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Clonal dissemination of VanA-type glycopeptide-resistant *Enterococcus faecalis* between hospitals of two cities located 100 km apart

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Abstract

Nosocomial dissemination of glycopeptide-resistant enterococci represents a major problem in hospitals worldwide. In Brazil, the dissemination among hospitals in the city of São Paulo of polyclonal DNA profiles was previously described for vancomycin-resistant *Enterococcus faecium*. We describe here the dissemination of VanA phenotype *E. faecalis* between two hospitals located in different cities in the State of São Paulo. The index outbreak occurred in a tertiary care university hospital (HCUSP) in the city of São Paulo and three years later a cluster caused by the same strain was recognized in two patients hospitalized in a private tertiary care hospital (CMC) located 100 km away in the interior of the state. From May to July 1999, 10 strains of vancomycin-resistant *E. faecalis* were isolated from 10 patients hospitalized in the HCUSP. The DNA genotyping using pulsed-field gel electrophoresis (PFGE) showed that all isolates were originated from the same clone, suggesting nosocomial dissemination. From May to July 2002, three strains of vancomycin-resistant *E. faecalis* were isolated from two patients hospitalized in CMC and both patients were colonized by the vancomycin-resistant *Enterococcus* in skin lesions. All isolates from CMC and HCUSP were highly resistant to vancomycin and teicoplanin. The three strains from CMC had minimum inhibitory concentration >256 μg/ml for vancomycin, and 64 (CMC 1 and CMC 2) and 96 μg/ml (CMC 3) for teicoplanin, characterizing a profile of VanA resistance to glycopeptides. All strains had the presence of the transposon *Tn*1546 detected by PCR and were closely related when typed by PFGE. The dissemination of the *E. faecalis* VanA phenotype among hospitals located in different cities is of great concern because *E. faecalis* commonly colonizes the gastrointestinal tract of patients and healthy persons for periods varying from weeks to years, which, together with the persistence of vancomycin-resistant *Enterococcus* in hospital rooms after standard cleaning procedures, increases the risk of the dissemination and reservoir of the bacteria.

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Nosocomial infections caused by glycopeptide-resistant *Enterococcus* spp are a growing problem in hospitals worldwide. Since the first cases of vancomycin-resistant *Enterococcus* (VRE) were described in 1988 (1,2) an increasing number of cases have been reported in several hospitals in the world (3,4), including Brazil (5). The molecular epidemiology of VRE has shown that a single predominant strain was recovered in hospital settings right after the first identification of VRE (6,7) and a polyclonal profile was reported in hospitals where VRE had been present for some time (8,9). The VanA phenotype VRE strains are typically highly resistant to vancomycin and moderately to highly resistant to teicoplanin (10). Inducible resistance to high levels of vancomycin and teicoplanin is mediated by the transposon *Tn*1546 or closely related elements (11). In the present investigation, we studied the inter-hospital dissemination of VanA-type *E. faecalis* strains that occurred between two hospitals located in two different cities 100 km apart in the State of São Paulo, Brazil.

Hospitals settings. Centro Médico de Campinas (CMC) is a private tertiary care hospital located in Campinas, SP, and 100 km from the capital of the State of São Paulo. Hospital das Clínicas of the University of São Paulo (HCUSP) is a tertiary care teaching hospital associated with the University Medical School with approximately 2000 beds and is the main reference hospital in São Paulo, a city of approximately 11 million inhabitants.

E. faecalis vancomycin-resistant clinical isolates. From May to July 1999, 10 vancomycin-resistant strains of *E. faecalis* were isolated from 10 patients admitted to HCUSP. Vancomycin-resistant *E. faecalis* was isolated from urine (N = 6), from a central venous catheter (N = 1), from feces (N = 1), and from bronchoalveolar lavage (N = 1). The DNA genotyping by pulsed-field gel electrophoresis (PFGE) showed that all isolates originated from the same clone, suggesting nosocomial dissemination (12).

