Changing trends in vancomycin-resistant enterococci in French hospitals, 2001–08

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Objectives: Unprecedented outbreaks of vancomycin-resistant enterococci (VRE) have occurred in French hospitals since 2004. The aim of this study was to provide a picture of the spread and control of VRE in France and to characterize the isolates.

Methods: Notification of VRE cases to Institut de Veille Sanitaire has been mandatory since 2001. Isolates of VRE were sent to the National Reference Centre for species and vancomycin-resistance gene identification. Isolates were tested for antimicrobial susceptibility and typed by PFGE and multilocus sequence typing.

Results: Five hundred and four VRE notifications from 195 hospitals were recorded, corresponding to 2475 cases of infection (n=243) or colonization (n=2232) and 74 episodes of clustered cases. Outbreaks were controlled by implementation of infection control measures, although the number of new hospitals reporting isolation of VRE was increasing. The majority of 902 VRE isolated from 2006 to 2008 were Enterococcus faecium (94.8%) with the vanA or vanB gene. No isolate was resistant to linezolid, tigecycline or fusidic acid. PFGE analysis showed 161 different patterns. Generally a few predominant clones and several minor clones spread in a single hospital. In a subset of 46 representatives of PFGE clones, 13 different sequence types were characterized, all belonging to clonal complex CC17, while the esp and hyl genes were inconsistently detected.

Conclusions: The national mandatory notification of unusual nosocomial events allowed rapid identification of VRE outbreaks and early implementation of control measures that have proved effective. However, VRE continue to emerge in a growing number of hospitals.

Keywords: VRE, genotyping, surveillance

Introduction

Enterococci are facultative anaerobic Gram-positive cocci, which are part of the resident flora of the gastrointestinal tract of humans and animals. In spite of their weak virulence, these microorganisms may be responsible for a variety of community- and hospital-acquired infections, such as endocarditis, bacteraemia, meningitis and wound and urinary tract infections, and are associated with intra-abdominal infections. It is noteworthy that enterococci are among the top causes of nosocomial infections worldwide. For 80%–90% and 5%–10% of isolates, respectively. For the last two decades, these microorganisms, especially E. faecium, have worryingly shown increasing resistance to many antimicrobial agents, including penicillins, aminoglycosides (high-level resistance) and glycopeptides (vancomycin-resistant enterococci (VRE)), thus limiting antimicrobial therapeutic options.

Glycopeptide resistance may be due to six acquired genes (vanA, vanB, vanD, vanE, vanG and vanJ), whereas vanC1 and vanC2/3 are responsible for intrinsic resistance in Enterococcus gallinarum and Enterococcus casseliflavus, respectively. VanA and VanB types are the most prevalent in Europe, as well as in the USA, and are mostly detected in E. faecium, which...
constitutes the main reservoir in humans.\textsuperscript{5,6} VanA is characterized by high-level resistance to vancomycin (MICs from 64 to >1024 mg/L) and teicoplanin (MICs from 16 to 512 mg/L), whereas VanB displays lower levels of resistance to vancomycin (MICs from 4 to >32 mg/L) and susceptibility to teicoplanin (MICs = 0.5 or 1 mg/L).

Since their first description in 1988,\textsuperscript{7,8} VRE have become a major threat to public health worldwide, a major risk being the transfer of plasmid-mediated VanA resistance to methicillin-resistant Staphylococcus aureus, already reported in the USA.\textsuperscript{9} Clinical isolates of VRE were initially reported in Europe and shortly after in the USA, but in different epidemiological backgrounds.\textsuperscript{10} In the USA, VRE became rapidly epidemic and then endemic in many hospitals, while faecal carriage was nearly absent in the community.\textsuperscript{11} By contrast, in Europe, outbreaks of VRE in hospitals were uncommon in spite of a large VRE reservoir in the healthy population in the community. The European community reservoir was putatively generated by the use of avoparcin (a glycopeptide) as a growth promoter in animal husbandry.\textsuperscript{12} After the ban of avoparcin in Europe in 1997, the prevalence of VRE in the faeces of food animals declined, at least in certain countries, whereas prevalence of infections in humans due to VRE remained low, but only for a few years.\textsuperscript{13} Indeed, since 2000, VRE rates in clinical isolates have increased in several European countries and peaked, whereas glycopeptide resistance had already declined in the non-hospital reservoir.\textsuperscript{14}

