Outbreak of hospital-adapted clonal complex-17 vancomycin-resistant Enterococcus faecium strain in a haematology unit: role of rapid typing for early control

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Objectives: To describe the investigation and molecular characterization of a vancomycin-resistant Enterococcus faecium (VREF) strain responsible for a nosocomial outbreak in the haematology unit of a tertiary-care university hospital.

Patients and methods: Two patients admitted to the haematology unit developed infection/colonization with VREF over a 3 month period when compared with none in the 2 previous years. On the basis of the identification of a clonal link between these two strains, weekly rectal screening was implemented for all patients in the haematology unit and contact precautions were extended to VREF carriers. In the following 6 month period, 11 patients colonized with VREF were detected. No further case was detected in the following 1 year period.

Results: VREF isolates from the haematology unit carried the vanA gene and were multiresistant to antimicrobial agents, including high-level resistance to vancomycin, teicoplanin and ampicillin. This resistance profile restricted the choice of antimicrobial therapy to linezolid or investigational drugs such as tigecycline. Molecular analysis showed that 11 of 13 (85%) VREF isolates belonged to pandemic clonal complex-17 carrying the esp and hyl virulence genes.

Conclusions: Rapid typing and infection control measures, including early reinforcement of barrier precautions combined with weekly rectal surveillance cultures, were followed by control of nosocomial spread of this VREF clone.

Keywords: ST16, epidemic, ampicillin resistance, vanA

Introduction

Vancomycin-resistant Enterococcus faecium (VREF) are recognized as emerging pathogens, which are associated with nosocomial outbreaks in the USA, Europe and more recently in Western Australia.1–3 VREF hospital outbreaks have usually involved high-risk patient populations such as haematology–oncology patients, transplant recipients and critically ill patients in intensive care units (ICUs).4–6 These outbreaks have adverse impact on treatment of patients and can disrupt care. Infection control (IC) measures including patient screening, isolation and cohorting are often necessary to interrupt the spread of epidemic VREF.7

A large European prevalence study of vancomycin-resistant enterococci conducted in 1997 showed a low prevalence (0.4%) of VREF among clinical isolates of enterococci.9 In this survey, the highest prevalence of vanA enterococci (2.7%) was noted in the UK, whereas in Belgium, a VREF prevalence of 1% was found.9 In the past 5 years, the incidence of VREF among E. faecium blood culture isolates in the European Antimicrobial Resistance Surveillance System (EARSS) increased to reach
high prevalence rates in 2004 in Portugal (42.3%), Italy (21.2%), Greece (20.3%) and Germany (10.6%). EARSS data also showed an increased incidence of VREF among E. faecium isolates from blood culture in Belgium (4.8%) in 2004 when compared with the previous years (www.ears.rivm.nl). However, VREF nosocomial outbreaks remain infrequently reported in Europe.3,4,10

In the present study, we describe how we investigated and managed an outbreak of ampicillin-resistant VREF carrying the vanA gene in the haematology unit of a tertiary-care hospital in Belgium.

Materials and methods

Setting and patient population

Erasme Hospital is an 858 bed tertiary-care academic hospital located in Brussels, Belgium. The haematology unit included a total of 27 beds with a bed occupancy of 7827 patient-days in 2004.

Case definition

A case of VREF was defined as any hospitalized patient infected or colonized with an isolate of vancomycin- and ampicillin-resistant E. faecium carrying the vanA gene.

Identification and phenotypic susceptibility testing

All VREF isolates were identified by Rapid ID32Strep (bioMérieux, Marcy l’Étoile, France) and mobility test at 35°C. Antimicrobial susceptibility to ampicillin, gentamicin, streptomycin, clindamycin, tetracycline and linezolid was determined using the disc diffusion method (Rosco Neo-Sensitabs, Taastrup, Denmark). All strains were tested on vancomycin screen agar containing 6 mg/L of vancomycin (Becton Dickinson, Heidelberg, Germany) and incubated for a full 24 h at 35°C. MICs were determined for ampicillin, vancomycin, teicoplanin, gentamicin and tigecycline by the Etest method (AB Biodisk, Solna, Sweden) according to the CLSI (formerly NCCLS) guidelines.11

