Outbreak in a haematology unit involving an unusual strain of glycopeptide-resistant *Enterococcus faecium* carrying both *vanA* and *vanB* genes

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**Objectives:** To report an outbreak due to an unusual strain of *Enterococcus faecium* containing both the *vanA* and *vanB* genes, in France, where the rate of glycopeptide-resistant enterococci (GRE) is below 1%.

**Methods:** Cases were patients infected or colonized with GRE on the haematology ward. Contact patients were screened by real-time PCR performed on rectal swabs. Clinical features were compared for GRE-positive and GRE-negative patients. GRE isolates were characterized by phenotypic and molecular methods including PFGE. Conjugation experiments were performed to identify *van* genetic support.

**Results:** After the index patient presented a bacteraemia with *vanA/vanB* E. faecium, 56 contact patients were screened, 7 of whom were found to be GRE positive: 6 additional cases with *vanA/vanB* E. faecium and 1 with GRE carrying *vanA* only. PFGE confirmed the clonal relationship of the seven *vanA/vanB* E. faecium strains, whereas the *vanA* isolate was distinct. Only the *vanA* gene could be transferred to enterococcal recipients by conjugation, and it was probably localized on a mobile genetic element. All isolates were resistant to vancomycin (MIC > 256 mg/L) and teicoplanin (MIC = 24–32 mg/L), but were susceptible to tigecycline (MIC = 0.09 mg/L), linezolid (MIC = 0.75 mg/L) and daptomycin (MIC = 1–2 mg/L). Significant differences (P < 0.001) between carriers and non-carriers of GRE were observed for the median duration of hospitalization (57 days versus 16.5 days) and of neutropenia (40 days versus 6 days), the median number of antibiotics used (5 versus 1.5) and the duration of glycopeptide treatment (14.5 days versus 0 days).

**Conclusions:** *vanA/vanB* E. faecium strains, although rare, can emerge in the absence of previous outbreaks of vanA-GRE or vanB-GRE.

**Keywords:** vancomycin-resistant enterococci, febrile neutropenia, nosocomial infections, contact precautions, GeneXpert VRE assay

**Introduction**

The incidence of glycopeptide-resistant enterococci (GRE) has recently increased in Europe, with >30% of strains isolated from blood cultures in Ireland and Greece.1 The dissemination of GRE is a matter of concern because GRE infections have been associated with a high mortality in some settings2 and vancomycin resistance genes can be transferred to methicillin-resistant *Staphylococcus aureus.*3 The prevalence rate of GRE remains low (<5%) in France, where a national programme relying on the notification of all cases to regional health authorities and strict infection control measures was implemented in 2005.4

Apart from the natural resistance of some enterococcal species (*vanC* genes in *Enterococcus gallinarum* and *Enterococcus casseliflavus*), glycopeptide resistance is conferred by various genes (*vanA/B/D/E/G/L/M/N*), resulting in the production of peptidoglycan...
precursors with a reduced affinity for glycopeptides. The acquisition of vanA or vanB resistance genes is the most frequently reported. vanA is responsible for a high level of resistance to both vancomycin (MIC ranging from 16 to >1000 mg/L) and teicoplanin (MIC ranging from 4 to 512 mg/L), but vanB confers only a lower level of resistance to vancomycin (MIC ranging from 4 to 32 mg/L) and susceptibility to teicoplanin is conserved (MIC = 0.5–1 mg/L). The vanA and vanB genes are associated with transposons (Tn1546 and Tn1549/Tn5382, respectively) present either on the chromosome or on transferable plasmids, and they transfer between enterococcal isolates by plasmid conjugation or transposition.

Most GRE reported in Europe and the USA are Enterococcus faecium strains containing vanA. In France, from 1 January 2006 to 31 December 2006, the National Reference Centre (NRC) for the resistance of enterococci (http://www.cnr-resistance-antibiotiques.fr/) analysed 2081 vancomycin-resistant E. faecium strains and reported that 71.4% contained vanA, 23.6% vanB and 5% other van genes, mostly vanD. None of them contained both the vanA and the vanB genes.

We report here an outbreak due to an unusual strain of E. faecium containing both the vanA and vanB genes. We describe the bacteriological and genetic characteristics of this strain, and also the epidemiological investigation and clinical features of the cases.

Methods

Description of the outbreak

Saint-Louis Hospital is a 630 bed university hospital in Paris, France. Despite no previous GRE epidemic in this hospital, a strain of E. faecium containing the vanA and vanB genes was isolated from the blood culture of a patient hospitalized for acute myeloid leukaemia in a 24 bed haematology unit.

