Enterococcus faecalis Resistant to Vancomycin and Teicoplanin (VanA Phenotype) Isolated from a Bone Marrow Transplanted Patient in Brazil

Rosangela F. Cereda, Helio S. Sader, Ronald N. Jones, Lilian Sejas, Antônia M. Machado, Yara P. Zanatta, Sinaida T. M. S. Rego and Eduardo A. S. Medeiros

Special Clinical Microbiology Laboratory (LEMC) and Nosocomial Infection Control Committee, Infectious Disease Division, Federal University of São Paulo, São Paulo, Brazil; Medical Microbiology Division, Department of Pathology, University of Iowa College of Medicine, Iowa City, Iowa; Central Laboratory, São Paulo Hospital, São Paulo, Brazil

We report for the first time in Brazil, a patient from whom an *Enterococcus faecalis* VanA phenotype was isolated. Glycopeptide resistance is not commonly observed in *Enterococcus faecalis*, so this finding is of great concern since this species is responsible for 90% of enterococcal infections in Brazil. The isolate was recovered from a surveillance rectal swab culture from a patient with acute lymphocytic leukemia (ALL). Identification to the species level was performed by conventional biochemical tests and Vitek GPI cards. Antimicrobial susceptibility testing was evaluated by use of broth microdilution and Etest (AB BIODISK, Solna, Sweden) methods. The isolate was identified as *E. faecalis* and was considered resistant to both vancomycin (MIC, > 256 μg/mL) and teicoplanin (MIC, 256 μg/mL). The isolate also showed high level resistance to gentamicin and streptomycin (MICs, > 1024 μg/mL), but was considered susceptible to ampicillin (MIC, 4 μg/mL). Although the frequency of enterococcal infections is very low in most Latin America countries, the finding of glycopeptide (*Van*A) resistance in *E. faecalis* increases concern about apreading antimicrobial resistance in this region.

Key words: *Enterococcus faecalis*, glycopeptide resistance, *van*A, initial case report, transplant.

Glycopeptide-resistant enterococci, best known as vancomycin-resistant enterococci (VRE), have emerged as an important human pathogen responsible for serious systemic infections, especially in debilitated hosts with lowered defense mechanisms [1]. The problem is complicated by the inherent drug resistance of this pathogen. *Enterococcus faecalis*, the most commonly occurring species in this genus, has acquired high-level aminoglycoside resistance, and glycopeptide resistance, although ampicillin resistance is still rarely found.

E. faecium, the second most frequent enterococcal species, is inherently more resistant to many antimicrobial agents. In Brazil, approximately one half of clinical *E. faecium* isolates are resistant to ampicillin. In addition, high-level aminoglycoside resistance and glycopeptide resistance are usually much higher among this species when compared to *E. faecalis* [2].

Three different genotypes have been described in *E. faecium* and *E. faecalis*: *van*A, *van*B, and *van*D. These genes encode either high-, intermediate-, or low-level resistance to glycopeptides. In addition of these genotypes, VRE is categorized in two general

Received on 23 June 2000; revised 4 October 2000.
Address for correspondence: Helio S. Sader, M.D. Laboratório Especial de Microbiologia Clínica - Infectious Disease Division - Federal University of São Paulo. Rua Botucatu, 740. São Paulo, SP – Zip Code: 04023-063 – Brazil. Phone: + (55 11) 5571-5180 / 5576-4393 / 5081-2819. Fax: + (55 11) 543-3013 / 571-5180. E-mail: heliosader@uol.com.br

The Brazilian Journal of Infectious Diseases 2001;5(1):40-46

1413-8670

phenotypes: 1) VanA or Class A – high level resistance to vancomycin and teicoplanin; and 2) VanB or Class B – the isolates are resistant to vancomycin but remain susceptible to teicoplanin (MIC, ≤ 8 μg/mL). Although *E. faecalis* is by far the most frequently isolated enterococcal species, VanA phenotype is rarely found in this species. A fourth genotype, *van*C, is intrinsic for *E. gallinarum* and *E. casseliflavus*, and encodes resistance to vancomycin (low-level) but not to teicoplanin [3, 4].

