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Editorials

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

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

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

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

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

The relentless increase in resistance to third-generation cephalosporins and fluoroquinolones in Enterobacteriaceae such as *Escherichia coli* and *Klebsiella pneumoniae* in Europe [2] has led to increasing use of carbapenems in hospitals, one of the most potent class of antibiotics against Gram-negative bacilli infections. Outbreaks due to metallo-beta-lactamase (MBL) producing, thus carbapenem-resistant, *K. pneumoniae* is therefore of great concern [11]. Isolates of Gram-negative bacilli simultaneously containing plasmids encoding various ESBLs, MBLs or AmpC beta-lactamases are now increasingly being reported in Europe. The acronym

XDR, which was originally coined for extensively drug-resistant *Mycobacterium tuberculosis*, is now used, though with various definitions, to describe such multidrug-resistant Gram-negative bacilli isolates for which only one or two antibiotic alternatives are available for therapy [12,13]. This increasing number of reports of XDR Gram-negative bacilli is particularly worrisome, especially because it has not been paralleled by development and availability of alternative therapeutic options. There are very few new antibiotics with a novel mechanism of action in the pharmaceutical industry research and development pipeline.

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

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

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

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

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Setting up an enhanced surveillance of newly acquired hepatitis C infection in men who have sex with men: a pilot in London and South East region of England

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We describe the implementation of an enhanced surveillance pilot for newly acquired hepatitis C (HCV) in men who have sex with men (MSM) in London and the South East region of England. Preliminary findings suggest ongoing HCV transmission among MSM infected with human immunodeficiency virus (HIV) and that enhanced surveillance for newly acquired HCV in MSM is feasible.

Background

Acute hepatitis C (HCV) among people living with human immunodeficiency virus (HIV) is increasingly being recognised as an emerging problem in developed countries following the wide introduction of anti-retroviral therapy (ART) [1]. In recent years there has been a rise in reports of newly acquired HCV infection among HIV-infected men who have sex with men (MSM) without a history of injecting drug use (IDU). Mucosal trauma during high-risk sexual practices has been suggested as the most likely route of transmission among MSM [2-5].

In 2006, a retrospective study estimated an average annual rise of 20% in the incidence of newly diagnosed HCV among HIV-positive MSM attending genito-urinary medicine (GUM) clinics in London and Brighton since 2002. 389 cases were reported among HIV-positive MSM, but only six cases were reported in HIV-uninfected MSM for the four and a half year period. [6] The findings reinforced the need for routine and repeat HCV testing of HIV-infected individuals, in line with guidelines of the British HIV Association (BHIVA) [7].

To better understand the burden of HCV infection and associated behavioural risk factors among MSM, the Health Protection Agency (HPA), in collaboration with BHIVA and the British Association for Sexual Health and HIV (BASSH), is piloting enhanced surveillance of newly acquired HCV in MSM (SNAHC) in London and South East region of England. We describe the set-up of the SNAHC pilot and report preliminary findings for the period from 1 January to 30 September 2008.

Methods

The SNAHC project was developed under the guidance of a multidisciplinary steering group [8]. In February 2008, all 35 GUM clinics in London and five major clinics in the South East region of England were invited to participate by email and a postal letter. The pilot was also announced at the annual BHIVA spring conference in 2008. Full project information was made available on a dedicated page on the HPA website (see link at the end of the article). Starting from January 2008, participating clinics were required to report all cases of newly acquired HCV in MSM using a standard reporting form requesting the following: HCV antibody and RNA test results, HCV genotype, reason for HCV testing, HIV status and date of HIV diagnosis, most recent CD4 count, ART, history of IDU, sexually transmitted infections (STI), sexual practices and recreational drug use. Monthly reminder emails to report new cases are sent to identified clinic contacts.

The surveillance case definitions for newly acquired HCV are:

- Confirmed case: HCV-antibody sero-conversion within the previous 36 months;
- Probable case: HCV-RNA-positive and HCV-antibody-negative or equivocal.