From May to July 2002, 3 strains of vancomycin-resistant *E. faecalis* were isolated from 2 patients hospitalized at CMC and both patients were colonized by VRE in skin lesions. All isolates were tested for susceptibility to vancomycin and teicoplanin using the Etest® and minimum inhibitory concentrations (MIC) were determined according to the NCMLS document M100-S10(M7) 2000 (13).

Control strains of E. faecalis. During the period from 1999 to 2002, 60 strains of vancomycin-susceptible *E. faecalis* stored in 10% skim milk at -20ºC were included in the present study as control strains for DNA typing by PFGE. These strains were isolated from patients with bloodstream infections admitted to CMC.

*Detection of transposon Tn*1546. *Tn*1546 was detectd by PCR based on the method of Martineau et al. (14). The isolates were cultured in BHI broth and 2 µl of 0.5 McFarland solution of the bacterial suspension was transferred to a PCR solution with a 50-µl final volume (2.5 mM $MgCl_2$, 0.1% Triton X-100, 2.5 mM dNTP, 2.5 U Taq polymerase, 50 pmol of each primer: 5'TTA TAA CCG TTC CCG CAG AC and 5'GAA ACC CAG ATT GA). PCR conditions were: 3 min at 92ºC, 30 cycles of denaturation at 92ºC for 1 min, annealing temperature at 60ºC and extension at 72ºC for 1 min, and a final extension at 72ºC for 3 min. PCR products were electrophoresed on 1.5% agarose gel. The primers were designed on the basis of the *Tn*1546 sequence deposited in Genbank under accession number M97297.

Genomic DNA typing by PFGE. DNA extraction was performed as previously described (15). The genetic relatedness among the strains was interpreted by the method of Tenover et al. (12) and isolates with identical or related PFGE patterns were considered to be derived from a common clone. The genetic relationship among PFGE patterns was also analyzed by computer soft-

ware after capturing the autoradiographic images with an IS-1000 digital imaging system (Bio-Capt MW, version 99; M&S Instruments Trading Inc., Marne-la Valle, France). The dendrogram of the PFGE patterns was generated using the Dice coefficient (Biogene software; Vilbert-Loumart, Marne-la-Valle, France).

All isolates from CMC and HCUSP were highly resistant to vancomycin and teicoplanin. The three strains from CMC had MIC >256 μg/ml for vancomycin, and 64 (CMC 1 and CMC 2) and 96 μg/ml (CMC 3) for teicoplanin, characterizing a profile of VanA resistance to glycopeptides.

The genomic DNA profiles obtained by PFGE revealed that all strains from HCUSP had the same profile, which was closely related (derived from the same clone, according to Tenover et al., 12) to that of the strains from CMC (Figure 1). Comparison of the *E. faecalis* strains from the two hospitals showed that the CMC strains were closely related to those from HCUSP, suggesting that they came from the same clone. The relationship among the *E. faecalis* strains shown in the dendrogram (Figure 2) demonstrated that the strains from the hospitals were 95% related. Among the *E. faecalis* control strains, we found one clone with three strains possibly related to the VanA-resistant strains.

All vancomycin-resistant strains were submitted to PCR amplification of the 168-bp fragment corresponding to *Tn*1546 (Figure 3), characterizing the VanA phenotype of the strains from both hospitals. The three vancomycin-susceptible isolates possibly related to the VRE clone had no amplification of the 168-bp fragment.

Since the identification of cases of VanA-resistant *Enterococci* in Brazil (16,17), subsequent cases and nosocomial dissemination of VRE were described in the city of São Paulo, especially related to the *E. faecium* VanA phenotype (5). Zanella et al. (18) described the characterization of a large group of VRE in Brazil during the investigation of a nosocomial outbreak in a general hospital in the city of São Paulo. However, inter-hospital dissemination of the *E. faecalis* VanA phenotype seemed to be less common than dissemination of the *E. faecium* VanA phenotype.