Molecular characterization

Multiplex PCR for detection of van genes was performed, as described previously.12 Resistance genes encoding tetracycline efflux pump system [tet(K)] and ribosomal protection protein [tet(M)] and aminoglycoside-modifying enzymes encoded by aac(6′)-aph(2′) were tested by PCR.13 Typing was performed on all VREF isolates by macrorestriction analysis (Smal) resolved by PFGE using following separation conditions: 0.5–8 s for 10 h and 10–20 s for 10 h. PFGE patterns were analysed using the Dice coefficient with version 2.5 of BioNumerics software (Applied Maths, Kortrijk, Belgium). The classification criteria for PFGE analysis include type, designated by a capital letter (e.g. A), and patterns showing zero to six DNA fragment differences. Any variant was indicated by a numeral suffix (e.g. A1). Multifocus sequence typing (MLST) analysis was performed on two strains belonging to the epidemic PFGE type A, as developed by Homan et al.14 and sequence type (ST) was assigned via the MLST web site (www.mlst.net). Multiplex PCR for the detection of asa1, gelE, clyA, esp and hyl virulence genes was performed on a selected sample of VREF isolates, as described previously.15

Rectal screening for VREF carriage

From 29 June 2004 to 20 September 2006, rectal screening was implemented and performed weekly for VREF carriage in patients hospitalized in the haematology unit. After 20 September 2006, screening was performed every 2 weeks. Rectal swabs were incubated (i) on enterococcus agar medium (Becton Dickinson, Le Pont de Claire, France) containing 6 mg/L of vancomycin (Oxoid, Basingstoke, UK) and (ii) in enrichment broth containing 7.5% NaCl at 35°C for 18 h. From September 2004, meropenem (4 mg/L) (Oxoid) was added to the enrichment broth to inhibit Enterococcus gallinarum growth and enhance selectivity for E. faecium.

IC measures

Before 29 June 2004, patients infected with VREF were hospitalized in individual rooms that were cleaned daily with aldehyde-containing disinfectant (Melsitt, Braun Medical AG, Emmenbrücke, Germany). Contact precautions included wearing gloves and gowns. Since 29 June 2004, screening of rectal carriers was implemented and contact precautions were extended to VREF-colonized patients. Room cleaning was reinforced by increasing the frequency of surface decontamination with Melsitt.

Results

Outbreak description and intervention

During the first semester of 2004, one patient developed bacteremia and a second patient became colonized with VREF in the haematology unit (Table 1). The infected patient was cured with linezolid therapy. In July 2004, based on typing results indicating clonal relatedness of these isolates, an outbreak warning was issued and the infection control team implemented weekly rectal screening of all patients admitted to the haematology unit and extended contact precautions to all carriers of the epidemic VREF. The incidence density of VREF nosocomial acquisition in the haematology unit was 12/7827 patient-days, compared with 0/8042 in 2003 (P < 0.001). Retrospective analysis of all VREF isolates detected in 2004 among hospitalized patients showed five additional cases among patients admitted to ICUs (n = 3) and other units (n = 2) (Table 1).

Monitoring of infection control measures

From July 2004 to December 2005, a total of 1161 rectal swabs (1–25 swabs/patient) were obtained from 307 patients admitted to the haematology unit. VREF was recovered from 35 (3%) swabs from 11 patients (3.6%). After implementation of infection control measures, no additional case of VREF infection was found in the unit, but 11 VREF rectal carriers were detected (Table 1). In other wards, one case of VREF infection was detected during the post-intervention period (Table 1). During the period January–December 2006, no case of VREF infection and no VREF rectal carrier was detected in 299 rectal swabs from 129 patients hospitalized in the haematology unit.

Origin of VREF isolates

Table 1 listed characteristics of the 20 VREF isolates recovered from patients hospitalized at Erasme Hospital from January 2004 to December 2005. Except for two community-acquired
### Table 1. Characteristics of vanA glycopeptide-resistant *E. faecium* (VREF) isolates recovered at Erasme Hospital in 2004 and 2005