In order to close the surveillance gap between the previous study in mid-2006 and the start of SNAHC, clinics were asked to retrospectively report the aggregate number of cases seen in 2006 and 2007. HCV incidence estimates for HIV-positive MSM were calculated using as denominator person years (PY) of MSM with diagnosed HIV infection attending care, consistent with the study by Giraudon *et al.* [6].

Results

Sixty percent (25/40) of invited clinics in the targeted regions agreed to participate, including all major HIV treatment centres, covering 91% of MSM attending for HIV care in London and 57% in the South East region [8].

Retrospectively reported HCV cases in HIV-infected MSM 2006 and 2007

A total of 200 cases in HIV-infected MSM were reported retrospectively from all participating clinics for 2006 and 2007 (91 cases in 2006, 109 in 2007).

HCV cases and estimated incidence in diagnosed HIV-infected MSM 2002-2007

For the sixteen clinics that had reported cases since 2002 [6], the annual cases and the estimated annual incidence for diagnosed HIV-infected MSM in the period from 2006 to 2007 has shown no obvious trend, with 84 cases in 2006 (incidence: 7.4/1,000 PY) and 101 cases in 2007 (8.2 per 1,000 PY) (Figure). In 2007, these sixteen clinics provided care for 85% of diagnosed HIV-infected MSM in London and 56% in Brighton.

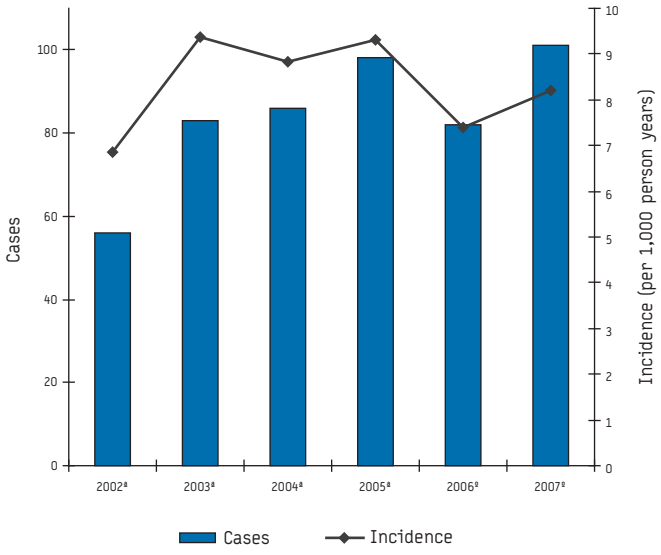
Case numbers reported for 2006 and 2007 (nil cases) from one large London clinic included in the Figure were inconsistent and are being further investigated. The case numbers and incidence for 2006 and 2007 are therefore probably underestimates of the true figures. Despite this missing information the number of reported cases has remained high.

Prospectively reported cases since January 2008

In the first nine months of the pilot, a total of 29 cases were reported from 11 of the 25 participating clinics. This is less than 40% of the number of cases expected for this period based on numbers reported retrospectively in the same clinics during 2006 and 2007. The median lead time between serological diagnosis and completed report was around two months. Ten forms were insufficiently completed, not allowing classification as confirmed or probable cases. After follow-up of missing information and strict application of the case definition the steering group verified 17 as confirmed and 12 as probable cases. All but one man had a known HIV-positive status at the time of HCV infection. The sections on behavioural risk factors were well completed. IDU was denied by all but three cases (two of which reported use within the last three months, one reported last use eight years previously); 72% of cases (21/29) gave information regarding fisting practices, 57% of those (12/21) denied any fisting in the previous three months.

FIGURE

Numbers of cases and estimated incidence of newly acquired HCV in diagnosed HIV-infected MSM reported by HIV clinics in London and Brighton, 2002-2007



[a] Reported in reference [6],
[b] Report from one clinic outstanding
HCV: hepatitis C virus; HIV: human immunodeficiency virus; MSM: men who have sex with men.