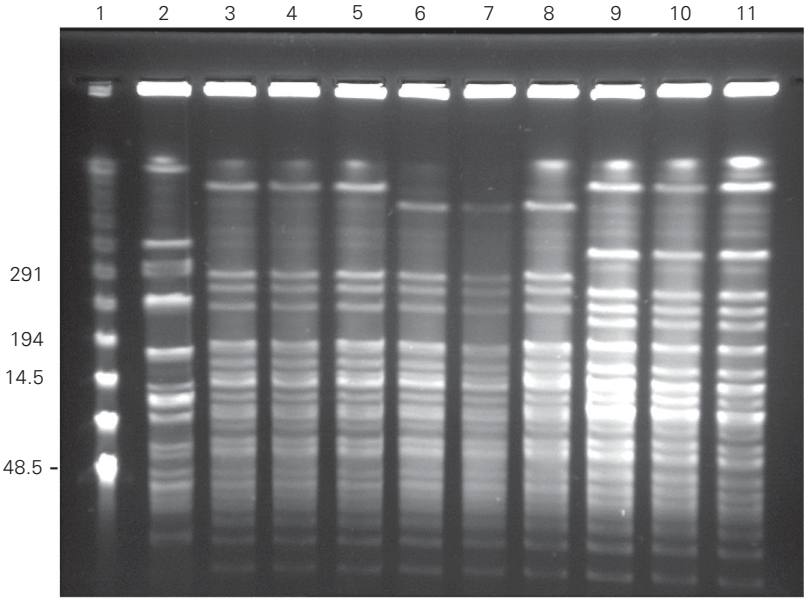

Figure 1. PFGE of *Enterococcus faecalis*. *Lane 1*, Molecular weight lambda ladder size marker (kb); *lane 2*, *E. faecalis* ATCC 29212; *lanes 3* to *5*, vancomycin-resistant *E. faecalis* isolated from Hospital das Clínicas, Universidade de São Paulo; *lanes 6* to *8*, vancomycin-resistant *E. faecalis* from Centro Médico de Campinas; *lanes 9* to *11*, vancomycin-susceptible *E. faecalis* isolated from bloodstream infections at Universidade Estadual de Campinas (control strains).

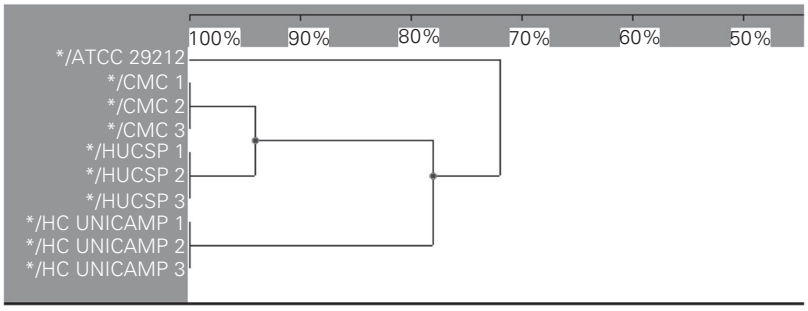

Figure 2. Dendrogram of *Enterococcus faecalis* VanA phenotype. The values were generated from the Dice coefficient showing the similarity of *E. faecalis* vancomycin-resistant strains from Centro Médico de Campinas (CMC), Hospital das Clínicas, Universidade de São Paulo (HCUSP) and vancomycin-susceptible *E. faecalis* from Universidade Estadual de Campinas (UNICAMP).

