

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 penicillin/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* (VRE_{fm}). Ampicillin- and/or vancomycin-resistant *E. faecalis* (VRE_{fs}) 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]

phenotype	Acquired resistance						Intrinsic resistance
	VanA	VanB	VanD	VanE	VanG	VanL	VanC
ligase gene	<i>vanA</i>	<i>vanB</i> ²	<i>vanD</i> ²	<i>vanE</i>	<i>vanG</i> ²	<i>vanL</i>	<i>vanC</i>
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	<i>E. faecium</i> <i>E. faecalis</i> <i>E. durans</i> <i>E. hirae</i> <i>E. gallinarum</i> ¹ <i>E. casseliflavus</i> ¹ <i>E. raffinosus</i> <i>E. avium</i> <i>E. mundtii</i>	<i>E. faecium</i> <i>E. faecalis</i> <i>E. durans</i> <i>E. gallinarum</i> ¹	<i>E. faecium</i> <i>E. faecalis</i> <i>E. raffinosus</i>	<i>E. faecalis</i>	<i>E. faecalis</i>	<i>E. faecalis</i>	<i>E. gallinarum</i> : <i>vanC1</i> <i>E. casseliflavus</i> : <i>vanC2/3</i>

¹ acquisition of *vanA* or *vanB* cluster in addition to *vanC1* or *vanC2/3* genes – rare event

² subtypes exist (*vanB1-3*, *vanD1-5*, *vanG1-2*); 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 VRE_{fm} 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 promoter avoparcin in animal husbandry, significant animal reservoirs of VRE_{fm} 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* (VSE_{fm}) 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 VSE_{fm} 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

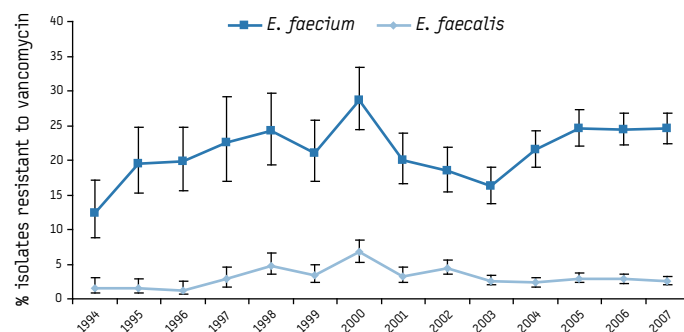
Prevalence levels of vancomycin-resistant enterococci (VRE) among enterococcal bacteraemia cases as reported by three different surveillance programmes with varying amounts of region coverage and participation, United Kingdom, 2001–2007

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 $\leq 1\%$ 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.](http://www.search.ifik.unibe.ch/de/index.shtml)

[search.ifik.unibe.ch/de/index.shtml](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 VREfm 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 VREfm 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 (Tn1546) 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 Sanità 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 Tn1546 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 Tn1546-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. Tn1546 transposon typing confirmed the role of horizontal spread of resistance determinants among these strains and suggested several independent acquisitions of different Tn1546 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.

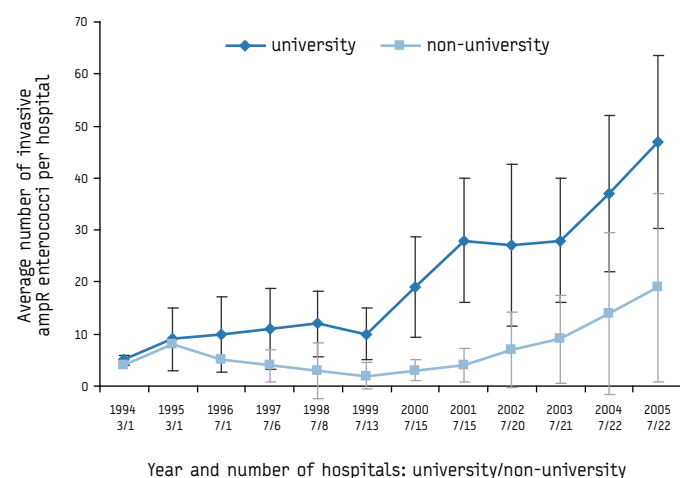
The Netherlands – an example of a low prevalence country

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

FIGURE 2
Average number of invasive ampicillin-resistant enterococci per hospital in university and non-university hospitals*, the Netherlands, 1994-2005



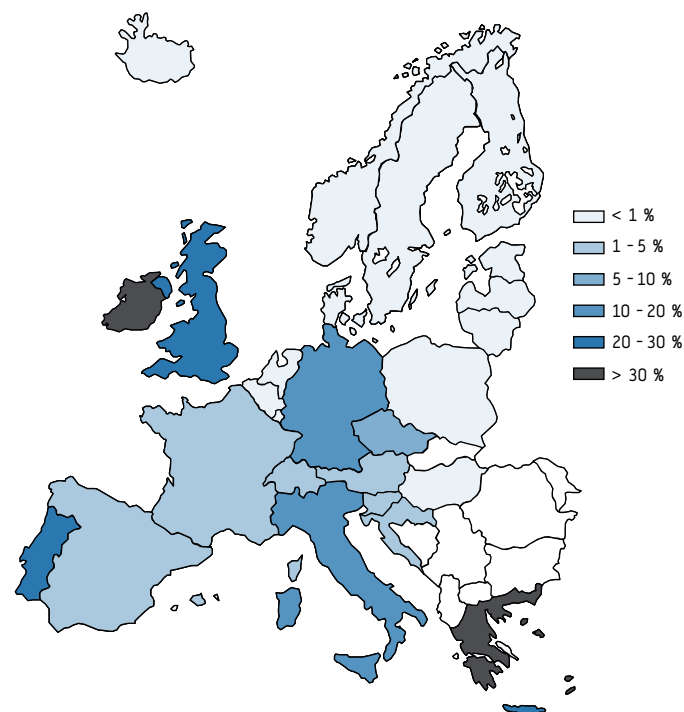
Note: Error bars denote standard deviations.
*For each year, the numbers of hospitals that provided data are indicated.
Adapted from [85].

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 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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