Discussion

The pilot shows the feasibility of enhanced surveillance of newly acquired HCV in MSM. The preliminary findings suggest ongoing HCV transmission among HIV-infected MSM in London and the South East region of England. The clinics that agreed to participate in the surveillance scheme provide care for the majority of diagnosed HIV-infected MSM in those regions and should therefore be representative of the population of interest. The low number of reported cases in HIV-uninfected MSM is consistent with previous studies [6]. However, the surveillance may be biased towards HIV-infected MSM, as regular HCV testing is recommended for individuals with diagnosed HIV infection in the United Kingdom while no such recommendation currently exists for other MSM.

Incompleteness of information and completion errors highlighted design weaknesses of the case definition section of the reporting form; the form is now being amended. Secondly, fewer reports than expected have been received for the period compared to the numbers reported in previous years. Apart from a genuine fall in incidence, likely reasons include under-reporting and reporting delay due to the need to complete laboratory, clinical and behavioural information. To address the latter issue, the steering group has suggested asking clinicians to report new cases at the time of the initial diagnosis, with subsequent follow-up for behavioural information and additional serology.

A comprehensive report will be prepared at the end of the 12 months pilot phase of SNAHC, allowing sufficient time for possible reporting delays. Preliminary results suggest that SNAHC will be able to provide useful information for the epidemiology and control of newly acquired HCV in MSM.

With around 100,000 MSM aged 16-44 years and nearly 12,000 HIV-positive MSM accessing care in 2007 [8,9], London has a substantial MSM population at risk. The city also constitutes a hub for national and international sexual and infection networks of MSM. We welcome knowledge of similar surveillance schemes for newly acquired HCV in MSM in other European countries. Further information on SNAHC, protocol and reporting forms are available at: http://www.hpa.org.uk/web/HPAweb&HPAwebStandard/HPAweb_C/1204186211416 or under www.hpa.org.uk, keyword 'SNAHC'.

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Emergence and spread of vancomycin resistance among enterococci in Europe

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

Introduction

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

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

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

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

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

Table 1

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

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

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

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

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

Description of the epidemiological situation in Europe

Northern Europe

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

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

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

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

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

United Kingdom and Ireland

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

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

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

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

The majority of Trusts reporting >10 cases were acute teaching Trusts. VRE is not a high profile cause of invasive infection in the UK; VRE is eclipsed by more profuse pathogens with, for example, 4,438 MRSA bacteraemias [41] and 50,392 Clostridium difficile cases reported by the Department of Health's mandatory reporting schemes over the same time period [42]. In consequence, VRE does not "enjoy" the same degree of political and press attention as MRSA and C. difficile.

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

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

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

France

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

Table 2

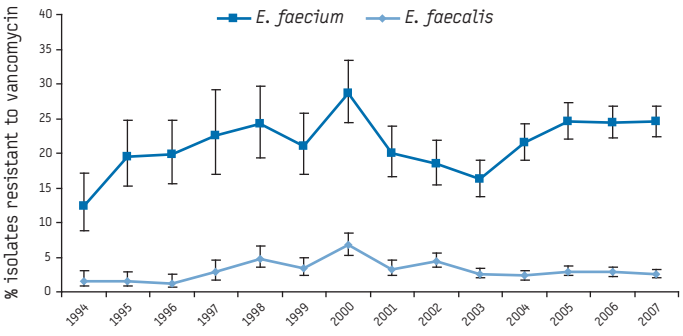
Prevalence levels of vancomycin-resistant entrococci (VRE) among enterococcal bacteraemia cases as reported by three different surveillance programmes with varying amounts of region coverage and participation, United Kingdom, 2001-2007

