0.33
Wounds	18/1,293 (1.4)	156/1,167 (13.4)	<0.001	6/355 (1.7)	22/308 (7.1)	0.09
Age group (years)	P[b]<0.001			P[b]=0.432		
<2	9/860 (1.0)	27/857 (3.2)	0.018	3/122 (2.5)	6/120 (5.0)	0.737
2–15	12/1,204 (1.0)	68/1,300 (5.2)	<0.001	2/62 (3.2)	4/75 (5.3)	0.660
15–45	26/4,054 (0.6)	223/5,909 (3.8)	<0.001	2/311 (0.6)	20/452 (4.4)	0.016
45–65	30/3,070 (1.0)	313/4,983 (6.3)	<0.001	6/453 (1.3)	68/1,454 (4.7)	0.017
>65	80/6,281 (1.3)	794/11,582 (6.9)	<0.001	10/1,122 (0.9)	55/1,389 (4.0)	0.002
Sex[e]	P[b]<0.001			P[b]=0.138		
Female	111/11,738 (1.0)	958/18,825 (5.1)	<0.001	14/1,305 (1.1)	75/2,238 (3.4)	0.010
Male	46/3,725 (1.2)	467/5,800 (8.1)	<0.001	9/764 (1.2)	78/1,252 (6.2)	<0.001

[a] Significance level indicating the probability that the slope of the linear regression using data of all years between 2004 and 2011 equals 0.

[b] Significance level indicating the probability that the slopes in the subgroups are equal by chance.

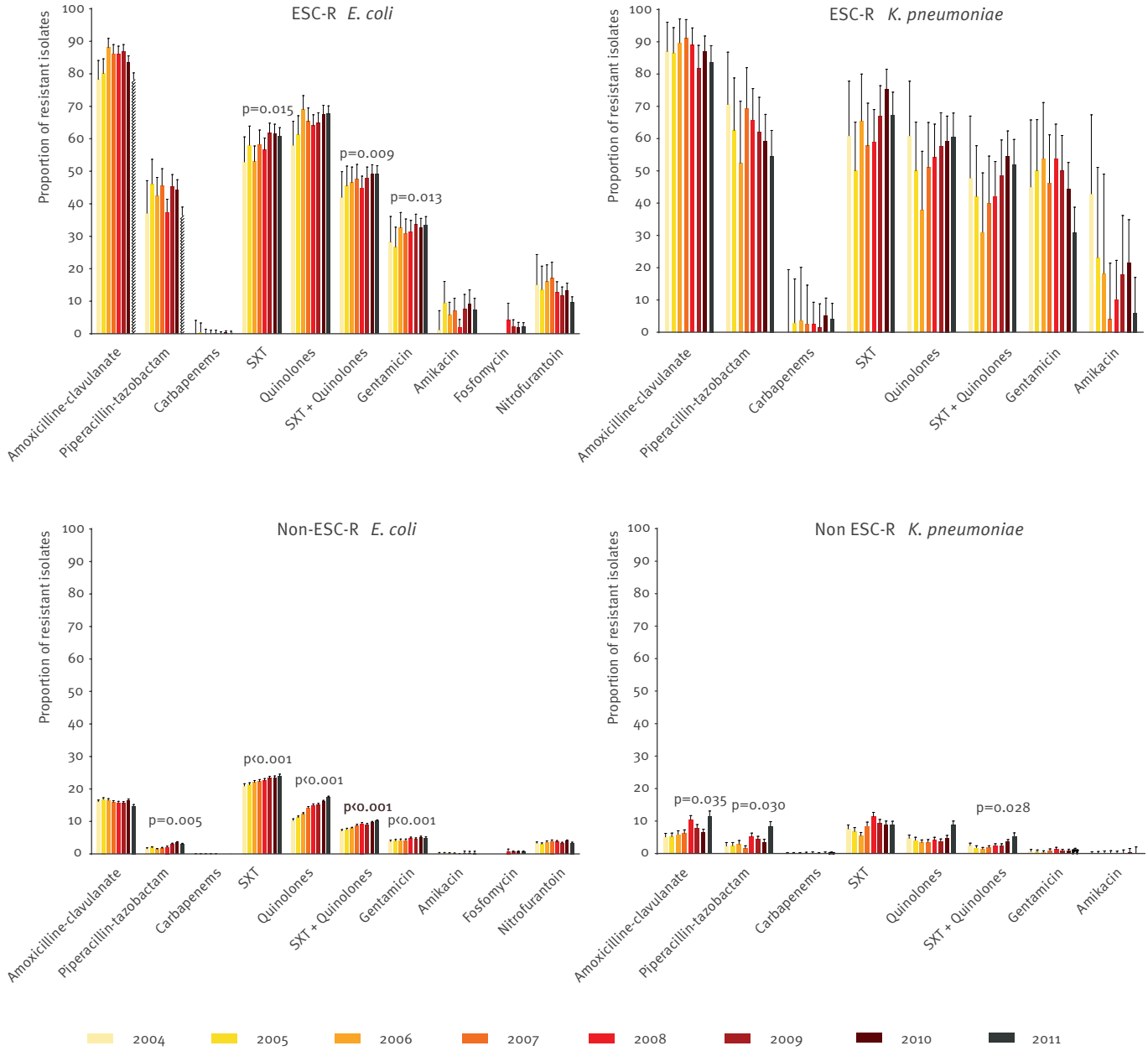
[c] South-western Switzerland includes the cantons Geneva, Vaud, Neuchâtel, Jura, Fribourg, Valais and Ticino; eastern Switzerland includes all other cantons.

