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ORIGINAL RESEARCH

Antibiotic Resistance And Genotyping Of Gram-Positive Bacteria Causing Hospital-Acquired Infection In Patients Referring To Children's Medical Center

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Introduction: A serious problem affecting human society is the development of bacterial resistance. The purpose of the current study was to evaluate the antibiotic resistance of Gram-positive bacteria (GPB) and genotyping of common GPB causing hospital-acquired infections (HAIs) in patients who were referred to Children's Medical Center during a 6-month period by random amplified polymorphic DNA (RAPD) and enterobacterial repetitive intergenic consensus sequence polymerase chain reaction (ERIC-PCR).

Methods: During the 6-month period, antimicrobial resistance profiles of GPB isolates recovered from patients in Children's Medical Center were determined using the Kirby–Bauer disk diffusion and MIC. Typing of common GPB was performed and the results were analyzed by gel compare software.

Results: In this cross-sectional study, 6524 cultures were performed and 138 Ggram-positive bacteria were isolated (2%). *Staphylococcus aureus* strains showed the highest antibiotic penicillin resistance (96.3%). Twenty-six per cent of the strains were methicillin-resistant *S. aureus* (MRSA) and no resistance was found against vancomycin. All isolates of *Enterococcus faecium* were resistant to ciprofloxacin (100%). The resistance to vancomycin was very high (67%) and no resistance was observed to linezolid. The results of genotyping analysis of *S. epidermidis* strains showed the presence of two clones with a genetic relationship of over 80%. All of the *S. aureus* strains were in one cluster and half of the *E. faecium* strains were in a cluster with a genetic predilection of over 80%.

Conclusion: This study indicated frequent occurrence of antimicrobial resistance, especially in *Enterococcus* spp. isolates. Rapid spreads of MRSA and VREF from a clonal origin require implementing careful isolation and infection control measures.

Keywords: antibiotic resistance, Gram-positive bacteria, hospital-acquired infection

Introduction

The administration and non-prescribed consumption of antibiotics has grown at a considerable rate, sometimes irrationally, in Iran. This process has become increasingly significant in recent years.[1,2] According to several studies, the controlled consumption of antibiotics in patients suffering from bacterial infections leads to improved microorganism susceptibility, and the emergence of resistant strains are rarely observed.[3,4] Among the Gram-positive bacteria, vancomycin-resistant *Staphylococcus aureus* (VRSA), vancomycin-resistant *Enterococcus* (VRE), methicillin-resistant *S. aureus*

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(MRSA) and beta-lactamase-resistant *Streptococcus* are considered the most important groups in terms of antibiotic resistance.[5,6] Due to their importance, antibiotic consumption policies should be taken and implemented in the agenda of the infection control committees of hospitals.[7] Generally, studies covering the antibiotic profile of Gram-positive bacteria (GPB) applied a disk diffusion of a range of antibiotics according to the standard Clinical and Laboratory Standards Institute (CLSI) patterns for sensitivity.[8] Among these antibiotics, we can note penicillin, methicillin, ampicillin, trimethoprim-sulfamethoxazole, rifampin, clindamycin, ciprofloxacin, and gentamicin.[9] In addition, it is essential to understand pathogen distribution and relatedness to determine the epidemiology of hospital-acquired infections (HAIs) and aid to design the rational pathogen control methods.[10]

Nowadays, researchers use various DNA-based, amplification-based and sequencing-based typing methods for genotyping of GPB.[11,12] These techniques include RFLP (restriction fragment length polymorphism),[13] ERIC-PCR (enterobacterial repetitive intergenic consensus sequence polymerase chain reaction),[12] AP PCR (arbitrary primers-PCR),[14] ribotyping,[15] examination of plasmid profile,[16] RAPD (random amplified polymorphic DNA),[17] and PFGE (pulse field gel electrophoresis).[18] The purpose of the current study was to evaluate the antibiotic resistance of GPB and genotyping of common GPB causing HAI in the patients who were referred to Children's Medical Center during 6 months by RAPD and ERIC-PCR.