As recommended, contact precautions were immediately implemented for the index case, including isolation measures and enhanced hand hygiene using alcohol-based hand rubs. Contact patients, defined as patients whose hospitalization stay overlapped with the stay of a patient with GRE in the same unit, were screened for colonization of the gut with GRE. Patients with three repetitive negative results were included in the GRE-negative group. GRE-colonized patients and contacts were cohorted in two distinct areas with dedicated nursing staff. Transfers to other wards were banned and new admissions were stopped. The infection control team regularly gathered with medical and nursing staff to manage this reorganization. Barrier precautions were explained and particular attention was paid to patients with diarrhea, who are common in haematology wards. The antimicrobial policy in case of neutropenic sepsis was revised during the time of the outbreak to limit the use of vancomycin. When necessary, linezolid was used rather than vancomycin.

Hospitalization data, clinical features and previous therapeutic regimens were compared for the GRE-positive and GRE-negative patients. Categorical variables were compared using Fisher’s exact test, while the Student’s t-test was used to compare continuous data. All statistical analyses were performed with EpiInfo v.6. A P value of $p \leq 0.05$ was considered to be statistically significant.

Phenotypic and genotypic characteristics of the GRE isolates

Patients were screened by performing the GeneXpert VRE® assay (Cepheid, Toulouse, France) on rectal swab specimens, according to the manufacturer’s instructions. In the case of a positive PCR result (vanA+ or vanB+), the rectal swab was cultivated onto selective chromID VRE agar (bioMérieux, Marcy-l’Étoile, France) for the GRE strain being isolated. Identification and van genotypes were determined on strains using the GenoType® VRE DNA strip assay (Biocentric, Bandol, France) following the manufacturer’s instructions. Identification was confirmed by mass spectrometry (Bruker Biotequipment®, Bruker Daltonics, Bremen, Germany).

Antibiotic susceptibility was tested by the agar diffusion method on Mueller–Hinton agar (Bio-Rad, Marnes-la-Coquette, France) and MICs were determined by Etest (bioMérieux). The results were interpreted according to the EUCAST recommendations for critical breakpoints.

Clinical features of GRE-positive patients

After the index patient had been diagnosed with a bloodstream infection due to GRE, 56 contact patients were screened, 7 of whom were detected as being colonized with GRE. The GeneXpert® PCR assay yielded positive results for vanA and vanB for six patients, and a vanA-positive but vanB-negative result for the seventh. These seven patients did not show infection with GRE.

For the patients carrying GRE isolates, no history of hospitalization in another clinical ward or transfer from another country was identified except for Patient 2, who had previously been hospitalized in Ireland. Epidemiological investigations showed that when the index case was diagnosed, this patient was hospitalized in the same ward as Patient 2 for more than a month (Figure 1a). As has already been described in case–control studies on risk factors for GRE colonization during outbreaks, GRE carriers had received more antibiotics (notably glycopeptides) and had a longer history of hospitalization and duration of neutropenia than GRE-negative patients (Table 1).

All cases were identified during the 15 first days of investigations and no secondary transmission occurred after control measures were implemented. However, as cohorting measures were maintained until all GRE carriers were discharged, 2 months elapsed before normal activity could be resumed on the haematology ward and new patients admitted (no cases, no contacts).

Phenotypic and genotypic characteristics of the GRE isolates

All isolates displayed a high level of resistance to $\beta$-lactams and glycopeptides (Table 2). Resistance phenotypes were identical for
Figure 1. Clinical and molecular epidemiology of patients and GRE isolates. (a) Timeline of the GRE-positive patients. Horizontal lines represent the presence of the patient in the haematology ward, framed by the hospitalization dates; stars and diamonds represent the detection of GRE positivity in the clinical and screening samples, respectively, with the isolation date indicated above. (b) PFGE of *E. faecium* isolates. MM, molecular marker; SPC, sporadic previous case; P1 to P8, Patient 1 to Patient 8; 1A, 1B, 1C and 2, PFGE profiles. The white arrows show the bands differentiating the profiles.
all isolates containing the vanA and vanB genes, except resistance to gentamicin. Antibiotics that were still active were linezolid, tigecycline, quinupristin/dalfopristin, and nitrofurantoin. Although there are no enterococcal EUCAST breakpoints for daptomycin and rifampicin, the MICs of these antibiotics (1.5 mg/L and 6 mg/L, respectively) were in the range of MIC distribution shown for wild-type strains (http://www.eucast.org/mic_distributions/).

The strains containing only the vanA gene, including the sporadic strain, had different resistance phenotypes. PFGE showed clonal identity of the seven vanA/vanB isolates with regard to the Tenover criteria (Figure 1b). However, the profiles of the isolates differed by one or two bands, distinguishing three profiles among the seven isolates. The epidemic profile was different from that of the vanA strains and was also different from 92.