The main source or reservoir of VRE remains unclear, but the acquisition of the strain may follow previous colonization of the patient (endogenous acquisition) or it may be acquired from other patients (exogenous acquisition) [3, 4]. The acquisition of VRE via the food chain has been proposed in some studies, since *van*A *E. faecium* has been isolated from farm animals and from animal-derived food products [5, 6]. The gastrointestinal tract is considered an important reservoir from which dissemination of resistant strains of enterococci may occur [7]. Nosocomial outbreaks have been associated with gastrointestinal colonization by VRE in patients with prolonged hospitalization and prolonged use of broad-spectrum antimicrobial agents [8].

Once VRE becomes established in a clinical unit or medial center, it may cause a range of infections associated with high mortality. Moreover, there is a potential for glycopeptide resistance genes to spread to other, more virulent organisms [3]. Since there is no widely accepted therapy for infections due to multiresistant VRE, it is essential to limit the spread of these microorganisms.

Although a few cases of infections due to vancomycin-resistant *E. faecium* have recently been described in Brazil [9], this is the first case involving an *E. faecalis* strain.

Case Report

A 23-year-old woman with acute lymphocytic leukemia (ALL) was hospitalized at the São Paulo Hospital (Federal University of São Paulo, São Paulo, Brazil) in December, 1997. She had several previous hospitalizations and antimicrobial treatment with glycopeptides, cephalosporins, carbapenem, and fluoroquinolones. On January 9, 1998, after receiving acyclovir, gancyclovir, fluconazole, cotrimoxazole, enduxan, and methotrexate for 7 days, she received a bone marrow transplant. A Hickman catheter was placed for drug administration. In addition, the patient had a catheter placed for bladder irrigation for 3 days. No other device was used during hospitalization. On January 12, the patient had an elevated temperature and mucositis. Because of the fever, ceftazidime and amikacin were initiated. After 24 hours, the amikacin was replaced with vancomycin and meropenem. Four days later, the patient was still febrile and the mucositis was grade III in intensity. Amphotericin B was introduced at this time. After 5 days, the patient became afebrile and asymptomatic. On January 30, the patient's white cell count was 900/mm^3 and all antimicrobial agents were withdrawn. Four days later she was discharged.

A surveillance rectal swab culture collected on January 14, grew *E. faecalis*. The swab was plated on a blood agar screen plate containing vancomycin 6 μg/mL, aztreonam 60 μg/mL, and nystatin 12.5 μg/mL. After incubation at 37 °C for 24 hours, Gram's stain was performed and Gram-positive cocci were detected and subcultured on non-selective sheep blood agar. Identification to the species level was performed by conventional methods proposed by Fackland and Collins [10]. Vancomycin, teicoplanin, ampicillin, gentamicin, and streptomycin sensitivity were evaluated by the Etest (AB BIODISK, Solna, Sweden) method [11]. The MIC results were as follows: vancomycin > 256 μg/mL (resistant), teicoplanin 256 μg/mL (resistant), ampicillin 4 μg/mL (susceptible), gentamicin and streptomycin > 1,024 μg/mL (high-level resistance). The results for other antimicrobial agents are shown as in Table 1. A double zone of inhibition was observed for vancomycin and teicoplanin. The strain was sent to the University of Iowa (Iowa City, IA, USA) for further characterization. Local results were confirmed and the same double zone phenomenon was observed. In addition, the strain demonstrated a double zone for ampicillin (Table 2). Colonies in the Etest double

Table 1. Antimicrobial susceptibility pattern of the original strain evaluated by Etest and broth microdilution methods

Antimicrobial agents	MIC (mg/mL)	
	Etest	Broth microdilution
Ampicillin	4	4
Cefazolin	-	> 16
Cefotaxime	-	> 32
Ciprofloxacin	-	> 2
Clindamycin	-	> 16
Chloramphenicol	-	> 16
Doxycycline	-	8
Erythromycin	-	> 16
Gentamicin	>1024	> 500
Imipenem	> 32	-
Levofloxacin	> 32	-
Meropenem	> 32	-
Oxacillin	-	> 8
Penicillin	> 32	-
Quinupristin/dalfopristin	-	> 16
Streptomycin	>1024	-
Teicoplanin	> 256	-
Vancomycin	> 256	-