<table>
<thead>
<tr>
<th>Unit</th>
<th>Patients</th>
<th>Date</th>
<th>Source</th>
<th>Diagnosis</th>
<th>Phenotype of resistance</th>
<th>Resistance genes</th>
<th>Virulence genes</th>
<th>PFGE type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haematology</td>
<td>P3</td>
<td>March 2004</td>
<td>blood</td>
<td>I</td>
<td>AMP, VAN, TEC</td>
<td>vanA</td>
<td>ND</td>
<td>A</td>
</tr>
<tr>
<td>Haematology</td>
<td>P5</td>
<td>May 2004</td>
<td>decubitus sore</td>
<td>C</td>
<td>AMP, VAN, TEC, GEN</td>
<td>vanA, aac(6')-aph(2''), tet(M)</td>
<td>ND</td>
<td>A</td>
</tr>
<tr>
<td>Haematology</td>
<td>P8</td>
<td>July 2004</td>
<td>rectal swab</td>
<td>C</td>
<td>AMP, VAN, TEC</td>
<td>vanA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haematology</td>
<td>P9</td>
<td>July 2004</td>
<td>rectal swab</td>
<td>C</td>
<td>AMP, VAN, TEC</td>
<td>vanA, aac(6')-aph(2''), tet(M)</td>
<td>esp, hyl</td>
<td>A</td>
</tr>
<tr>
<td>Haematology</td>
<td>P10</td>
<td>September 2004</td>
<td>rectal swab</td>
<td>C</td>
<td>AMP, VAN, TEC, GEN</td>
<td>vanA, aac(6')-aph(2''), tet(M)</td>
<td>esp, hyl</td>
<td>A</td>
</tr>
<tr>
<td>Haematology</td>
<td>P11</td>
<td>September 2004</td>
<td>rectal swab</td>
<td>C</td>
<td>AMP, VAN, TEC, GEN</td>
<td>vanA, aac(6')-aph(2''), tet(M)</td>
<td>esp, hyl</td>
<td>A</td>
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<tr>
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<td>P12</td>
<td>October 2004</td>
<td>rectal swab</td>
<td>C</td>
<td>AMP, VAN, TEC, GEN</td>
<td>vanA, aac(6')-aph(2''), tet(M)</td>
<td>esp, hyl</td>
<td>A</td>
</tr>
<tr>
<td>Haematology</td>
<td>P13</td>
<td>October 2004</td>
<td>rectal swab</td>
<td>C</td>
<td>AMP, VAN, TEC, GEN</td>
<td>vanA, aac(6')-aph(2''), tet(M)</td>
<td>esp, hyl</td>
<td>A</td>
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<tr>
<td>Haematology</td>
<td>P14</td>
<td>October 2004</td>
<td>rectal swab</td>
<td>C</td>
<td>AMP, VAN, TEC, GEN, TET</td>
<td>vanA, aac(6')-aph(2''), tet(M)</td>
<td>esp, hyl</td>
<td>L</td>
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<tr>
<td>Haematology</td>
<td>P15</td>
<td>December 2004</td>
<td>rectal swab</td>
<td>C</td>
<td>AMP, VAN, TEC, GEN</td>
<td>vanA, aac(6')-aph(2''), tet(M)</td>
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<td>Haematology</td>
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<td>August 2005</td>
<td>rectal swab</td>
<td>C</td>
<td>AMP, VAN, TEC, GEN</td>
<td>vanA, aac(6')-aph(2''), tet(M)</td>
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<td>Haematology</td>
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<td>August 2005</td>
<td>rectal swab</td>
<td>C</td>
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<tr>
<td>Haematology</td>
<td>P20</td>
<td>December 2005</td>
<td>rectal swab</td>
<td>C</td>
<td>AMP, VAN, TEC, GEN, TET</td>
<td>vanA, aac(6')-aph(2''), tet(M)</td>
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<tr>
<td>Cardiology</td>
<td>P1</td>
<td>February 2004</td>
<td>skin wound</td>
<td>C</td>
<td>AMP, VAN, TEC, GEN</td>
<td>vanA, aac(6')-aph(2''), tet(M)</td>
<td></td>
<td>ND</td>
</tr>
<tr>
<td>ICU</td>
<td>P2</td>
<td>February 2004</td>
<td>blood</td>
<td>I</td>
<td>AMP, VAN, TEC</td>
<td>vanA</td>
<td>esp, hyl</td>
<td>A</td>
</tr>
<tr>
<td>ICU</td>
<td>P4</td>
<td>March 2004</td>
<td>skin</td>
<td>C</td>
<td>AMP, VAN, TEC</td>
<td>vanA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICU</td>
<td>P6</td>
<td>June 2004</td>
<td>intra-abdominal</td>
<td>I</td>
<td>AMP, VAN, TEC</td>
<td>vanA, aac(6')-aph(2''), tet(M)</td>
<td>esp, hyl</td>
<td>A</td>
</tr>
<tr>
<td>ENT</td>
<td>P7&lt;sup&gt;f&lt;/sup&gt;</td>
<td>June 2004</td>
<td>skin wound</td>
<td>I</td>
<td>AMP, VAN, TEC</td>
<td>vanA, aac(6')-aph(2''), tet(M)</td>
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<tr>
<td>ICU</td>
<td>P16</td>
<td>March 2005</td>
<td>pleural fluid</td>
<td>I</td>
<td>AMP, VAN, TEC, GEN</td>
<td>vanA, aac(6')-aph(2''), tet(M)</td>
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<tr>
<td>Medicine</td>
<td>P17</td>
<td>July 2005</td>
<td>abdominal fluid</td>
<td>C</td>
<td>AMP, VAN, TEC, GEN, TET</td>
<td>vanA, aac(6')-aph(2''), tet(M)</td>
<td></td>
<td>R</td>
</tr>
</tbody>
</table>

