First outbreak of vancomycin-resistant enterococci in a tertiary hospital in Turkey

Dilek Colak1,2, Thierry Naas2*, Filiz Gunseren3, Nicolas Fortineau2, Dilara Ogunc1, Meral Gultekin1 and Patrice Nordmann2

1Clinical Microbiology and 2Infectious Diseases Departments, Akdeniz University School of Medicine, Antalya, Turkey; 2Service de Bactériologie-Virologie, Hôpital de Bicêtre, 78 rue du Général Leclerc, Assistance Publique/Hôpitaux de Paris, Faculté de Médecine Paris-Sud, 94275 Le Kremlin-Bicêtre Cedex, France

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Twenty multidrug-resistant vancomycin-resistant Enterococcus faecium strains of the VanA phenotype were isolated over a 1 year period from five patients in the intensive care unit at the University Hospital of Antalya, Turkey. Molecular investigation showed that these strains belonged to five different pulsotypes and that the vanA gene was carried by a Tn1546-like transposon inserted onto a self-transferable plasmid of approximately 200 kb. One patient was infected by two different strains, suggesting horizontal gene transfer within that patient. This is the first documented outbreak of VRE in Turkey with concomitant spread of plasmid and strains.

Keywords: Enterococcus faecium, VRE, outbreak

Introduction

Over the last decade Enterococcus spp. have emerged as important nosocomial pathogens.1 Since their initial recovery from patients in France and the UK, vancomycin-resistant enterococci (VRE) have been reported in many countries worldwide.1,2 While in the USA, hospital outbreaks of clonally related VRE have been documented,1 the prevalence of these isolates remains low in European hospitals.2

Six different glycopeptide resistance phenotypes (VanA to VanE and VanG) have been described.1 VanA and VanB are of greatest clinical relevance.1,2 The VanA phenotype is inducible by glycopeptides and determined by a highly conserved gene cluster, vanRSHAX, located on active Tn1546-type transposons.3 These transposons are often carried by plasmids, which facilitates their horizontal spread among enterococcal strains.3–5 Nosocomial VRE outbreaks, attributed either to horizontal transfer of resistance determinants, to clonal dissemination of epidemic strains, or both, have been reported.1,4,5

Here we present a detailed molecular analysis of VRE isolated at the Akdeniz University Hospital in Antalya, Turkey, during an outbreak from April 1998 to March 1999. This is the first outbreak caused by vanA-positive enterococci characterized in Turkey.

Materials and methods

Bacteria, antimicrobial agents and MIC determination

Twenty vancomycin-resistant Enterococcus faecium (VRE) isolates were isolated from five different patients and from a sphygomanometer (Table 1). VRE isolation from clinical specimens was carried out using 5% sheep blood and bile esculin agar plates (bioMérieux, Marcy l’Étoile, France). Bile esculin agar plates supplemented with 6 mg/L vancomycin were used for VRE isolation from rectal swabs. Enterococci were identified to the species level by sequencing the manganese-dependent superoxide dismutase gene,6 in addition to conventional tests7 and the API ID 32 STREP test (bioMérieux). The rifampicin and fusidic acid-resistant E. faecium BM4105-RF was used as a recipient strain in conjugation experiments.5,8

Routine antibiograms and MICs were determined on Mueller–Hinton agar by the disc diffusion method and by the Etest method (AB Biodisk, Solna, Sweden), respectively.
### Table 1. Clinical strains used in this study

<table>
<thead>
<tr>
<th>Origin</th>
<th>Sex</th>
<th>Age</th>
<th>Isolate</th>
<th>Date of isolation (day.month.year)</th>
<th>Source</th>
<th>MIC (mg/L)</th>
<th>PFGE group</th>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>SAM</td>
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<td></td>
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<td></td>
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<tr>
<td>Patient 1&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>11 months</td>
<td>1</td>
<td>22.04.98</td>
<td>pleu. flu.</td>
<td>128</td>
<td>64</td>
</tr>
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<tr>
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<td>M</td>
<td>11 months</td>
<td>2</td>
<td>23.04.98</td>
<td>pleu. flu.</td>
<td>128</td>
<td>64</td>
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<tr>
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<td>42 years</td>
<td>3</td>
<td>19.12.98</td>
<td>CSF</td>
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<td>64</td>
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<td>42 years</td>
<td>4</td>
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<td>64</td>
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<td>5</td>
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<td>7</td>
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<td>urine</td>
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<td>64</td>
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<tr>
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<td>urine</td>
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<td>64</td>
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<td>17</td>
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<td>20</td>
<td>08.03.99</td>
<td>sphyg.</td>
<td>32</td>
<td>32</td>
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</table>