zones (DZ) of ampicillin, teicoplanin, and vancomycin were collected and tested separately. Two of 3 DZ isolates demonstrated identical susceptibility testing results by Etest, but the isolate subcultured from the DZ on the ampicillin strip (isolate number 3) was susceptible to both vancomycin and teicoplanin (Table 2). The pulsed-field gel electrophoresis (PFGE – Figure 1)[12] and ribotype (Riboprinter, E. I. duPont the Nemours, Wilmington, DE, USA) [13] were identical for all 3 isolates and the original strain, and the *van*A gene was identified in the original strain and both DZ isolates with the *Van*A phenotype (vancomycin DZ isolate and teicoplanin DZ isolate). Additional PCR tests [14] confirmed high-level gentamicin resistance and the species identifications, e.g. *E. faecalis* (Table 2). A plasmid analysis was performed and the *van*A samples presented an identical profile (Figure 2) [15]. In spite of repeating the test several times and using different enzymes, we were not able to evaluate the plasmid analysis on isolate number 3 (ampicillin DZ isolate).

Discussion

Enterococcal infections can be associated with significant morbidity and mortality because they often occur in critically ill patients, especially those receiving organ transplants [1, 3]. If the strain reported here had caused a systemic infection, the treatment would have been very difficult. Isolates with high-level resistance to aminoglycosides are refractory to the synergistic effects that occur when these compounds are associated with cell-wall active drugs, such as β-lactams and glycopeptides. Multiple risk factors are related to infection by VRE, and colonization (like the case reported here) usually precedes the infection [16]. VRE carriage

Table 2. Characterization of the samples by Etest, Ribotype, PCR and Vitek GPI

Test		**Original Strain**[a]	**Isolate (1)**[b]	**Isolate (2)**[b]	**Isolate (3)**[b]
Etest (μg/mL)	Vancomycin	> 256 (2)[c]	> 256	> 256 (2)[c]	1.5
	Teicoplanin	64 (0.25)[c]	> 256 (0.25)[c]	> 256	0.38
	Ampicillin	4 (1)[c]	4	4	4
	Streptomycin	96	512	256	256
	Gentamicin	> 2048	> 2048	> 2048	> 2048
Ribotype	723-3	723-3	723-3	723-3	
PCR	*van* gene	*van*A	*van*A	*van*A	*van* neg
	High-level streptomycin resistance	negative	negative	negative	negative
	High-level gentamicin resistance	positive	positive	positive	positive
	Species	*E. faecalis*	*E. faecalis*	*E. faecalis*	*E. faecalis*
E. faecalis	GPI Identification	*E. faecalis*	*E. faecalis*	*E. faecalis*	*E. faecalis*
	Profile #	77767630610	77767630610	77767630610	77767630610

[a] Original strain;

[b] Subcultures from Etest double zone (DZ) colonies:

(1) = vancomycin strip (DZ vancomycin),

(2) = teicoplanin strip (DZ teicoplanin),

(3) = ampicillin strip (DZ ampicillin).

[c] MIC results when reading the internal zone are in parenthesies.

Figure 1. Pulsed field gel electrophoresis (PFGE) of chromosomal DNA digested with *Sma*I. Column 1: original strain; column 2: isolate from the ampicillin double zone; column 3: isolate from the teicoplanin double zone; column 4 vancomycin double zone