[d] Other sample locations are not shown, therefore figures do no sum up to the total number.

[e] Sex was not specified for six *E. coli* isolates.

Figure 1

Co-resistance rates for ESC-R and non-ESC-R *Escherichia coli* and *Klebsiella pneumoniae* isolates, Switzerland, 2004–11

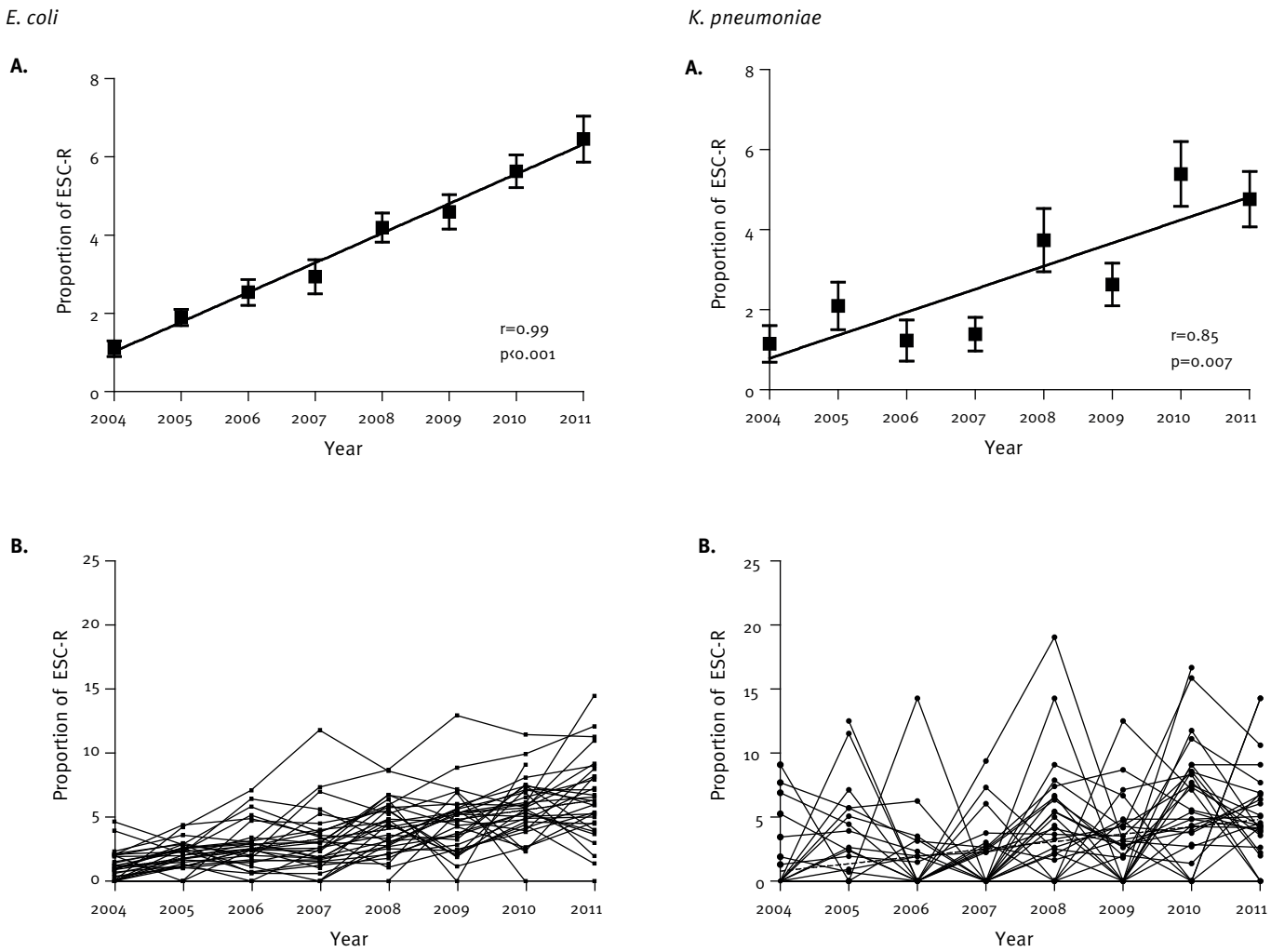


ESC-R: extended-spectrum cephalosporins-resistant; SXT: trimethoprim-sulfamethoxazole.

Resistance rates and 95% confidence intervals for different antibiotics for ESC-R and non-ESC-R *E. coli* and *K. pneumoniae* for