This unexpected change is still not fully understood. However, it is now considered that acquisition of vancomycin resistance by a distinct subpopulation consisting of hospital-adapted, ampicillin-resistant \textit{E. faecium} strains has been a key factor in the successful spread of VRE in hospitals and further dissemination of vancomycin resistance among the hospital \textit{E. faecium} population. Molecular epidemiological surveys have shown that this subpopulation belongs to a particular clonal complex (CC), designated CC17,\textsuperscript{10} delineated by DNA sequence typing (multilocus sequence typing (MLST))\textsuperscript{12} and phylogenetic analysis. Clones belonging to the CC17 lineage are mostly characterized by ampicillin and fluoroquinolone resistance,\textsuperscript{10} and possess a pathogenicity island harbouring the putative virulence genes \textit{esp} and \textit{hyl}.

A comprehensive national programme for prevention of nosocomial infections was gradually set up in France during the period from 1993 to 2004.\textsuperscript{17} This programme included various achievements. In particular, a mandatory notification system for sentinel infectious events was implemented by law in 2001 to promote early outbreak investigation and control, to target infection control recommendations and to identify emerging infections, including emerging multidrug-resistant bacteria.\textsuperscript{17,18} The list of events to be notified included identification of VRE in clinical samples and whether they were responsible for outbreaks, sporadic infections or colonizations. Since isolation of VRE was at that time unusual in French hospitals, an outbreak was defined as two or more cases clustered in time and space where transmission was suspected to have occurred in that setting. Therefore, in hospitals without previous isolation of VRE, two VRE cases could be considered an outbreak. Notifications were made by the clinician or by the clinical microbiology laboratory to the practitioner in charge of hygiene in the hospital, to local and regional health authorities and to the French Institute for Public Health Surveillance (Institut de Veille Sanitaire (InVS)). Following the first notified VRE outbreaks in 2005, this early warning system was complemented for VRE by diffusion of specific recommendations for control of VRE.\textsuperscript{19} In addition, clinical microbiology laboratories were asked to send the VRE isolates to the reference laboratory for enterococci, funded in 2006, which is part of the National Reference Centre for Antimicrobial Resistance (coordinator: Prof. P. Courvalin, Institut Pasteur, Paris, France).

The aim of this study was to provide a picture of the emergence, spread and control of VRE outbreaks in France since 2001 and to investigate phenotypic and genotypic characteristics of VRE isolates received at the National Reference Centre for Enterococci (NRC-E) between 2006 and 2008.

**Materials and methods**

**Bacterial isolates**

From January 2006 to December 2008, among the bacterial isolates received at the NRC-E, a total of 902 non-duplicate (one isolate per patient) human isolates of enterococci with acquired resistance to vancomycin were included for this study. VRE were collected from 112 different French hospital laboratories. All isolates and vancomycin resistance genes were identified by molecular techniques. Antimicrobial susceptibility to major anti-Gram-positive agents was tested by disc diffusion on Mueller–Hilton agar according to the recommendations of Comité de l’Antibiogramme de la Société Française de Microbiologie (CA-SFM; www.sfm.asso.fr; releases of January 2006, 2007 and 2008).\textsuperscript{20} MICs of glycopeptides were determined by using the Etest method according to the manufacturer’s recommendations (bioMérieux, La-Balme-les-Grottes, France).

A subset of 755 isolates (83.7%) was selected for analysis by using PFGE. Only isolates from outbreaks and single isolates from public health facilities located in the vicinity of hospitals facing outbreaks were studied. On the basis of PFGE patterns, one isolate per cluster was further selected irrespective of cluster size ($n$ = 28) or unique isolates were selected ($n$ = 18). This subset of 46 vancomycin-resistant \textit{E. faecium} isolates was analysed by MLST and tested for the presence of virulence genes (esp and \textit{hyl}).

