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MOLECULAR CHARACTERIZATION OF VANCOMYCIN-RESISTANT *Enterococci* STRAINS EIGHT YEARS APART FROM ITS FIRST ISOLATION IN SÃO PAULO, BRAZIL

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SUMMARY

E. faecium was the first reported VRE species, carrying the *vanA* gene in Brazil. In spite of this, vancomycin-resistant *E. faecalis* has become the predominant species in Brazilian hospitals. The aim of this study was to evaluate the genetic relatedness of VREs isolated in a Brazilian teaching hospital eight years apart from its first isolation. We analyzed 38 VRE strains obtained from 81 surveillance cultures of patients admitted to the four largest intensive care units in Hospital São Paulo in February, 2006. Presence of the *vanA* gene was assayed by PCR and PFGE analysis was used for molecular characterization. All VRE strains carried the *vanA* gene. Two distinct clonal groups were observed among vancomycin-resistant *E. faecalis*. Vancomycin-resistant *E. faecium* belonged to five distinct clones were demonstrated by molecular typing. All of these clones were different from the first vancomycin-resistant enterococci clone isolated eight years ago in our hospital.

KEYWORDS: VRE; *E. faecalis*; *E. faecium*; PFGE ("Pulsed-Field Gel Electrophoresis").

INTRODUCTION

Enterococci became an important nosocomial pathogen due to their increasing role as opportunistic agents and their special ability to acquire resistance to antimicrobial drugs, including vancomycin[18]. Vancomycin-resistant enterococci (VRE) are a common cause of nosocomial infections and are important agents of gastrointestinal colonization. As the prevalence of VRE in hospitalized patients continues to increase, implementation of appropriate infection control measures requires routine surveillance of VRE transmission patterns[19].

The first cases of human infections associated with VRE were detected in the late 1980s in Europe[16] and in the United States[15]. Since then, VRE isolation has been reported from different parts of the world[4] and surveillance programs for early VRE detection are currently recommended in order to prevent VRE spreading[19]. Vancomycin-resistant enterococci have been an emerging problem in some Brazilian hospitals since the late 1990s. The first report of a VRE infection in Brazil occurred in 1996, in Curitiba[10]. Since 1997, the occurrence of VRE outbreaks has been documented in hospitals from other Brazilian cities[1,8,9,12,13,22,25,26]. Vancomycin-resistant enterococci have been isolated in Hospital São Paulo (HSP) since 1998. Since then, a surveillance program has been carried out by the Infection Control Committee in order to identify patients harboring VRE and to implement contact precautions. Despite this intervention, vancomycin-resistant *Enterococcus faecalis* has became endemic in our setting thereafter. On the other hand, studies on the clonality of vancomycin-resistant enterococci (VRE) are still limited in Brazil, mainly due to territory dimension, the differences in the population characteristics, and the number of nosocomial institutions over the country. The aim of this study was to determine the phenotypic and genotypic characteristics of VRE isolates eight years apart from its first isolation in our setting.

MATERIALS AND METHODS

Hospital São Paulo is a 750-bed university-affiliated hospital with an average of 177,000 patient-days per year. It comprises nine intensive care units (ICUs) and 41 medical and surgical wards. Since 1998, the Infection Control Committee has recorded the VRE incidence rate throughout the hospital. Surveillance through active rectal swab has been performed weekly in the four ICUs (Medical-Surgical, Medical, Pneumonology and Emergency) that have maintained higher rates of VRE incidence in the hospital. This incidence has varied from 17.4 isolates per 1,000 patient-days at Medical-Surgical ICU to 50.3 isolates per 1,000 patient-days at Emergency ICU. A total of 81 rectal swabs (one for each patient) obtained from patients admitted to those ICUs in February 2006 were examined. Only patients admitted for more than 48 hours were eligible for the study. All the rectal swabs were collected by the infection control team. Fecal samples were inoculated onto NaCl 0.9% and, after one hour, they were cultivated in VREBAC medium (PROBAC, Brazil) and incubated at 35 °C for 18-24 hours. Suspected VRE colonies were submitted to identification using conventional biochemical tests and antimicrobial susceptibility[11].