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

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

Figure 1

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



Source: LabBase voluntary laboratory reporting [36]

first six months of 2008. Overall, it is assumed that the Reference Centre has analysed isolates from the major outbreaks occurring in France since 2005. Among 507 isolates analysed, 27 were obtained from blood cultures, 30 from various suppurations (mostly intra-abdominal), 10 from intravenous catheters, 68 from urine and 372 from rectal swabs. The latter isolates were obtained during faecal screening, which is part of infection control measures. This distribution confirms the low ratio of infection versus colonisation for VRE. The vast majority of isolates were *E. faecium* and *E. faecalis* and contained the *vanA* or *vanB* genes; *vanA E. faecium*, *vanB E. faecium*, *vanA E. faecalis* and *vanB E. faecalis* represented 78.2%, 18%, 3% and 0.8% of isolates, respectively.

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

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

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

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

Central Europe

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

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

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

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

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

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

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

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

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

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

Southern Europe

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

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

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

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

laboratories in 2006 and 49 in 2002), which currently cover around 10% of the population. VREfm rates increased from 15% in 2001 to 24% in 2003, but decreased to 11% in 2007. The frequency of VREfs has increased but has remained below 5% during the entire period (from <1% in 2002 to 4% in 2006) [8]. The first clonal outbreak caused by VRE in Italy was reported in an ICU in 1996 and since then clonal outbreaks have been reported in different hospitals [50;58;59]. Nationwide spread of an *E. faecium vanA* strain causing infections in different cities from 2001 to 2003 was also described [50]. Most VREfm strains associated with human infections which were characterised since 1993 have been multidrug-resistant and have clustered with hospital-acquired clonal types [19;50;60]. Horizontal transfer of Tn*1546* also seemed to contribute to the recent spread of VRE in Italy [61].

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

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

Eastern and south-eastern Europe

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

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

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

The Netherlands – an example of a low prevalence country

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

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

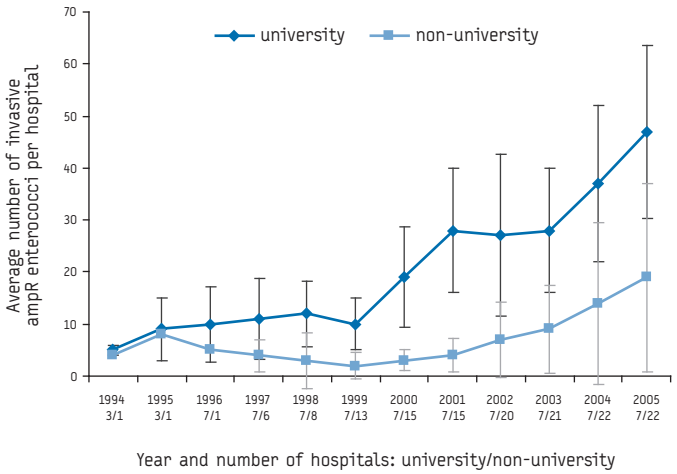
The Dutch Working party "Infection Prevention" has developed guidelines with measures to prevent transmission of highly resistant microorganisms (HRMO), including *E. faecium* (http://www.wip.nl/). In these guidelines *E. faecium* is considered an HRMO when the strain is resistant to both ampicillin and vancomycin and isolation of patients is indicated only in those cases. Ampicillin-susceptible VREfm isolates are considered animal derived. Isolation of patients with these strains is not indicated, because these strains do not spread in hospitals and ampicillin can still be used as the first choice drug to treat these strains.