**Molecular techniques**

Genomic DNA was extracted using the InstaGene\textsuperscript{®} matrix kit (Bio-Rad, Marnes-la-Coquette, France) in accordance with the manufacturer’s recommendations. Identification of \textit{E. faecalis} and \textit{E. faecium} species and determination of the presence of vancomycin resistance genes was carried out by a multiplex PCR assay described previously.\textsuperscript{21} A first screening step was performed by using the four sets of specific primers for the genes \textit{ddl} (\textit{E. faecalis}), \textit{ddl} (\textit{E. faecium}), \textit{vanA} and \textit{vanB}. If there was a negative result, other van genes (\textit{vanC1}, \textit{vanC2/3}, \textit{vanD}, \textit{vanE}, \textit{vanG} and \textit{vanJ}) were amplified separately.

Detection of \textit{esp} and \textit{hyl} genes was performed by PCR amplification as previously described, with specific primers.\textsuperscript{22}

MLST assays based on seven housekeeping genes (\textit{atpA}, \textit{ddl}, \textit{gdh}, \textit{purK}, \textit{gyd}, \textit{psST} and \textit{adk}) were performed as previously described.\textsuperscript{23} Different sequences were assigned allele numbers and different allelic profiles were assigned sequence types (STs) based on the \textit{E. faecium} MLST database (http://efaecium.mlst.net).

PFGE was performed as previously described.\textsuperscript{24} Briefly, agarose plugs containing genomic DNA were digested with Smal (Amersham Biosciences, Orsay, France) according to the supplier’s recommendations. Electrophoresis was performed using a CHEF-DRII apparatus (Bio-Rad)
and the Enterococcus program (ramped pulse times of 5 s and 35 s at 200 V for 21 h). Molecular weight markers (lambda ladder) were included in each electrophoresis gel. The PFGE patterns were analysed by using Fingerprinting II software (Bio-Rad). Similarity matrices and dendrograms were obtained by the unweighted pair group method using arithmetic averages (UPGMA). Similarity coefficients were calculated according to the Dice method. Isolates clustering above 85% similarity (corresponding generally to a difference of ≤3 bands) were considered the same clone.

Results

Notification of VRE

From August 2001 to December 2008, 504 VRE notifications from 195 hospitals were received at InVS, corresponding to 2475 cases of infection \( (n=243) \) or colonization \( (n=2232) \) (Figure 1). The infection/colonization ratio was 0.11. Seventy-four episodes of clustered cases of infections/colonizations due to VRE were notified (2–461 cases per episode). As shown in Figure 1, the number of notified cases was low from 2001 to 2003 and then dramatically increased. Not only were more cases notified, but also large outbreaks were reported. In particular, in 2004–05, three unprecedented outbreaks occurred in hospitals from three distant regions, in Paris, Clermont-Ferrand and Nancy \(^{7,25,26} \) (Figure 2). These large outbreaks prompted the French authorities to recommend in 2005 and 2006 reinforcement of strict infection control measures. \(^ {19,27,28} \) The outbreaks were considered as fully controlled in 2007 in the hospitals in Paris and Clermont-Ferrand and in 2009 in the hospitals in Nancy. In 2008, other hospitals in the north and east of France faced the sudden emergence of outbreaks, which have been controlled recently. Globally, the numbers of notifications and the number of isolates sent to the NRC-E increased. Although the number of large outbreaks tended to decrease, the number of hospitals reporting isolation of VRE and small outbreaks increased from 20 in 2006 to 37 in 2007 and 84 in 2008.

Clinical isolates

A total of 902 VRE isolated from humans during a 3 year period were analysed; there were 93 in 2006, 137 in 2007 and 672 in 2008 (Table 1). Forty-six percent of isolates were from outbreaks (≥2 cases) and 54% from sporadic cases. Only 6.4% of isolates were considered responsible for infections; the remainder corresponded to colonization or faecal carriage during outbreaks. Sites of isolation of the 902 clinical isolates were faeces or rectal swabs (81.2%), urine (9.3%) and blood (3.4%), including one endocarditis, and 6.1% were from other origins, mostly intra-abdominal.