M, male; AMP, ampicillin; SAM, ampicillin–sulbactam; IPM, imipenem; VAN, vancomycin; TEC, teicoplanin; GEN, gentamicin; STR, streptomycin; CIP, ciprofloxacin; pleu. flu., pleural fluid; Environ., environmental sample; sphyg., sphygomomanometer; CSF, cerebrospinal fluid.

<sup>a</sup>Patients 1 and 2 died from the severe enterococcal infection.
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(Table 1). The results were interpreted according to the 2001 guidelines of the French Society for Microbiology (http://www.sfmi.asso.fr/Sect4/atbUK.html). Cefinase discs were from BD Diagnostic Systems (Le Pont-de-Claiux, France).

Plasmid content and conjugation assays

Plasmid DNA were prepared using an alkaline lysis protocol.8 and transconjugants were selected onto TS agar plates containing rifampicin (64 mg/L; Aventis, Paris, France), fusidic acid (64 mg/L; Léo, St Quentin en Yveline, France) and vancomycin (32 mg/L; Lilly, St Cloud, France).

Detection and sequencing of vanA genes

Whole-cell DNA was purified as described previously.9 The PCR conditions and primers used to detect vanA and vanB genes have been described previously.10 The 730 bp amplicons were purified using the Qiagen PCR purification kit (Qiagen) before sequence determination using an ABI PRISM 3100 sequencer (Applied Biosystem, Les Ulis, France). Nucleotide sequence analyses were carried out at the National Center for Biotechnology Information website (http://www.ncbi.nlm.nih.gov).

Amplification of Tn1546-like transposons and vanRSHAX regions by long range (LR)-PCR and restriction fragment length polymorphism (RFLP) analysis

Tn1546-like elements were amplified using the Long Range PCR kit (Perkin-Applied Biosystems) and whole-cell DNA of the isolates as templates. A single oligonucleotide, primer 1,48 complementary to the transposon-flanking inverted repeats was used to amplify the entire Tn1546-like structures. Primers 2 and 3 were used to amplify the vanRSHAX gene-containing regions. The PCRs were performed as described previously.5 The LR-PCR products containing Tn1546-like elements were digested either with EcoRI or DdeI, and the vanRSHAX amplicons were digested with DdeI.5 Restriction endonucleases were from Amersham Pharmacia Biotech (Orsay, France).

PFGE typing and hybridization

Smal-restricted whole-cell DNA preparations were separated in a 1% agarose gel (Bio-Rad) using a CHEF DRII system (Bio-Rad), according to the manufacturer’s instructions. Electrophoresis was run for 20 h at 14°C, with a 6 V/cm current, a switch angle of 120° and switch times of 0.1–20 s. PFGE results were analysed according to the criteria of Tenover et al.11

After migration, the gel was stained with ethidium bromide (0.5 mg/L) prior to the DNA transfer onto a Hybond N+ membrane (Amersham Pharmacia Biotech).9

Southern hybridizations were performed with the ECL non-radioactive kit (Amersham Pharmacia Biotech) using a 730 bp PCR-generated intragenic vanA probe.

Results

Hospital setting, clinical isolates and surveillance study

Akdeniz University Hospital is a 600 bed teaching hospital with a combined single 40 bed room polyvalent intensive care unit (ICU). Two VRE isolates were obtained from pleural fluid samples of a paediatric patient who had been hospitalized in the ICU in April 1998 (Table 1). Eight months later, 12 VRE strains were recovered from clinical samples and four from stool cultures of three patients in this ICU from December 1998 to March 1999. Patients 1 and 2 died as a result of their severe enterococcal infections.