Concluding remarks

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

Figure 2

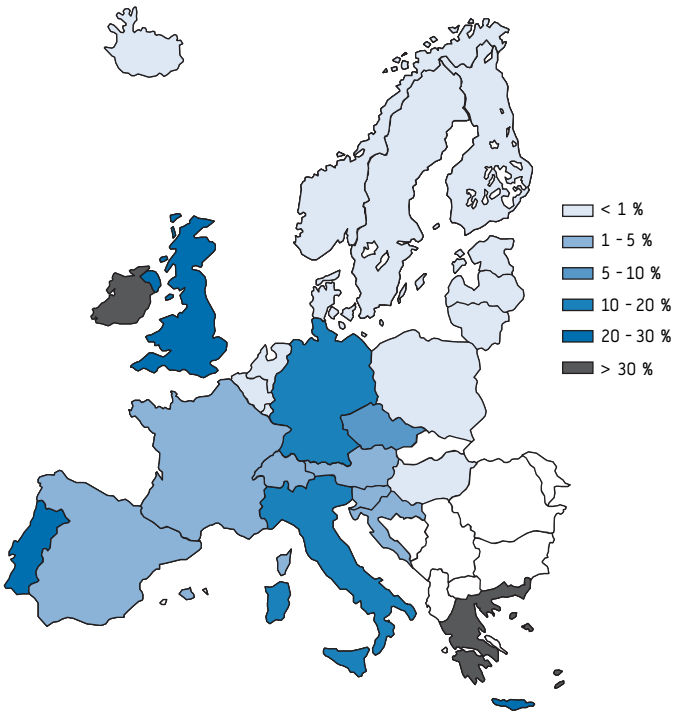
Average number of invasive ampicillin-resistant enterococci per hospital in university and non-university hospitals*, the Netherlands, 1994-2005



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

Figure 3

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



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

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

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

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Increasing prevalence of ESBL-producing Enterobacteriaceae in Europe

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

Introduction

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

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

Table 1

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

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

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

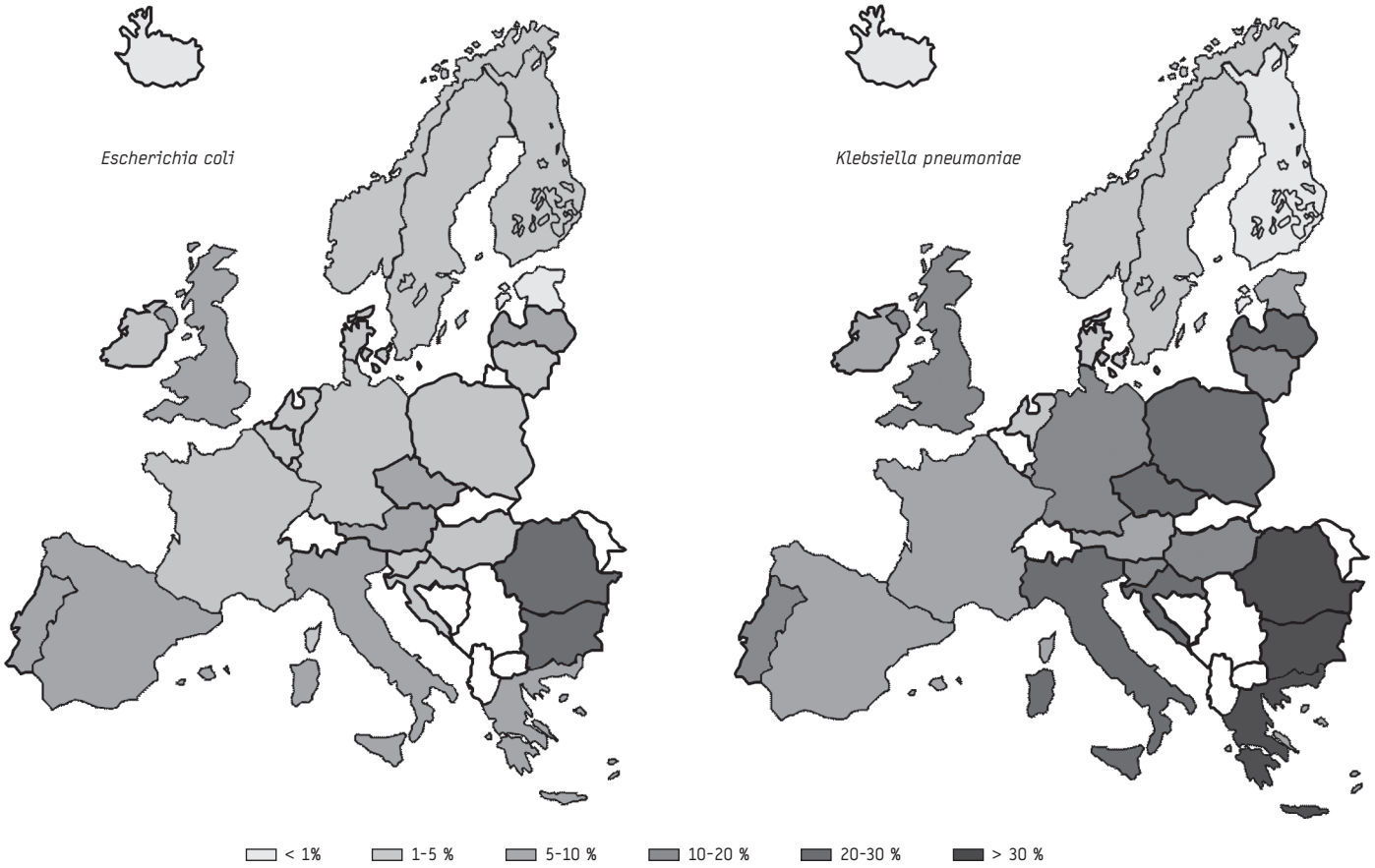
(MBL) and KPC carbapenemases, or to cephamycins, such as CMY enzymes, have more recently emerged and are often associated with ESBLs (see section *Epidemiology of ESBL in Europe*).