Species distribution was as follows: 855 \( E. \) \( \text{faecium} \) (94.8%); 41 \( E. \) \( \text{faecalis} \) (4.5%); 3 \( \text{Enterococcus avium} \) (0.3%); 2 \( \text{Enterococcus durans} \) (0.2%); and 1 \( \text{Enterococcus hirae} \) (0.1%).

van genotyping and antimicrobial susceptibility

Analysis of van genotypes of the 855 \( E. \) \( \text{faecium} \) isolates showed that 563 (65.8%), 290 (33.9%) and 2 (0.2%) contained the vanA, vanB and vanD genes, respectively. Over the 3 year period, the prevalence of van genotypes in \( E. \) \( \text{faecium} \) was as follows: 86 vanA, 2 vanB and 1 vanD in 2006; 91 vanA and 37 vanB in 2007; and 386 vanA, 251 vanB and 1 vanD in 2008 (Table 1). The marked increase in the prevalence of \( E. \) \( \text{faecium} \) vanB in 2008 was related to outbreaks occurring in the north of France. The vanA and vanB genes were detected in 34 \( E. \) \( \text{faecalis} \) (82.9%); 4 in

![Figure 1](https://academic.oup.com/jac/article-abstract/66/4/713/723449/715)
Table 1. Prevalence of van genotypes according to enterococcal species from 2006 to 2008

<table>
<thead>
<tr>
<th>Enterococcal species</th>
<th>vanA</th>
<th>vanB</th>
<th>vanD</th>
<th>vanA</th>
<th>vanB</th>
<th>vanD</th>
<th>vanA</th>
<th>vanB</th>
<th>vanD</th>
<th>total</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. faecium</td>
<td>86</td>
<td>2</td>
<td>1</td>
<td>91</td>
<td>37</td>
<td>0</td>
<td>386</td>
<td>251</td>
<td>1</td>
<td>855</td>
</tr>
<tr>
<td>E. faecalis</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>22</td>
<td>7</td>
<td>0</td>
<td>41</td>
</tr>
<tr>
<td>E. avium</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>E. durans</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>E. hirae</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>90</td>
<td>2</td>
<td>1</td>
<td>99</td>
<td>37</td>
<td>1</td>
<td>412</td>
<td>259</td>
<td>1</td>
<td>902</td>
</tr>
</tbody>
</table>

Figure 2. Geographical localization of VRE isolates in France. The size of the circle is proportional to the number of isolates.
Table 2. Percentages of VRE resistant to antibiotics (other than vancomycin) according to species and genotype

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>vanA (n=441)</th>
<th>vanB (n=161)</th>
<th>vanA (n=23)</th>
<th>vanB (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>93.7</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>51.5</td>
<td>77.4</td>
<td>54</td>
<td>57</td>
</tr>
<tr>
<td>Kanamycin (high level)</td>
<td>78.5</td>
<td>99.4</td>
<td>70</td>
<td>86</td>
</tr>
<tr>
<td>Gentamicin (high level)</td>
<td>21.8</td>
<td>23.6</td>
<td>61</td>
<td>57</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>2.5</td>
<td>1.2</td>
<td>30</td>
<td>29</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>63</td>
<td>4.7</td>
<td>87</td>
<td>86</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>99.1</td>
<td>100</td>
<td>96</td>
<td>71</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>95.5</td>
<td>95.7</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Pristinamycin</td>
<td>0.7</td>
<td>0</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>91.8</td>
<td>97.5</td>
<td>70</td>
<td>43</td>
</tr>
<tr>
<td>Linezolid</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Trimethoprim/sulfamethoxazole</td>
<td>67.6</td>
<td>90.7</td>
<td>61</td>
<td>43</td>
</tr>
<tr>
<td>Teicoplanin</td>
<td>100</td>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>8.8</td>
<td>1.9</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Fusidic acid</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Note: vanA and vanB are genetic markers that confer resistance to vancomycin.

2006, 8 in 2007 and 22 in 2008) and 7 E. faecalis (17.1%; all in 2008), respectively (Table 1). van genes were also detected in other enterococcal species: vanA (n=2) or vanD (n=1) in E. avium; vanA (n=2) in E. durans; and vanB (n=1) in E. hirae.