*Patients, indicated by P, were numbered in chronological order.
*C, colonized; I, infected.
*AMP, ampicillin; VAN, vancomycin; TEC, teicoplanin; GEN, high-level resistance to gentamicin (MIC > 256 mg/L); TET, tetracycline.
*ND, not done.
*ENT, ear, nose, throat.
*Strain not conserved.
isolates (patients P4 and P20), VREF isolates were considered to be nosocomially acquired (detected >48 h after hospital admission). Thirteen VREF isolates were collected from the haematology unit, 4 from ICU and the remaining 3 isolates from other units (Table 1). Five VREF isolates (25%) were collected from infected patients and 15 (75%) from colonized patients, including 11 isolates from rectal screening samples (Table 1).

Antimicrobial susceptibility testing
All VREF isolates presented high-level resistance (HLR) to vancomycin (MIC ≥ 256 mg/L), teicoplanin (MIC 24–256 mg/L) and ampicillin (MIC ≥ 16 mg/L). VREF isolates were either resistant or intermediate to streptomycin, resistant to clindamycin and susceptible to linezolid and tigecycline (MIC ≤ 0.125 mg/L). Thirteen isolates (65%) showed HLR to gentamicin (MIC > 256 mg/L) and three of these isolates (15%) were also resistant to tetracycline (Table 1).

Resistance and virulence gene distribution
All VREF isolates carried the vanA gene and all high-level gentamicin-resistant isolates carried the aac(6’)-aph(2’’)) gene (Table 1). The gene encoding the bifunctional aminoglycoside-modifying enzyme was also found in two VREF isolates showing low-level gentamicin resistance (4 mg/L) (Table 1). The tet(M) gene was detected in all tetracycline-resistant VREF isolates and in two other isolates that appeared susceptible to tetracycline by the disc diffusion method (Table 1). In contrast, the tet(K) gene was not found. Multiplex PCR for the detection of aal1, gelE, cylA, esp and hyl virulence genes demonstrated the presence of esp and hyl genes in all isolates tested (Table 1).

Molecular typing
PFGE typing clustered 14 isolates (74%) in the same epidemic PFGE type A. This PFGE type A included three variants A1 (n = 11), A2 (n = 1) and A3 (n = 2), showing one to five DNA fragment differences (data not shown). The remaining five isolates showed sporadic PFGE types that were unrelated to type A (18–25 DNA fragment differences) (Table 1). The epidemic curve of patients (n = 13) admitted to the haematology unit from January 2004 to September 2006 demonstrated that a prolonged clonal outbreak occurred during the 2004–05 period, with two peaks of transmission (Figure 1). Typing of VREF isolates found in other units revealed the presence of the epidemic PFGE type A in ICU patients (n = 3 isolates) and sporadic types in other units (n = 4 isolates) (Table 1). MLST analysis of two representative VREF isolates belonging to epidemic PFGE type A identified these as ST-16, which belongs to clonal complex-17 (CC-17). Isolates included in the epidemic clone PFGE type A/ST-16 were multiresistant to antibiotics and characterized by the presence of the vanA resistance gene and esp and hyl virulence genes and were also frequently (71%) associated with the presence of the aac(6’)-aph(2’’)) resistance gene.

