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

M Souli[1], I Galani[1], H Giamarellou (hgiama@ath.forthnet.gr)[1]
1. Fourth Department of Internal Medicine, Athens University Medical School, Athens, Greece

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

Introduction

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

Definitions

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

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

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

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

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

Acinetobacter baumannii

Clinical relevance

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

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

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

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

Resistance mechanisms

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

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

Proportion of resistant strains

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

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

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

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

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

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

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

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

Table 1

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

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

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

Risk factors for resistance

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

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

Pseudomonas aeruginosa

Clinical relevance

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

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

Resistance mechanisms

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

Proportion of resistant strains

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

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

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

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

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

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

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

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

Table 2

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

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

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

Risk factors for resistance

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

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

Enterobacteriaceae

Clinical relevance

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

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

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

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

Resistance mechanisms

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

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

Proportion of resistant strains

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

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

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

Risk factors for resistance

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

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

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

Current therapeutic options

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

TABLE 3

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

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

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

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

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

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

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

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