Susceptibility testing was performed for 632 (70.1%) of the 902 isolates, including 602 E. faecium and 30 E. faecalis. According to the CA-SFM guidelines, all studied isolates were susceptible to linezolid, tigecycline and fusidic acid (Table 2). The vast majority of E. faecium isolates were resistant to ampicillin, erythromycin, clindamycin and levofloxacin, whereas only a few isolates were resistant to chloramphenicol, pristinamycin and rifampicin (Table 2). A few isolates were susceptible to ampicillin, as shown in previous studies. These isolates were presumed to be of animal origin. As expected, all E. faecalis isolates were resistant to both clindamycin and pristinamycin, but susceptible to ampicillin (Table 2). About 20% of E. faecium and 60% of E. faecalis were resistant to clindamycin, whereas the majority of isolates were resistant to trimethoprim/sulfamethoxazole and doxycycline, with the exception of the E. faecium vanB isolates, which were often susceptible to doxycycline (Table 2). This difference between the vanA and vanB E. faecium was unexplained, but might relate to the co-existence on the same plasmids circulating in enterococci of the vanA operon and of a tetracycline resistance determinant.

PFGE patterns

Molecular epidemiological investigations of outbreaks and clusters of infections in French hospitals showed clonal spread of different epidemic E. faecium strains (data not shown). Generally, a few (one to four) predominant clones and several minor clones (up to 26) spread in a single hospital. Among the 731 E. faecium isolates, 125 of 532 vanA-type VRE and 36 of 199 vanB-type VRE were clonally related.

Generally, each hospital had specific clones, although spread between neighbouring hospitals (<50 km) could be observed. In four cases, strains with identical PFGE patterns were identified in remote hospitals. An epidemiological link was found only in one hospital, which was facing an outbreak. In this case, the index case of the outbreak was a patient colonized with VRE who had been transferred from another hospital with VRE outbreak, 210 km away.

Eighteen vanA- and 6 vanB-positive E. faecalis strains were analysed by PFGE. Eight and five had different banding patterns, respectively.

MLST analysis and virulence markers of E. faecium isolates

On the basis of PFGE patterns, a subset of 46 vancomycin-resistant E. faecium isolates (32 vanA and 14 vanB), either representative of clusters (n=28) or unique isolates (n=18), was selected for MLST typing. Based on allelic profiles, 13 different STs were defined. Analysis with the Eburst software clustered all strains within CC17 (Table 3). Among E. faecium vanA, the most frequently isolated STs were ST280 (17.4%), ST78 (15.2%) and ST18 (13.0%), followed by ST17, ST187 and ST262 (4.3% each), and then ST25, ST64, ST202, ST203 and ST294 (2.2% each). Among the 14 E. faecium vanB isolates tested, ST192 and ST203 were predominant (8.7% each) and other STs were ST17 and ST78 (4.3% each) and ST202 and ST323 (2.2% each). As previously shown for isolates belonging to CC17, all isolates possessed purk allele 1.

The presence of virulence genes (esp and hyl) was far from being constant, even in epidemic isolates; they were detected in 45.7% (n=21) and 52.2% (n=24) of isolates, respectively. The two genes were associated in 15 isolates (32.6%). Other combinations of putative virulence factors were also detected: esp alone for 13.0% (n=6) of the VRE isolates; hyl alone for 19.6% (n=9); and none for 34.8% (n=16).

All isolates belonging to ST202 (n=2) and ST192 (n=4) possessed the esp and hyl genes, all ST280 (n=8) had the hyl gene, all ST18 (n=6) were negative for the esp gene and all ST17 (n=4) possessed the esp gene. Most ST203 (4/5) isolates were negative for both genes.