Discussion
VREF first emerged as important nosocomial pathogens in the USA during the 1990s. In Europe, fewer nosocomial outbreaks have been reported, but the incidence of VREF appears to be increasing in the last 5 years in many countries.2–4,10,16,17 Recently, genetic population studies have shown that a majority of VREF strains associated with nosocomial infections worldwide are part of the same lineage called CC-17.16–19 Its founder type, ST-17, was recovered in most hospital outbreaks and clinical VREF isolates.16,17,19 CC-17 strains are generally resistant to ampicillin and carry genes for the virulence factors enterococcal surface protein (esp) and hyaluronidase (hyl) and, in most cases, the putative pathogenicity island.16,18–21 The hyl gene was found with higher frequency in clinical versus colonizing stool isolates of VREF.22 In clinical VREF isolates, this gene ranged in frequency from 12% to 16% among patients from continental Europe, to 39% and 71% among these from the USA and UK, respectively.15,23 In European hospitals, rates of infection with VREF have been rising since 2001, suggesting a situation similar to that described in the USA but with a 10 year delay.16 In the present study, epidemic VREF strains were characterized as clonal by PFGE typing and belonged to ST-16 clone by MLST. This clone is a ddl variant allele of ST-17 that has been previously reported among nosocomial strains from other European countries including Germany, The Netherlands, UK, Spain and Greece during the 1998–2004 period.17 In our study, the epidemic strain showed an antibiotic resistance profile and virulence genes similar to those described in other clinical strains of CC-17. This hospital-adapted complex-17 was shown to have spread worldwide during the last decade.16,19 We also observed the presence of esp and hyl virulence genes in VREF isolates displaying divergent PFGE types, as shown previously among clinical VREF isolates belonging to the CC-17.17,23 Multiresistant VREF strains belonging to CC-17 that are resistant to linezolid have been described in Germany.24 Nosocomial spread of linezolid-resistant VREF was recently detected in the USA.25 These findings underline the importance of monitoring the occurrence of epidemic-virulent CC-17 VREF strains by advanced surveillance systems to prevent their spread in hospitals, especially if they become resistant to linezolid, which is the only active drug available. In addition, the
emergence of vancomycin-resistant Staphylococcus aureus harbouring the vanA gene as a result of transmission from Enterococcus species to methicillin-resistant S. aureus (MRSA) underscores the danger of coexistence of both pathogens in the hospital setting. As MRSA is already endemic in many European hospitals, prevention of VREF dissemination is a public health priority.39

This study describes the first outbreak of ampicillin-resistant VREF in our tertiary-care hospital. This outbreak was related to clonal spread of a multiresistant strain showing HLR to vancomycin, teicoplanin and ampicillin, generally combined with HLR to gentamicin. This broad-spectrum co-resistance to antibiotics restricted the choice of antimicrobial therapy to linezolid as the drug of last resort for treating patients with infection caused by this strain. An alternative therapy could have been tigecycline. Tigecycline belongs to the glycycycline class of compounds and is derived by modification of the 9-position of minocycline. In vitro data have shown that it is active against Gram-positive bacteria, including VREF.30 In this study, tigecycline presented an excellent in vitro activity against multiresistant VREF strains irrespective of the presence of the tet(M) gene.

Patients with haematological malignancies appear at high risk for acquisition of VREF as indicated by the many nosocomial outbreaks involving these patients.3,31 The need for implementing step-wise measures to control the epidemic has been described, including surveillance cultures of patients, allocation of dedicated nurses for cohorted care and reinforcement of hand hygiene practices.3,10 More drastic measures including temporarily closing the unit to new admissions are sometimes necessary to stop the spread of epidemic VREF strains.10 A recent report described a significant reduction in the acquisition of vancomycin-resistant Enterococcus after routine environmental cleaning measures.32 In our hospital, the enhanced detection of VREF carriers by rectal selective culture and broader application of genotyping results contributed to control of the outbreak, as described elsewhere.33

This is the first clonal outbreak due to the hospital-adapted CC-17 VREF reported from Belgium. Infection control measures including weekly surveillance cultures and increase in use of barrier precautions appeared useful in controlling its nosocomial spread. Rapid recognition of this epidemic ST-16 VREF strain can be based on screening for HLR to ampicillin associated with the presence of the vanA gene, followed by molecular typing and detection of virulence genes. This approach contributed to avoid dissemination of this epidemic strain in our hospital. Advanced surveillance systems must be developed for early detection of epidemic hospital-adapted strains of VREF in European healthcare institutions.

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Transparency declarations

None to declare.

References