### Table 1. Characteristics of GFE-positive case patients and GFE-negative patients present in the haematology ward

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>GRE-positive patients (n=8)</th>
<th>GRE-negative patients (n=48)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Underlying disease, n (%)</td>
<td>6 (75)</td>
<td>28 (58)</td>
<td>0.5</td>
</tr>
<tr>
<td>Intensive chemotherapy, n (%)</td>
<td>2 (25)</td>
<td>20 (42)</td>
<td>0.06</td>
</tr>
<tr>
<td>Duration of hospitalization (days)</td>
<td>57</td>
<td>19-103</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Neutropenia, n (%)</td>
<td>8 (100)</td>
<td>28 (58)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Duration of neutropenia (days)</td>
<td>16.5</td>
<td>1-49</td>
<td>0.002</td>
</tr>
<tr>
<td>Fever, n (%)</td>
<td>8 (100)</td>
<td>28 (58)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Number of antibiotics used during the hospital stay, median (range)</td>
<td>5</td>
<td>1-9</td>
<td>0.001</td>
</tr>
<tr>
<td>Glycopeptides, n (%)</td>
<td>7 (87.5)</td>
<td>10 (41)</td>
<td>0.04</td>
</tr>
<tr>
<td>Glycopeptides (days), median (range)</td>
<td>25 (14)</td>
<td>0-28</td>
<td>0.001</td>
</tr>
</tbody>
</table>

R, resistant; I, intermediate; S, susceptible; AMP, ampicillin; STR, streptomycin; KAN, kanamycin; GEN, gentamicin; TET, tetracycline; ERY, erythromycin; CLI, clindamycin; PRI, pristinamycin; SXT, co-trimoxazole; NIT, nitrofurantoin; VAN, vancomycin; TEC, teicoplanin; TGC, tigecycline; LZD, linezolid; Q/D, quinupristin/dalfopristin; DAP, daptoycin; RIF, rifampicin; FUS, fusidic acid; ND, not done; SPC, sporadic previous case. The numbers in parentheses below the antibiotic name correspond to EUCAST clinical breakpoints (mg/L) where available.
French epidemic clones collected by the NCR for the resistance of enterococci since 2006. Positive results for IS16 PCR concluded that the clonal strains belonged to the clonal complex CC17.

GRE detection on rectal swabs using the Cepheid Xpert® vanA/ vanB assay has previously been described, with higher performances for vanA than vanB detection.\textsuperscript{15,16} False-positive vanB results are likely to be due to digestive flora present on rectal swabs, as it is known that anaerobic species probably constitute a reservoir for vancomycin resistance genes, particularly vanB.\textsuperscript{17}

In our report, the dual vanA/vanB genotype of the isolates was confirmed by a PCR hybridization method performed on all seven E. faecium isolates, as well as by in-house PCR at NRC. vanA/vanB E. faecium isolates were first described in England in 1997. Woodford et al.\textsuperscript{18} showed that the genotype of the epidemic strain switched from vanB to vanA through an intermediate isolate containing each gene on distinct plasmids. Since then, two outbreaks involving vanA/vanB E. faecium have been described. In Australia, Dendle et al.\textsuperscript{19} described a clonal outbreak that emerged against the background of vanB GRE in a ward where patients colonized with vanB GRE and vanA GRE were in close contact, suggesting horizontal exchanges of the vanA and vanB genes. In Finland, Suppola et al.\textsuperscript{20} reported the acquisition of vanA and vanB genes by an endemic ampicillin-resistant vancomycin-susceptible E. faecium strain during concomitant outbreaks involving either vanA- or vanB-containing strains. More recently, vanA/vanB E. faecium isolates have been reported in Saudi Arabia, exhibiting either a vanA or a vanB phenotype.\textsuperscript{21}

The vanA gene, but not the vanB gene, was transferred by conjugation from E. faecium to E. faecalis at high frequencies, ~10^{-14} per donor cell. The transfer of resistance was accompanied by the transfer of a large plasmid (>40 kb). These results suggested that vanA and vanB genes were localized on different genetic supports, probably plasmidic for vanA. Transconjugants displayed high-level resistance to kanamycin and erythromycin, and PCR experiments showed that the erm(B) and aph(3′)′ genes had co-transferred with vanA.

Because the prevalence of GRE is low in France and high in Ireland,\textsuperscript{13,14} and because no other risk factors for GRE were found except for Patient 2, we assume that the clonal strain was imported from Ireland. This hypothesis is strengthened by molecular results from Ireland,1,4 and because no other risk factors for GRE were found. We are grateful to Fabienne Meunier for excellent technical assistance in molecular methods, and to Sandra Fournier for help in implementing outbreak control measures.

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\textbf{Transparency declarations}

None to declare.

\textbf{References}


Outbreak involving GRE vanA+/vanB+