the years 2004 to 2011. Fosfomycin and nitrofurantoin were not tested against *K. pneumoniae* isolates. Because of low numbers data are not shown for fosfomycin for the years 2004 to 2007. P values are given for significant trends only (p<0.05).

Figure 2

Increase in ESC-R rates in *Escherichia coli* and *Klebsiella pneumoniae* in 34 hospitals in Switzerland, 2004–11



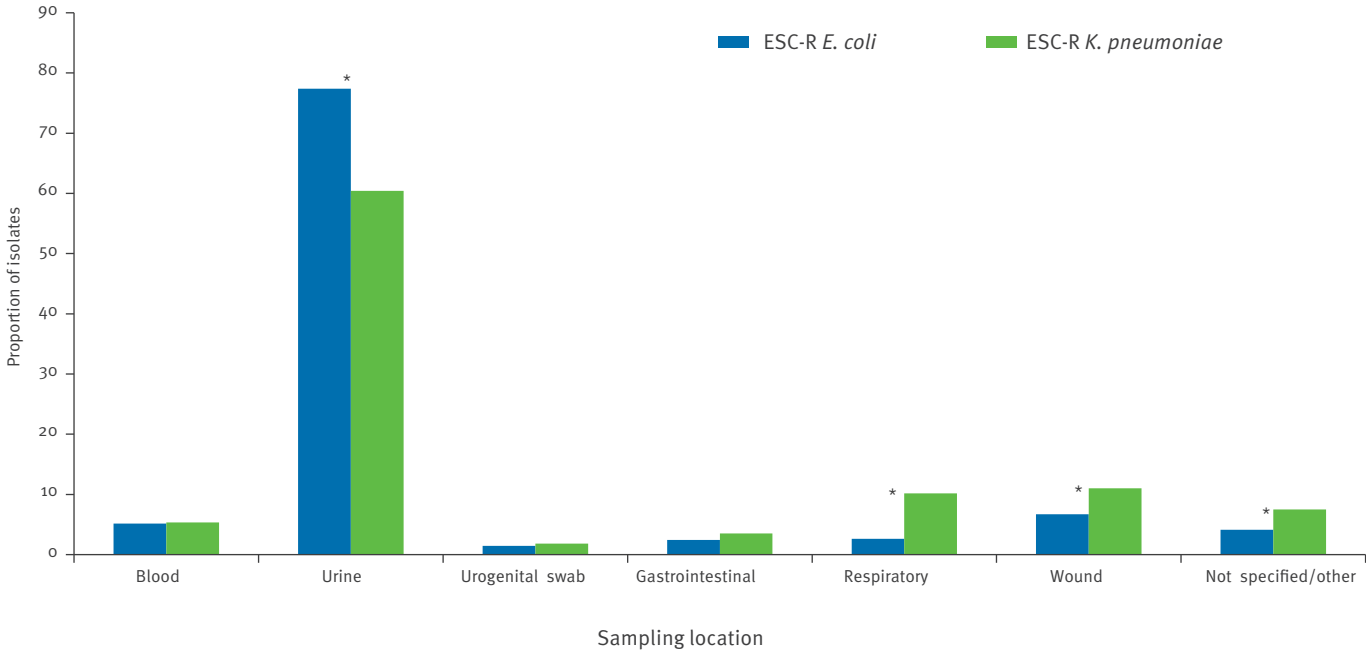
ESC-R: extended-spectrum cephalosporins-resistant; p: significance for correlation; r: Pearson's correlation index.