Discussion

In Europe, the European Antimicrobial Resistance Surveillance System (EARSS) revealed that the percentage of resistance to glycopeptides in E. faecium isolated from blood cultures dramatically increased after 2000 in several European countries, leading to a situation resembling that in the USA. However, reports showed a heterogeneous picture for VRE over Europe. Several European countries, such as Ireland, Germany and Greece, experienced an increasing VRE trend over time. By contrast, in other countries, VRE prevalence is still low, e.g. countries in Northern Europe, or is decreasing (Austria, Portugal and Ireland).
### Table 3. MLST, antibiotic resistance and putative virulence genes in selected VRE (2006–08)

<table>
<thead>
<tr>
<th>Genotype (%)</th>
<th>ST</th>
<th>No. of strains (%)</th>
<th>MIC (mg/L)</th>
<th>Co-resistances&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Markers of virulence&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>VAN</td>
<td>TET</td>
<td>KAN</td>
</tr>
<tr>
<td>VanA (69.6)</td>
<td>78</td>
<td>7 (15.2)</td>
<td>96</td>
<td>8</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td>&gt;256</td>
<td>32</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td>64</td>
<td>12</td>
<td>x</td>
<td>x</td>
<td>x</td>
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<tr>
<td></td>
<td>16</td>
<td>8</td>
<td>x</td>
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</tr>
<tr>
<td></td>
<td>&gt;256</td>
<td>32</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td>&gt;256</td>
<td>24</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td>&gt;256</td>
<td>32</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>VanB (30.4)</td>
<td>17</td>
<td>2 (4.3)</td>
<td>12</td>
<td>0.75</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td>78</td>
<td>2 (4.3)</td>
<td>12</td>
<td>0.5</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>0.75</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td>202</td>
<td>1 (2.2)</td>
<td>8</td>
<td>0.75</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td>203</td>
<td>4 (8.7)</td>
<td>8</td>
<td>1.5</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td>96</td>
<td>0.38</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td>&gt;256</td>
<td>0.75</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>1.5</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td>&gt;256</td>
<td>0.75</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td>323</td>
<td>1 (2.2)</td>
<td>16</td>
<td>0.5</td>
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<td>192</td>
<td>4 (8.7)</td>
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<td>64</td>
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CL, clindamycin; DOX, doxycycline; ERY, erythromycin; GEN, gentamicin; KAN, kanamycin; LVX, levofloxacin; RIF, rifampicin; SXT, trimethoprim/sulfamethoxazole; TET, tetracycline; VAN, vancomycin.

<sup>a</sup>x, resistant; all isolates were resistant to ampicillin and susceptible to chloramphenicol, linezolid and fusidic acid.

<sup>b</sup>All isolates contained purK allele 1.
Spread of VRE in France

Italy). In France, the proportion of invasive VRE clinical isolates has not significantly increase since 2002 (<5%). However, European surveillance targets blood cultures, which may not reflect the epidemiological burden for VRE since these microorganisms are mostly responsible for colonization and non-invasive infections. However, surveillance data from the French national healthcare-associated multidrug-resistant bacteria surveillance network (BMR-Raisin) confirmed very low incidence rates of VRE from various clinical samples in 2007 (0.0021 per 1000 patient-days).

The French mandatory notification of unusual nosocomial events probably helped the rapid identification of major VRE outbreaks in France, as this pathogen was not targeted by any national surveillance system, suggesting a good early warning capacity, as has been shown for other emerging pathogens, such as Acinetobacter and Clostridium difficile. However, under-notification to health authorities is likely. In 2008, 41 districts addressed notifications to InVS and isolates to NRC-E (out of an overall total of 100 counties in France). For 27 counties (65.9%), notifications and isolates were addressed to InVS and to NRC-E, respectively, whereas for 6 (14.6%) only notification was done and for 8 (19.5%) only isolates were sent to NRC-E. Therefore, the picture displayed by VRE notification was probably not exhaustive. Possibly, the increase in numbers of VRE isolates received between 2006 and 2008 might be related to laboratories improving their compliance with recommendations. Overall, to the best of our knowledge, the major outbreaks have been notified and the corresponding isolates sent to NRC-E.

Forty-six percent of the VRE received at NRC-E were isolated during the 74 outbreaks notified to InVS and the remainder were from sporadic cases. The low ratio (0.11) of infection/colonization is consistent with previous findings from various countries. For instance, in a report of a 6 year study in Canada, 94% of VRE were colonizers. In our experience, the most common site of infection was the urinary tract, which is similar to other previous studies in Korea and Portugal. Isolates from blood cultures were rare, which is consistent with the EARSS data for France.