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

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

Figure 1

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



EARRS: European Antibiotic Resistance Surveillance System

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

Epidemiology of ESBL in Europe

General surveillance studies

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

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

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

Faecal colonisation surveillance studies

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

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

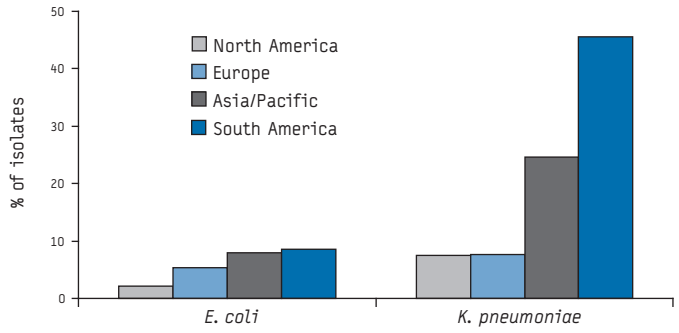
Geographic differences and ESBL types circulating in European hospitals

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

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

Figure 2

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



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

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

Northern European countries

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

Southern countries

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

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

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

United Kingdom

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

Eastern countries

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

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

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

Predominant ESBLs circulating in Europe

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

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

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

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

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

ESBLs in non-humans hosts

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

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

Table 2

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

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

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

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

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

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

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

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

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

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

Clonal expansion of ESBL-producing Enterobacteriaceae

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

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

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

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

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

The impact of plasmid transfer on ESBL-producing Enterobacteriaceae

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

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

Multidrug-resistance profiles in ESBL-producing isolates

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

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

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

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

Concluding Remarks

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

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

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

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

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

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

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

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

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

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

Introduction

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

Definitions

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

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

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

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

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

Acinetobacter baumannii

Clinical relevance

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

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

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

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

Resistance mechanisms

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

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

Proportion of resistant strains

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

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

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

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

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

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

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

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

Table 1

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

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

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

Risk factors for resistance

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

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

Pseudomonas aeruginosa

Clinical relevance

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

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

Resistance mechanisms

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

Proportion of resistant strains

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

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

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

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

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

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

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

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

Table 2

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

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

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

Risk factors for resistance

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

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

Enterobacteriaceae

Clinical relevance

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

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

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

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

Resistance mechanisms

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

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

Proportion of resistant strains

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

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

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

Risk factors for resistance

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

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

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

Current therapeutic options

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

Table 3

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

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

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

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

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

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

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

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