A. Means and standard error of the means of ESC-R prevalence rates in 34 Swiss hospitals from 2004 to 2011. The linear regression showed significant increase over time for *E. coli* and *K. pneumonia* ($p<0.001$ for both), correlation was linear for *E. coli* ($p<0.001$) and *K. pneumoniae* ($p=0.007$).

B. Rates of ESC-R isolates for individual hospitals. While the increase of ESC-R rates was steady and comparable in all hospitals for *E. coli*, we observed high variability between different years and hospitals for *K. pneumoniae*.

Figure 3

Distribution of ESC-R *Escherichia coli* and *Klebsiella pneumoniae* isolates, by sampling location, Switzerland, 2004-2011



ESC-R: extended-spectrum cephalosporins-resistant.

* p<0.001.

ESC-R *E. coli* as well as ESC-R *K. pneumoniae* were isolated mostly from urinary samples. ESC-R *K. pneumoniae* were isolated 2.2 times more frequently from respiratory or wound samples than ESC-R *E. coli* (p<0.001).

This time trend was linear and without seasonality (data not shown). This increase in rates of ESC-R isolates was observed in all patient groups analysed and was significantly more pronounced in inpatients (p=0.027), in eastern Switzerland (p=0.037), in male patients (p<0.001), and those older than 45 years (p<0.001) (Table). There was no difference between patients under 15 and those 15–45 years of age, or between the age groups 45–65 years and over 65 years, although there was a trend for lower resistance in children younger than two years compared with those 2–15 years-old (p=0.055). We noted a significant increase of ESC-R isolates (from 0.6% in 2004 to 3.5% in 2011; p<0.001) in female patients aged between 15 and 45 years. In inpatients, we did not record differences between those with a nosocomial infection and those without (p=0.15).

The absolute number of BSIs due to ESC-R *E. coli* increased from 12 in 2004 to 68 in 2011. Extrapolated to the overall Swiss population with 7.95 million inhabitants at the end of 2011 [19], we would expect that BSIs due to ESC-R *E. coli* increased from 40 (0.5/100,000 inhabitants) to 227 (2.9/100,000 inhabitants) episodes per year.

The co-resistance rates to other antibiotic classes are shown in Figure 1. Rates were significantly higher for ESC-R than non-ESC-R *E. coli* isolates. Co-resistance did not differ between in- and outpatients (data not shown). For the non-ESC-R *E. coli* isolates, rates increased significantly over time for sulfamethoxazole-trimethoprim (SXT; 20.9% in 2004 versus 24.0% in 2011, p<0.001), quinolones (10.3% in 2004 versus 17.4% in 2011, p<0.001), the combined resistance to SXT and quinolones (7.1% in 2004 versus 10.1% in 2011, p<0.001), piperacillin-tazobactam (1.6% in 2004 v versus. 3.0% in 2011, p=0.005), and gentamicin (3.8% in 2004 versus 4.9% in 2011, p<0.001). For the ESC-R *E. coli*, we observed an increase in co-resistance to gentamicin (28.2% in 2004 versus. 33.6% in 2011, p=0.013), SXT (52.9% in 2004 versus. 60.1% in 2011, p=0.015), and combined resistance to SXT and quinolones (42.0% in 2004 versus 49.1% in 2011, p=0.009), and a non-significant increasing resistance rate for quinolones alone (58.0% in 2004 versus 67.8% in 2010, p=0.06).