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Susceptibility to five antimicrobials (ampicillin, high-level gentamicin, high-level streptomycin, teicoplanin, and vancomycin) was evaluated by disk diffusion test, according to the Clinical Laboratory Standards Institute (CLSI)[7]. Minimum inhibitory concentrations (MICs) of ampicillin, gentamicin, streptomycin, teicoplanin and vancomycin were also determined by the gradient diffusion test (E test; AB Biodisk, Solna, Sweden) and by the agar-dilution method according to the CLSI guidelines[7], in those isolates that presented resistance.

Detection of vancomycin-resistance genes was performed by polymerase chain reaction (PCR), using methods previously described[6]. The genetic relatedness of bacterial strains was evaluated by PFGE. Briefly, bacterial DNA was digested with *Sma*I and the restriction fragments were separated by electrophoresis on the CHEF DR III apparatus (BioRad, Hercules, CA). Running parameters were as follows: 200 V (6 V/cm); temperature, 13 °C; initial switch time 5' and final 60' for 23h. After the electrophoresis run was completed, the PFGE gel was stained in a 0.08 µg/mL ethidium bromide solution. Macrorestriction patterns were determined by visual inspection. The isolates were classified as identical if they shared the same band profile, and different when differing by seven or more bands[23]. Three reference VRE strains were used to compare profiles in this study (first *E. faecalis* from HSP, first *E. faecium* from HSP and first *E. faecalis* from Porto Alegre).

RESULTS

Vancomycin-resistant isolates were detected in 37 (45.7%) out of 81 fecal cultures. Vancomycin-resistant *E. faecium* was detected in 20 (54.1%) out of these 37 patients and vancomycin-resistant. *E. faecalis* in 17 (45.9%) out of these 37 patients. The proportion of colonized patients in ICUs was: Pneumonology - 41.6%; Medical-Surgical - 14.2%; Emergency - 15.3% and Medical - 40%. Two different VRE species were detected in one patient (*E. faecalis* and *E. faecium*). The species isolates were identified and had their antimicrobial susceptibilities determined. The strains presented MICs of 1024 µg/mL and 256 µg/mL for vancomycin and teicoplanin respectively, and they all harbored the *van*A gene. The MICs for gentamicin and streptomycin in *E. faecalis* were variable. 41.2% (7/17) and 29.4% (5/17) were gentamicin-resistant and streptomycin-resistant respectively. In *E. faecium,* gentamicin resistance and streptomycin-resistance were 15.0% (3/20) and 90.0% (18/20), respectively. None *E. faecalis* strain was resistant to ampicillin whereas 95.0% (19/20) of *E. faecium* strains were ampicillin-resistant. With regard to the phenotypic similarity of species isolates, only two isolates per intensive care unit were randomly selected to be analyzed by PFGE (Tables 1 and 2). PFGE revealed the presence of two distinct vancomycin-resistant *E. faecalis* clones (Fig. 1) and five distinct *E. faecium* clones (Fig. 2). Among *E. faecium,* one of these clones were

Table 1
Results of nine vancomycin-resistant *E. faecalis* typed by PFGE

Number	DD AMP/VANCO[1]	E-test vancomycin/teicoplanin µg/mL	ICU[2]	Profile of PFGE
1	S/R	1024/256	Medical-surgical	"A"
2	S/R	1024/256	Medical-surgical	"A"
3	S/R	1024/256	Medical-surgical	"B"
4	S/R	1024/256	Emergency	"B"
5	S/R	1024/256	Emergency	"B"
6	S/R	1024/256	Emergency	"B"
7	S/R	1024/256	Medical	"B"
8	S/R	1024/256	Medical	"B"
9	R/R	1024/256	First *E. faecalis* from HSP	"C"

[1]DD AMP/VANCO: disc diffusion test for ampicillin (10 µg) and vancomycin (30 µg); S: susceptible; R: resistant; [2]intensive care units.