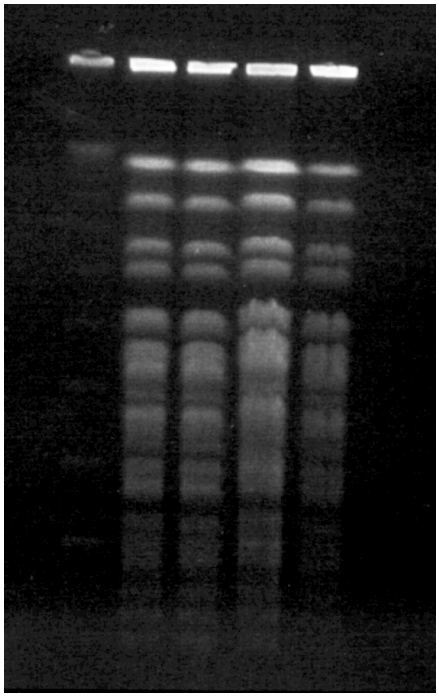
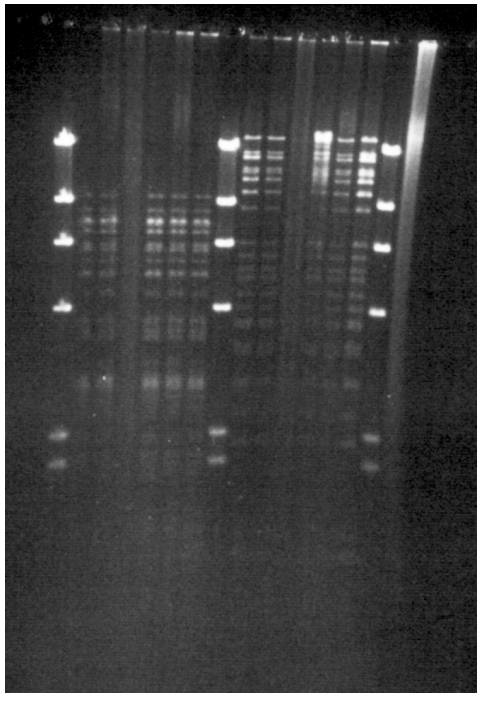


Figure 2. Electrophoresis of plasmid DNA digested with *Hind*III and *Eco*R1. Column 1: original strain; column 2: isolate from the ampicillin double zone; column 3: isolate from the teicoplanin double zone; column 4 vancomycin double zone; columns 5 and 6: susceptible controls



tends to be prolonged (from 19 to 303 days), and a patient whose gastrointestinal tract is colonized with VRE may function as a reservoir and facilitate the nosocomial dissemination of this pathogen. In addition, these patients will have a sustained risk for developing VRE infection [7, 17].

Over the last 3 years, our prospective VRE surveillance program has not identified any glycopeptide resistant strain [18]. Rectal swabs are obtained twice weekly from all ICU patients. The swab is plated on a selective agar screen plate. A total of 516 rectal swabs were cultured during a 17-month period and 37 vancomycin-intermediate enterococci isolates (MIC, 16 μg/mL) were recovered from 11 patients (1.2%). These isolates were identified as *E. gallinarum* (28 isolates), *E. casseliflavus* (5 isolates), *E. faecalis* (3 isolates), and *E. faecium* (1 isolate). *van*A, *van*B or *van*C genes were not identified among *E. faecalis* or *E. faecium* strains. Although our medical center has several important risk factors for the appearance of VRE (600-bed university hospital, vancomycin use of 245 defined daily dose/1000 bed-days in the intensive care unit), this is the first glycopeptide-resistant *E. faecalis* strain identified.

Despite the fact that one isolate had a distinct vancomycin susceptibility pattern by Etest, the PFGE and ribotyping results of all isolates analyzed were identical. The hypothesis of mixed culture was excluded because all isolates had identical PFGE and ribotyping patterns (Figure 1). However, as previously observed by Woodford, et al. [19], PFGE analysis and the resistance phenotype may not be sufficient to accurately define the epidemiology of enterococci. These results reinforce the difficulties found in the characterization of VRE isolates. The fact that one of these isolates separated from the Etest double zones (the ampicillin DZ isolate) had a vancomycin MIC distinct from the other isolates with identical chromosomal patterns, may be explained by the loss of the plasmid containing *van*A gene, since we could not detect plasmid in this vancomycin-susceptible isolate.

The detection of the present case, as well as other cases of colonization/infection due to VRE in Brazil, warns us of for the very recent appearance and dissemination of this important cause of nosocomial infections in our environment. A surveillance program is necessary to rapidly detect and control the appearance and dissemination of VRE on a nation-wide basis. The microbiology laboratories have an important role as the first line of defense by detecting these resistant strains accurately and quickly. Measures for preventing the spread of VRE in hospitals include the application of strict isolation precautions and the implementation of effective antimicrobial use control programs.

In summary, the clinical laboratories in Brazil must be prepared to detect VRE, and infection control personnel should be prepared to contain potential outbreaks before the pathogen becomes endemic.

Acknowledgements

We are very grateful to Ana C. Gales, Richard Hollis, Stacy Coffman and Steve Marshall (Medical Microbiology Division, Department of Pathology, University of Iowa College of Medicine, Iowa City, Iowa), who assisted with the characterization of this strain.