Klebsiella pneumoniae

Overall, from 2004 to 2011 ESC-R *K. pneumoniae* isolates increased significantly from 1.1% to 4.4% (p=0.002). The increase was comparable in all patient subgroups (Table) and significant and linear in inpatients (Table, Figure 2A). However, in contrast to ESC-R *E. coli*, the Pearson's correlation index was lower due to higher variability between different years in single hospitals (Figure 2B). Furthermore, ESC-R *K. pneumoniae* isolates were more frequently isolated from other sites than blood or urine than ESC-R *E. coli* (34.3% *K. pneumoniae* versus 17.4% *E. coli*, p<0.001). In particular, about two thirds of these non-blood, non-urine ESC-R *K. pneumoniae* were isolated from wounds or respiratory samples (Figure 3).

As shown in Figure 1, co-resistance in ESC-R *K. pneumoniae* exceeded 50% for all antibiotic classes tested except for aminoglycosides (gentamicin 30.8% and amikacin 6.0%) and carbapenems (4.1%) in 2011. These resistance rates were significantly higher in ESC-R than in non-ESC-R *K. pneumoniae* isolates. While there was no significant trend in co-resistance in ESC-R *K. pneumoniae*, we observed a significant increase in resistance in non-ESC-R *K. pneumoniae* for amoxicillin-clavulanate (5.1% in 2004 versus 11.5% in 2011, p=0.035), piperacillin-tazobactam (2.3% in 2004 versus 8.3% in 2011, p=0.030), and for double resistance to SXT and quinolones (2.5% in 2004 versus 5.3% in 2011, p=0.028).

Discussion

Increasing rates of ESC-R *Enterobacteriaceae* have been described worldwide, however most data rely on BSIs in inpatients [2,6,20]. Data on prevalence of ESC-R isolates among outpatients are rare and restricted to single years (e.g. France, 2006 [7]; Italy, 2003 [8]; Spain, 2006 [9]; Turkey, 2007 [10]). Only one of these studies compared rates of ESC-R *Enterobacteriaceae* among in- and outpatients [8], and data regarding temporal trends among outpatients are missing. Here, we present the temporal trends of ESC-R *E. coli* and *K. pneumoniae* among outpatients and compare the epidemiology of in- and outpatients from 2004 to 2011.

E. coli

According to EARSS data, rates of ESC-R *E. coli* isolated from BSIs increased from 2.7% in 2003 to 8.2% in 2009 [21]. Although such prevalence was lower in our population (1.5% in 2004 and 6.2% in 2011), the increasing trend was comparable with the above European data. We calculated an annual incidence of 2.9/100,000 cases of BSI due to ESC-R *E. coli* in 2011, which is in the same range as the average of 2.6/100,000 cases described in the 31 countries participating in EARSS in 2007 [21]. The societal and economic burden of infections due to ESC-R *E. coli* is remarkable. Taking into consideration the morbidity and mortality data available in the literature, we would estimate 58 additional deaths and 1,131 additional hospital days attributable to BSI due to ESC-R *E. coli* in 2011 in Switzerland [22]. Extrapolating to other clinical manifestations, such as lower respiratory tract infections, skin and soft tissue infections and UTIs, we would expect 838 infections leading to 131 deaths and 9,233 additional days of hospitalisation [23] in Switzerland during the year 2011.

Rates of ESC-R *E. coli* increased steadily in all patient subgroups analysed, but especially in patients older than 45 years, in male patients and inpatients. This observation probably reflects the higher prevalence of established risk factors for infections due to ESBL producers in these sub-populations, such as older age, diabetes mellitus, prostate disease, previous antibiotic use, indwelling catheters, recurrent UTIs, recent hospital admission and residence in long-term care facility [24-30].

Rates of ESC-R *E. coli* also increased significantly over time in lower risk populations, such as young women and outpatients. This correlates well with European studies demonstrating increasing faecal carriage rates of ESBL-producing *E. coli* in the outpatient setting [31-34]. In Switzerland, a recent study detected ESBL-producing *E. coli* in 5.8% of routine stool samples from staff members of meat-processing companies [35], whereas as many as 15.2% of pigs and 17.1% of cattle at slaughter carried ESBL-producing *E. coli*, indicating that there is an established reservoir of these organisms in farm animals in Switzerland [36].

Co-resistance in ESC-R *E. coli* is frequent and at least in part due to the fact that genes coding for the resistance to different antibiotics are located on the same plasmids [6]. In our study, roughly half of ESC-R *E. coli* were resistant to both SXT and quinolones. Resistance rates to these antibiotics increased over time, whereas resistance to nitrofurantoin and fosfomycin did not increase between 2004 and 2011, indicating that both antibiotics are still a valuable alternative for non-complicated UTIs [37]. For invasive infections, carbapenems are still a valuable option, but it is feared that spread of carbapenemase producers (e.g. KPC and NDM producers) will increase the incidence of MDR bacteria [38].