Table 2
Results of ten vancomycin-resistant *E. faecium* typed by PFGE

Number	DD AMP/VANCO[1]	E-test vancomycin/teicoplanin µg/mL	ICU[2]	Profile of PFGE
1	R/R	1024/256	Pneumonology	"A"
2	S/R	1024/256	Medical-surgical	"B"
3	R/R	1024/256	Medical-surgical	"C1"
4	R/R	1024/256	Medical-surgical	"D"
5	R/R	1024/256	Emergency	"C2"
6	R/R	1024/256	Medical	"B"
7	R/R	1024/256	Pneumonology	"B"
8	R/R	1024/256	Emergency	"B"
9	R/R	1024/256	Medical	"E"
10	R/R	1024/256	First *E. faecium* from HSP	"F"

[1]DD AMP/VANCO: disc diffusion test for ampicillin (10 µg) and vancomycin (30 µg); S: susceptible; R: resistant; [2]intensive care unit.

identified in four isolates from four different patients; each one from a different ICU. All of these clones were different from the first VRE clones detected eight years ago in our hospital.

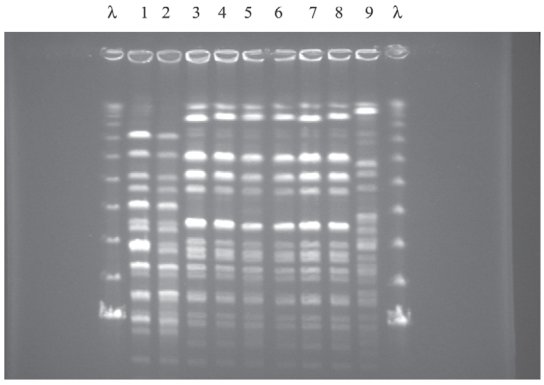

Fig. 1 - PFGE profiles of SmaI-digested chromosomal DNA of vancomycin-resistant *E. faecalis* isolates obtained from colonized inpatients from Hospital São Paulo, Brazil. Lanes l, molecular size markers (48.5kb). lane 1: clone "A"; lane 2: clone "A"; lanes 3-8: clone "B"; lane 9: clone "C".

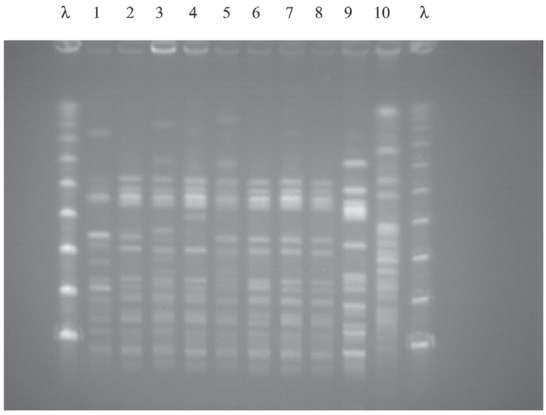

Fig. 2 - PFGE profiles of SmaI-digested chromosomal DNA of vancomycin-resistant *E. faecium* isolates obtained from patients from Hospital São Paulo, Brazil. l: lambda ladder (48.5 kb); lane 1: clone "A"; lane 2: clone "B"; lane 3: clone "C1"; lane 4: clone "D"; lane 5: clone "C2"; lane 6: clone "B"; lane 7: clone "B"; lane 8: clone "B"; lane 9: clone "E"; lane 10: clone "F".

DISCUSSION

Glycopeptide resistance is disseminated among *E. faecium* isolates around the world[14]. However, to date the first reported VRE outbreaks were attributed to *E. faecalis*[1,3,8,12,13,22], while occurrence of vancomycin-resistant *E. faecium* has been sporadic[10,25]. On the other hand, the first VRE isolated in Brazil was an *E. faecium* strain with the *van*D2 phenotype recovered from a patient with sepsis in Curitiba. This fact drew attention for a potential problem that might be coming along in our healthcare institutions thereafter[10]. In the following years, no additional cases of VRE were detected in Curitiba, although several cases of VRE identified as either *E. faecium* or *E. faecalis* harboring VanA phenotype were documented in São Paulo[25,26].

