Inter-hospital dissemination of glycopeptide-resistant Enterococcus faecalis in Brazil

J. C. R. Cordeiro, S. Silbert, A. O. Reis and H. S. Sader

Laboratório Especial de Microbiologia Clínica, Disciplina de Doenças Infecciosas e Parasitárias, Universidade Federal de São Paulo, Brazil

ABSTRACT

The antimicrobial susceptibility patterns of 73 glycopeptide-resistant Enterococcus faecalis isolates from nine hospitals in Brazil were analysed by the disk diffusion method and E-tests. Isolates were typed by pulsed-field gel electrophoresis (PFGE), and vancomycin resistance genes were detected by PCR. The isolates shared a single major PFGE pattern, with six subtypes, and all were positive for vanA. These results indicate the occurrence of inter-hospital dissemination of glycopeptide-resistant E. faecalis in São Paulo, and raise concerns about the rapid dissemination of this pathogen throughout Brazil.

Keywords Antimicrobial resistance, clonal dissemination, Enterococcus faecalis, glycopeptide resistance, nosocomial infections, PCR, typing

Original Submission: 17 December 2002; Revised Submission: 24 February 2003; Accepted: 8 April 2003

Clin Microbiol Infect 2004; 10: 260–262

Corresponding author and reprint requests: S. Silbert, Laboratório Especial de Microbiologia Clínica, Disciplina de Doenças Infecciosas e Parasitárias, Universidade Federal de São Paulo, EPM, Rua Leandro Dupret, 188 São Paulo, SP, CEP 04025-010, Brazil
E-mail: suzanesilbert@terra.com.br

Enterococci have been isolated with increasing frequency from nosocomial infections over the past two decades. These opportunistic pathogens represent a frequent cause of infection in patients hospitalised for long periods or in patients receiving multiple courses of antimicrobial therapy [1]. The percentage of nosocomial infections in the USA caused by glycopeptide-resistant enterococci (GRE) increased from 0.3% in 1989 to 25.9% in 1999 [2]. The SENTRY Antimicrobial Surveillance Program identified an increase in the prevalence of glycopeptide resistance among clinical isolates of enterococci in the USA and Latin America, from 14% and 0% in 1997, to 17% and 2% in 1999, respectively [3].

There are important differences in the epidemiology of GRE in the USA and Europe [4,5]. In the USA, the high incidence of infections with GRE has been attributed to the extensive use of vancomycin in hospitals [6]. In contrast, infections with GRE and outbreaks occur less frequently in most western European countries, although GRE have been identified among healthy individuals, as well as in raw sewage and farm animals [7]. These epidemiological differences might be associated with use of the glycopeptide avoparcin as a growth promoter for food animals in some European countries. However, this agent has not been approved for use as an animal growth promoter in the USA, and has not been used in Brazil since 1998 [4,7,8].

The first glycopeptide-resistant Enterococcus strain isolated in Brazil was a strain of E. faecium of the vanD genotype [9]. Later, several hospitals located in São Paulo and other Brazilian cities reported outbreaks and isolated cases of infection or colonisation with GRE [10,11]. Initially, most reports were of E. faecium, but dissemination of glycopeptide-resistant E. faecalis (GREF) has since occurred in some Brazilian cities [11,12]. The objective of the present study was to characterise GREF isolates collected in São Paulo hospitals and to evaluate the dissemination of GREF clones among Brazilian hospitals.

In total, 73 GREF isolates were collected from patients hospitalised at the following Brazilian hospitals: Hospital São Paulo (n = 36); Hospital do Rim e Hipertensão (n = 10); Hospital Oswaldo Cruz (n = 7); Hospital 9 de Julho (n = 6); Hospital Bandeirantes (n = 7); Hospital do Servidor Público Estadual (n = 4); Hospital Santa Paula (n = 1); Hospital Unimed Sorocaba (n = 1); and Hospital Santa Marcelina (n = 1). One isolate/patient was
included in the study. The GREf strains were isolated from the following body sites: urinary tract (19.1%); blood (13.7%); soft tissues (6.8%); lower respiratory tract (2.7%); upper respiratory tract (1.3%); anal swab (44%); and other specimens (8.3%). The isolates were identified to the species level with the conventional biochemical tests described by Facklam et al. [13].

All samples were investigated by disk diffusion [14] for resistance to ampicillin, teicoplanin, vancomycin, gentamicin, streptomycin, chloramphenicol and doxycycline. Production of β-lactamase was tested by the nitrocephin test (Becton Dickinson, Sparks, MD, USA). MICs of vancomycin, teicoplanin, ampicillin and linezolid were determined by Etest (AB Biodisk, Solna, Sweden) for isolates with vancomycin inhibition zones ≤16 mm. Isolates were categorised according to the National Committee for Clinical Laboratory Standards (NCCLS) breakpoints [14]. ATCC strains Staphylococcus aureus 29213 and E. faecalis 29212 were used as controls.