A prospective surveillance study was initiated in order to determine the epidemiology of VRE, and infection control measures were implemented according to the recommendations of the Hospital Infection Control Practice Advisory Committee. Surveillance cultures (rectal swab or stool cultures) were taken twice a week from all ICU-hospitalized patients during the 4 month study period. Staff, patient-care equipment and environmental surfaces were also screened for VRE colonization. One hundred and ninety-three rectal swabs from patients, 31 stool and 12 hand cultures from ICU staff, and 37 environmental cultures were performed. A fifth patient was found to be a carrier of VRE, and one VRE isolate was cultured from the sphygmonomanometer dedicated to patient 3. VRE was not isolated from any staff specimen. Twenty VRE isolates were recovered at the end of the study period (Table 1).

Antimicrobial susceptibility testing

All isolates were characterized by high MICs of vancomycin and teicoplanin, and all were of intermediate susceptibility to ciprofloxacin (Table 1). The E. faecium isolates had various levels of resistance to ampicillin, but none of them was cefinase positive (Table 1). Sixteen isolates were of the high-level gentamicin resistance phenotype, and four expressed a low-level aminoglycoside resistance phenotype. All the strains studied were resistant to erythromycin (MICs > 128 mg/L).

vanA gene detection

The vanA gene was detected by PCR in all the isolates. The sequence, determined for five of the 730 bp amplicons (derived from strains 1, 3, 9, 12 and 19), was identical to that published for the prototype vanA gene3,10 (GenBank acces-
sion number M97297). None of the strains was PCR positive for the vanB gene (data not shown).

**Plasmid extraction, mating experiments and PFGE typing**

The PFGE analysis distinguished five different PFGE types among the 20 E. faecium isolates (types A–E). The E. faecium isolates collected from patient 4 were closely related to that found in the environment (subtype D). The isolates from patients 2 and 3 were indistinguishable, except for strains 9 and 10, which were unrelated. All VRE isolates harboured an ∼200 kb plasmid (data not shown). This result was confirmed with a single hybridization signal for the Smal-restricted whole-cell DNA PFGE (Figure 1). For all isolates, vancomycin-resistant transconjugants were obtained at a frequency of $10^{-6}$–$10^{-7}$ and erythromycin was co-transferred (data not shown).

**RFLP analysis of the Tn1546-like transposon and the vanRS region**

LR-PCR of Tn1546-like elements produced the expected 11 kb fragment for all isolates. ClaI and EcoRI RFLP patterns of the amplified transposons from isolates 3 to 20 were indistinguishable from each other. The restriction pattern obtained for isolates 1 and 2 differed from this common pattern, but was identical to that of the prototype Tn1546. The DdeI restriction patterns of the 4.4 kb DNA amplicons encompassing the vanRS region were identical to that obtained for the homologous fragment of the prototype Tn1546 element.

**Discussion**

During a susceptibility survey performed in 2000 enterococci isolated in European hospitals, no VRE strains were detected in Turkey. Recently, one VRE-infected patient was reported in Ankara, Turkey. Here we present the isolation of several VRE strains isolated from different patients over a 1 year period. This study demonstrated that the VanA phenotype most likely derived from a similar plasmid, carrying Tn1546 or a minor variant of this transposon, which spread to at least four strains. The outbreak was polyclonal, as demonstrated by the diversity of the E. faecium PFGE types. The observed relatedness of some of the E. faecium isolates suggested cross-infection between two patients by strain B.

Previous reports have documented similar features of the epidemiological phenomena demonstrated by the present study, i.e. plasmid-mediated horizontal transfer and clonal dissemination of epidemic strains were revealed as major factors of vancomycin resistance spread in enterococcal populations. However, in most cases the parallel occurrence of all of these mechanisms was reported either in multicentre studies or studies in which isolates of both human and non-human sources were compared. Data presented here illustrate, as shown by others, the complexity of the epidemiological situation concerning VRE that may occur in a single medical centre, or even within a single patient.

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**Figure 1.** Analysis of Smal-digested genomic DNA by PFGE (a) and corresponding Southern hybridization with an internal vanA gene probe (b). Lanes 1–20 represent the 20 VRE strains studied. Lane 21 corresponds to an unrelated strain. Lane M, bacteriophage lambda concatamers were used as molecular size markers (Bio-Rad).

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References