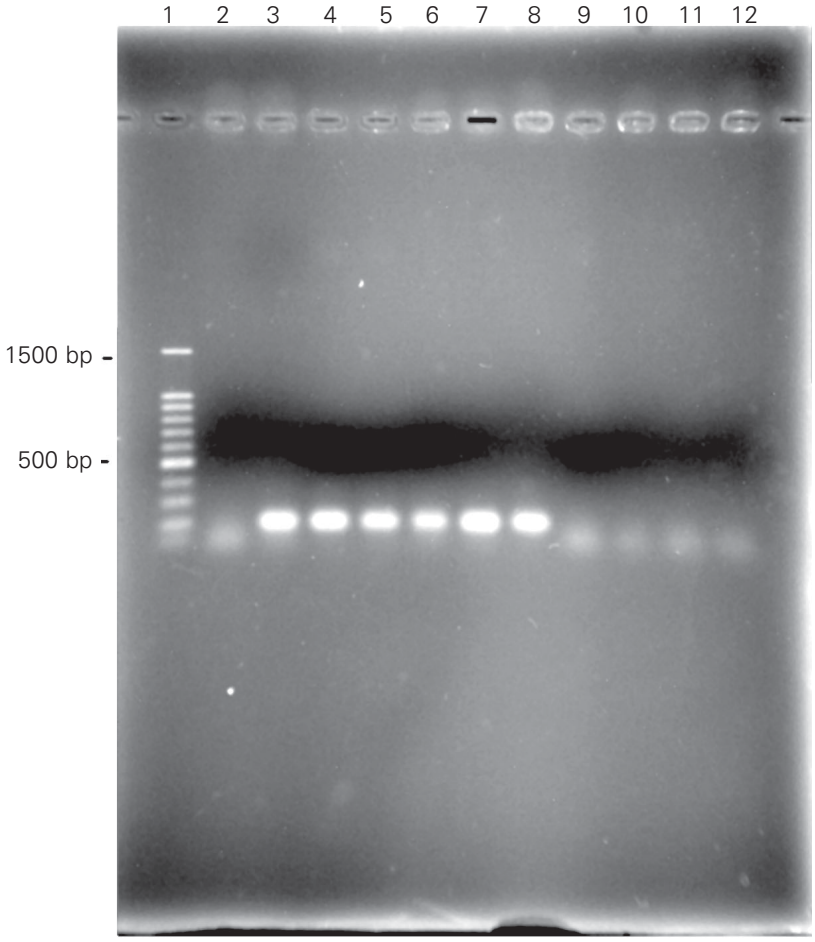

Figure 3. Demonstration of transposon *Tn*1546 in vancomycin-resistant isolates of *Enterococcus faecalis* by PCR. The 168-bp product corresponded to the amplification of *Tn*1546. *Lane 1*, Molecular weight in bp; *lane 2*, *E. faecalis* ATCC 29212; *lanes 3* to *5*, vancomycin-resistant *E. faecalis* from Hospital das Clínicas, Universidade de São Paulo; *lanes 6* to *8*, *E. faecalis* vancomycin resistant from Centro Médico de Campinas; *lanes 9* to *11*, *E. faecalis* vancomycin-susceptible from Universidade Estadual de Campinas; *lane 12*, negative control.

The molecular typing study of the VRE isolated in 1999 from HCUSP showed intra-hospital dissemination. The emergence of the two cases of VRE in the hospital in Campinas indicated a possible inter-hospital dissemination. DNA typing showed that the two clusters were closely related and strongly suggests an inter-hospital dissemination involving the spread of VRE in different cities in Brazil. Of note, the cluster in CMC occurred three years after the detection of the VRE cases in HCUSP.

The origin of VRE in CMC was unclear but appeared to have been imported from HCUSP. The strains were closely related genomically, suggesting intra-hospital dissemination. A previous study on intra-hospital VRE dissemination involved *E. faecium* strains from 6 medical centers in São Paulo, showing a polyclonal DNA profile and intra-hospital dissemination in the city of São Paulo (5). However, the dissemination of the *E. faecalis* VanA profile among hospitals involving different cities in Brazil has not been described previously. This event is of great concern because *E. faecalis* commonly colonizes the gastrointestinal tract of patients and healthy persons for periods varying from weeks to years (19); the persistence of VRE in hospital rooms after standard cleaning procedures increases the risk of the dissemination and reservoir of the bacteria (20).

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