VRE became increasingly problematic for institutions in which they now are endemic. Controlling the VRE spreading may become a less difficult and costly task if the epidemiology is better understood[21]. In these settings, colonized and infected patients and colonized environmental surfaces may serve as persistent reservoirs. The molecular epidemiology of VRE in a setting where it is endemic is more complex. Several patterns of VRE transmission in non-outbreak periods have been reported (eg. polyclonal, oligoclonal, monoclonal)[2,5,17,20,21,24]. Molecular-typing techniques can be important tools for efficiently identifying clonal VRE dissemination. The heterogeneity of nosocomial VRE in centers in which this organism is endemic likely reflects antimicrobial selective pressure at work in high-risk patient populations combined with the constant introduction of new strains by patients transferred from other institutions[21].

In our institution, eight years apart from its introduction, vancomycin-resistant *E. faecalis* has remained apparently oligoclonal in the ICUs. On the other hand, we have noted a regular appearance of *E. faecium* over the last years with its worse resistance profile. The widespread dissemination of these two species likely reflects the transfer of patients with undetected colonization between hospital wards. However, the reasons for the maintenance of this oligoclonal pattern in our institution are unclear. Vancomycin-resistant *E. faecalis* incidence has been maintained high but stable in the last three years in our setting. Thus, the infection control measures must be reinforced in order to reduce these high incidence rates.

All vancomycin-resistant *E. faecalis* clones identified in this study were distinct from the first one previously isolated in HSP. In addition, the major vancomycin-resistant *E. faecalis* clone isolated in our setting is similar to those previously isolated in Porto Alegre and Rio de Janeiro in 2001[22]. The predominant clone, denominated "B" has high-level of resistance to gentamicin and was isolated from three ICUs. This fact may suggest an inter-hospital dissemination of this clone among Brazilian states.

Faster VRE detection methods may control the nosocomial spread of this pathogen among new patients that may become index cases for dissemination[21]. In our study we used the PCR method, which provide results in less than 48 hours and proved to be an excellent method to identify this microorganism.

In conclusion, the present study demonstrated that the initial VRE clones (mainly *E. faecalis*) were replaced by new ones, eight years apart from its first isolation in our hospital. Certainly, the spread of a single clone via patient-to-patient transmission through healthcare professional hands represents the major concern for infection control team. Probably, VRE will continue to be a significant nosocomial pathogen and continuous efforts aimed to understanding their molecular epidemiology are warranted.

RESUMO

Caracterização molecular de espécies de *Enterococci* resistentes à vancomicina oito anos após seu primeiro isolamento em São Paulo, Brasil

E. faecium contendo o gene *vanA* foi a primeira espécie de VRE descrita, no Brasil. Apesar disto, *E. faecalis* resistente a vancomicina

tem se tornado a espécie predominante nos hospitais brasileiros.O objetivo desse estudo foi avaliar a relação genética de VREs isolados em um hospital de ensino brasileiro após oito anos de seu primeiro isolamento. Analisamos 37 isolados de VRE obtidos de 81 culturas de vigilância de pacientes admitidos nas quatro maiores Unidades de Tratamento Intensivo em Fevereiro de 2006. A presença do gene *vanA* foi analisada por PCR e a caracterização molecular por PFGE. Todas as amostras VRE carreavam o gene *van*A. Entre os *E. faecalis* vancomicina-resistentes, dois distintos grupos clonais foram observados. *E. faecium* resistente a vancomicina pertencentes a cinco clones distintos foram demonstrados por tipagem molecular. Todos esses clones foram diferentes do primeiro clone de enterococo resistente a vancomicina isolado oito anos atrás em nosso hospital.

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