E. faecium remained, as in many other countries, the most prevalent species among VRE (94.8% of isolates), which is similar to the results of North American, Australian and Italian studies, where prevalence ranged from 79.5% to 99%. Whereas initially most outbreaks were due to E. faecium containing the vanA gene, some recent outbreaks were due to E. faecium vanB. Large outbreaks with E. faecium vanB have previously been reported from Australia and Singapore. Therefore, from an epidemiological point of view, control measures should prioritize the targeting of cases of infections or colonizations due to vancomycin-resistant E. faecium, whether containing the vanA or the vanB gene.

As expected, the majority of isolates were multiply resistant to antimicrobials. Although around 95% of vancomycin-resistant E. faecium were resistant to ampicillin and erythromycin, only around 22% were resistant to high levels of gentamicin, which is lower than the frequencies reported by the EARSS for E. faecium from blood cultures in France, regardless of resistance to vancomycin (ranged from 21.4% to 29.9% between 2003 and 2008). So far, we have not observed any VRE resistant to linezolid, in contrast to other countries, such as Germany and USA, which have reported outbreaks due to linezolid-resistant VRE. All isolates were also susceptible to tigecycline.

PFGE typing of VRE isolates showed considerable diversity, with a total of 125 E. faecium vanA patterns and 36 E. faecium vanB patterns circulating in France between 2006 and 2008. Twenty of these clones spread in neighbouring hospitals. When VRE outbreaks occurred in a single healthcare facility, isolates tended to be polyclonal, with one or two major clones and several minor clones, suggesting a highly diverse population of hospital-acquired E. faecium strains. This picture can possibly be explained by exchanges of a mobile resistance determinant between various enterococci. Also, the efficient control of outbreaks may have favoured diversity of clones.

Global emergence of hospital-adapted E. faecium has been characterized by MLST. The allelic profile defining STs of the 46 studied isolates was heterogeneous, but all belonged to CC17. ST78, which accounted for 19.6% of STs, was displayed by four isolates considered to be representatives of isolates responsible for four outbreaks in 2006. Six strains with ST280 were representative of six outbreaks occurring in 2007 and 2008. Various other STs were identified (Table 3), including ST18, ST203, ST192 and ST17, and, more rarely, ST25, ST64, ST187, ST294, ST202, ST262 and ST323. Most of the STs in our study have also been reported in other countries, including Korea, Germany, Italy, China and the Netherlands. ST280 has only been reported in Germany and China. There was no relationship between a specific ST type, the susceptibility profile and the epidemic nature of the isolates.

The esp gene is part of a putative pathogenicity island and is considered to be a marker for epidemicity that could putatively contribute to the spread of vancomycin-resistant E. faecium isolates in hospitals. Our data showed a lower prevalence of esp-positive isolates than previously reported in isolates from the USA, the UK and Spain (45.7% versus 65%, 61% and 70%, respectively), but higher than reported from Portugal (33%). Most CC17 isolates were reported as containing another putative virulence gene, hyl (hyaluronidase), which was found to be enriched among clinical isolates. The presumed function of hyaluronidase in E. faecium is to contribute to the permeability of the matrix. Various frequencies of hyl-containing isolates have been reported in different countries (16% in Europe, 39% in the USA, 52.2% in our study and 71% in the UK). Overall, 32.6% of the 46 VRE analysed by MLST harboured both esp and hyl genes, whereas 34.8% of the strains harboured neither of the genes. No obvious link between the virulence profile and any ST type was demonstrated.

Efforts deployed to control outbreaks, including major outbreaks, were successful, confirming that active infection control intervention can eliminate the transmission of VRE in healthcare facilities. However, an increasing number of hospitals previously free of VRE reported isolation of VRE and sometimes outbreaks. VRE will probably be impossible to eradicate completely from French hospitals since nearly all isolates belong to CC17, composed of particularly well hospital-adapted strains. For this reason, both surveillance and early warning systems remain useful at local, regional and national levels, since the occurrence of outbreaks is always unexpected, cannot be predicted and requires rapid intervention.
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**Transparency declarations**

None to declare.

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