References

1. Sastry V., Brennan P.J., Levy M.M., et al. Vancomycin-resistant enterococci: an emerging pathogen in immunosuppressed transplant recipients. Transplant Proc **1995**;27:954-5.
2. Cereda R.F., Pignatari A.C., Hashimoto A., Sader H.S. *In vitro* antimicrobial activity against enterococci isolated in a university hospital in São Paulo, Brazil. Braz J Infect Dis **1997**;1:83-90.
3. Leclerq R., Courvalin P. Resistance to glycopeptides in enterococci. Clin Infect Dis **1997**;24:545-56.
4. Eliopoulos G.M. Vancomycin-resistant enterococci. Infect Dis Clin N Amer **1997**;11:851-65.
5. Bates J., Jordens J.Z., Griffiths D.T. Farm animals as a putative reservoir for vancomycin-resistant *Enterococcus* spp in sewage. J Antimicrob Chemother **1994**;33:553-61.
6. Sader H.S., Pfaller M.A., Tenover F.C., et al. Evaluation and characterization of multiresistant *Enterococcus faecium* from 12 U. S. medical centers. J Clin Microbiol **1994**;32:2840-2.

7. Lai K.K., Fontecchio S.A., Kelley A.L., et al. The epidemiology of fecal carriage of vancomycin-resistant enterococci. Infect Control Hosp Epidemiol **1997**;18(11):762-5.
8. Gordts B., Landuyt H., Ieven M., et al. Vancomycin-resistant enterococci colonizing the intestinal tracts of hospitalized patients. J Clin Microbiol **1995**;33:2842-6.
9. Costa L.M.D., Souza D.C., Martins L.T.F., et al. Vancomycin-resistant *Enterococcus faecium*: First case in Brazil. Brazilian J Infect Dis **1998** [in press].
10. Facklam R.R., Collins M.D. Identification of *Enterococcus* species isolated from human infections by a conventional test scheme. J Clin Microbiol **1989**;27:731-4.
11. Endtz H.P., Van Den Braak N., Belkum A., et al. Comparison of eight methods to detect vancomycin resistance in enterococci. J Clin Microbiol **1998**,36:592-4.
12. Pfaller M.A., Hollis R.J., Sader H.S. Molecular Biology - PFGE Analysis of Chromosomal Restriction Fragments. In: Isenberg H.D. Clinical Microbiology Procedures Handbook. Washington, ASM Press, **1992**: p.10.5.c.1-10.5.c.11.
13. Stull T.L., Lipuma J.J., Edlind T.D. A broad-spectrum probe for molecular epidemiology of bacteria: Ribosomal RNA. J Infect Dis **1988**;157:280-6.
14. Dutka-Malen S., Evers S., Courvalin P. Detection of glycopeptide resistance genotypes and identification to the species level of clinically relevant enterococci by PCR. J Clin Microbiol **1995**;30:1621-4.
15. Arbeit R.D. Laboratory procedures for the epidemiological analysis of microorganisms. In: Murray P.R., Baron E.J., Pfaller M.A., et al. [eds.]. Manual of Clinical Microbiology. 7th ed. Washington, American Society for Microbiology, **1999**: p.116-37.
16. Edmond M.B., Ober J.F., Weinbaum J.L., et al. Glycopeptide-resistant *Enterococcus faecium* bacteremia: Risk factors for infection. Clin Inf Dis **1995**;20:1126-33.
17. Whitman M.S., Pitsakis P.G., DfJesus E., et al. Gastrointestinal tract colonization with vancomycin-resistant *Enterococcus faecium* in an animal model. Antimicrob Agents Chemother **1996**;40:1526-30.
18. Cereda R.F., Vinagre A., Hashimoto A., et al. Low prevalence of patients colonized with vancomycin-resistant enterococci in spite of the high use of vancomycin. In: Abstract of The 98th General Meeting of the American Society for Microbiology. Atlanta, USA, **1998**. Abstract C-356.
19. Woodford N., Chadwick P.R., Morrison D., Cookson B.D. Strains of glycopeptide-resistant *Enterococcus faecium* can alter their *van* genotypes during an outbreak. J Clin Microbiol **1997**;35:2966-8.