All strains were typed by pulsed-field gel electrophoresis (PFGE). SmaI restriction digests of total DNA were analysed on the CHEF-DRIII system (Bio-Rad Laboratories, Richmond, CA, USA) in 0.5x TBE buffer (89 mM Tris, 89 mM boric acid, 2 mM EDTA) on an agarose 1% w/v gel. Electrophoresis was for 23 h at 200 V, with a switch interval ramped from 5 to 30 s. PFGE patterns were considered identical if every band was shared, similar if there were one to three band differences, and different if there were four or more band differences [15]. One isolate of glycopeptide-susceptible E. faecalis was included in each PFGE run as a control. Vancomycin resistance genotypes were determined by PCR with vanA- and vanB-specific primers [16,17] selected from published gene sequences.

Table 1. Distribution of PFGE patterns of the 73 isolates according to the medical centres evaluated

<table>
<thead>
<tr>
<th>Hospital (total number of isolates)</th>
<th>Number of isolates of each subtype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A1</td>
</tr>
<tr>
<td>Hospital do Rim e Hipertenção (10)</td>
<td>6</td>
</tr>
<tr>
<td>Hospital 9 Julho (6)</td>
<td>3</td>
</tr>
<tr>
<td>Hospital Bandeirantes (7)</td>
<td>5</td>
</tr>
<tr>
<td>Hospital do Servidor Público</td>
<td>4</td>
</tr>
<tr>
<td>Estadual (4)</td>
<td></td>
</tr>
<tr>
<td>Hospital Oswaldo Cruz (7)</td>
<td>6</td>
</tr>
<tr>
<td>Hospital Santa Paula (1)</td>
<td>–</td>
</tr>
<tr>
<td>Hospital Unimed Sorocaba (1)</td>
<td>1</td>
</tr>
<tr>
<td>Hospital São Paulo (36)</td>
<td>26</td>
</tr>
<tr>
<td>Hospital Santa Marcelina (1)</td>
<td>–</td>
</tr>
<tr>
<td>Total (73)</td>
<td>51</td>
</tr>
</tbody>
</table>

All isolates showed high-level resistance to vancomycin and teicoplanin, which is characteristic of the vanA phenotype. All isolates were susceptible to linezolid and ampicillin, and high-level resistance to streptomycin was not detected in any isolate. In contrast, all isolates showed high-level resistance to gentamicin. All isolates were negative for β-lactamase production.

All 73 isolates displayed a single major PFGE pattern, A, with six subtypes (Table 1). The glycopeptide-susceptible isolate had a completely different PFGE pattern. The most prevalent subtype was A1, which was found in 51 (70%) isolates from seven hospitals. The second most common subtype was A2, which was detected in 18 (26%) isolates from five hospitals. All isolates, except the negative control, were positive for vanA by PCR, in agreement with the phenotypic analysis.

GRE have emerged as an important cause of nosocomial and community-acquired infections, but were only recently described in South America [18]. However, since the first report, the frequency of isolation of GRE has increased significantly, especially in Brazil [11,12]. Treatment of infections with GRE has been extremely problematic, since these organisms are resistant to multiple classes of antimicrobial agents. Although the epidemic strain evaluated in the present study was fully susceptible to ampicillin and linezolid, it was fully resistant to gentamicin. Treatment of severe systemic infections caused by these isolates will therefore require use of streptomycin, a more toxic aminoglycoside, in order to obtain synergism.

Reis et al. [11] found several strains of vancomycin-resistant enterococci showing identical or similar PFGE patterns from different medical
centres in São Paulo, Brazil, which strongly suggests inter-hospital dissemination of this pathogen. Other studies have documented spread of a vancomycin-resistant Enterococcus clone among hospitals [19–21]. In the present study, molecular typing results indicated clonal dissemination of GREI in all the participating centres. The recognition of subtypes may reflect hospital-specific evolution of the main clone. The absence of a surveillance system for infected or colonised patients upon hospital readmission in Brazil might have contributed to this dissemination. However, further epidemiological studies, as well as studies on the use of antibiotics as animal growth promoters, are necessary to evaluate the precise mode of dissemination of glycopeptide resistance among E. faecalis isolates in Brazil. This report emphasises the need for appropriate infection control measures in hospitals and public-health communities. The findings also raise concerns about rapid dissemination of this pathogen throughout other Brazilian hospitals.

ACKNOWLEDGEMENTS

The authors thank A.C. Gales and A.L.C. Darini for important contributions. This study received financial support from Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP—processos 00/14198-7 and 2001/033497).

REFERENCES


© 2004 Copyright by the European Society of Clinical Microbiology and Infectious Diseases, CMI, 10, 255–266