K. pneumoniae

K. pneumoniae is the second most frequent cause of Gram-negative BSIs after *E. coli* and often affects patients with impaired immune system such as patients with diabetes, alcohol problems and hospitalised patients with indwelling devices [5]. In our population, BSIs due to *K. pneumoniae* in 2011 were eight times less frequent than those due to *E. coli*.

As for *E. coli*, prevalence rates of ESC-R *K. pneumoniae* are increasing worldwide. Between 2007 and 2010 rates of ESC-R *K. pneumoniae* increased significantly in nine of 28 countries participating in EARSS, leading to very different rates (from below 1% in northern countries to greater than 50% in some south-eastern countries) [6]. Restricting our analysis to blood cultures, we

observed an increase in ESC-R isolates from 1.4% in 2004 to 3.5% in 2011 (p=0.05), which is comparable to the northern countries of Europe [5].

As for *E. coli*, rates of ESC-R *K. pneumoniae* increased in all patient subgroups but, in contrast to *E. coli*, there were no differences by age or sex. Resistance to other antibiotic classes was even higher than in *E. coli*. Indeed, ESC-R K. pneumonia resistance rates in 2011 were above 50% for all antibiotics tested except aminoglycosides and carbapenems. Carbapenemase resistance in ESC-R *K. pneumoniae* increased from 0% to 4.1%, which was not significant (p=0.11). However, in view of the global epidemiology, this finding probably anticipates a worrisome expansion of these life-threatening pathogens [39].

In contrast to *E. coli*, infections with ESC-R *K. pneumoniae* are still mainly hospital-associated [40], with outbreaks more frequently reported for ESC-R K. pneumonia than for ESC-R *E. coli* [41,42]. This is supported by our analysis demonstrating that the increase of infections due to ESC-R *K. pneumoniae* was more pronounced in inpatients, which is mainly due to the accumulation of multiple small outbreaks in single hospitals (see Figure 2b). Several reasons for the higher frequency of outbreaks in *K. pneumoniae* have been postulated: (i) environmental contamination occurs significantly more often when the patient is carrying ESBL-producing *K. pneumoniae* compared with ESBL-producing *E. coli* [41]; (ii) *K. pneumoniae* has a higher ability to persist in the environment due to the formation of biofilms [43]; (iii) some antiseptics (e.g. chlorhexidine or hexamidine) are less effective against *K. pneumoniae* [44]. In addition, our data demonstrate that ESC-R *K. pneumoniae* were more frequently isolated from wounds or the respiratory tract, which may facilitate transmission, when standard hygiene precautions are not implemented.

Our study has several limitations. Because physicians frequently implement an empirical treatment for UTIs and provide a urine sample only if the infection does not improve, our analysis may overestimate the real incidence of ESC-R [45]. In addition, it is important to note that our study describes the epidemiology of ESC resistance, which includes ESBL, pAmpCs and cAmpCs. However, our data are consistent with those of the European Center for Disease Prevention and Control (ECDC), which also use ESC-R as a surrogate for ESBL production, demonstrating that 65 to 100% of ESC-R *E. coli* in Europe are ESBL producers [5]. We were not able to perform genetic analysis to confirm the presence of ESBL genes in our collection of isolates. Performing phenotypic ESBL confirmation tests in a subset of our samples, we speculate that about 93 to 96% of ESC-R isolates in Switzerland are true ESBL producers. However, the clinical impact of confirming ESBL production is debated, and newer guidelines even abstain from confirmatory tests and at least for clinical decisions completely rely on phenotypic resistance testing results [46,47].

In conclusion, we demonstrate a significant increase of ESC-R *E. coli* and *K. pneumoniae* isolates in the period from 2004 to 2011 in Switzerland. This increase is comparable to other European countries. Our data allowed us to demonstrate an increase in ESC-R in non-invasive and in outpatient samples and to estimate the burden of disease in Switzerland. National surveillance should be implemented and maintained to monitor the spread of life-threatening MDR pathogens and support physicians in the implementation of correct and efficacious antibiotic treatments in community and hospital settings.

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Conflict of interest

None declared.

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