Materials And Methods

In the present study, the isolates were collected from the referral hospital of Tehran Children ‘s Medical Center for a 6-month period from July 2017 to January 2018. Isolation of bacteria was undertaken as part of the routine hospital laboratory procedure. The GPB strains isolated from clinical specimens of blood, wound and sterile fluids (pleural effusion, peritoneal and cerebrospinal fluid [CSF)] of patients hospitalized in this center were included in the study. All isolates were re-identified using conventional confirmatory tests, such as Gram stain, catalase and coagulase production, DNase, and mannitol fermentation. These specimens include *S. aureus*, coagulase-negative *Staphylococcus*, *Streptococcus pneumoniae, Enterococcus spp*. and *Streptococcus viridans*.

The antibiotic susceptibility of the isolates was evaluated according to the guidelines published in 2016 by CLSI.[19] The measured antibiotics include penicillin (10 U), gentamicin (10 μg), ciprofloxacin (5 μg), cefoxitin (30 μg), trimethoprim/sulfamethoxazole (1.25/23.75 μg), erythromycin (15 μg), clindamycin (2 μg), ampicillin (10 μg), linezolid (30 μg), cefazolin (30 μg), and ampicillin-sulbactam (10/10 μg). All disks were purchased from Mast Co., UK. *S. aureus* ATCC 25923 was used for quality control of the test. The MICs of vancomycin were determined by E-test methods.

Following the assessment of antimicrobial resistance in each organism, the genotyping of the common hospital -cquired GPB (*S. aureus*, coagulase-negative *Staphylococcus*and *E. faecium*) was accomplished. To achieve this, bacterial genomic DNA extraction was performed using a DNA extraction kit (Bioneer, South Korea) according to manufacturer’s guidelines. RAPD-PCR was accomplished for *E. faecium* genotyping, whereas ERIC-PCR was done to assess the genotyping of *S. aureus* and *S. epidermidis*. Briefly, DNA amplification was performed on a thermo cycler (Bio Rad, USA) in a final volume of 25 mL. The amount of PCR materials for both sets was mentioned in Table 1.

The PCR products were loaded on a 1% (w/v) agarose gel containing 1/10,000 gel red (Biotium, USA), and were analyzed by gel electrophoresis and banding patterns were visualized and photographed in Gel-Documentation system (Uvitec, UK). A 100-bp Plus DNA ladder (Thermos Scientific, USA) was used as a molecular size standard. Comparison of PCR fingerprinting profile was performed using GelCompar II software, version 6.5 (Applied Maths, Sint-Martens-Latem, Belgium). Each gel photograph was inverted as TIFF images and then normalized using the reference marker. Similarity analysis of results was calculated using the Dice coefficient/unweighted pair-group method with arithmetic mean (UPGMA). The criterion for related clones was taken as profiles with 80% or more similar bands.

Statistical Analysis

Statistical analysis of the results was performed by the statistical package SPSS 13.0 (SPSS Inc. Chicago, IL, USA).

Results

In the current cross-sectional study, among a total of 6524 collected isolates (5243 blood culture, 889 CSF, 139 dialysis fluid, 48 pleural fluid, 60 ascites, 44 joint fluid, 66 wound and 35 CV line) over the course of the 6 months, 138 (2%) GPB were separated among 88 males patients (36.2%). Ninety-two children (66.7%) were under the age of one year, and only 20 patients (14.49%) were above 5 years of age. A little over half (55.1%) had a history of previous hospitalization.

Table 1 The PCR Components And Procedures Used For Each Species

Species	Primers	The Sequence Of Primers	Materials	Concentration	The Procedure
E. faecium	272	5'-AGCGGGCCAA-3'	Buffer DFS Taq DNA polymerase MgCl2 dNTP Primer (272) DNA Distilled water	1X 1/25 Unit 3 mM 0/25 mM 0/5 mM up to 50 ng up to 25µL	4cycles: 94°C 5 min, 36°C 5 min, 72°C 5 min, followed by 30 cycles: 94°C 1 min, 36°C 1 min, 72°C 1 min
S. aureus and *S. epidermidis*	Eric-1	5'-ATGTAAGCTCCTGGGGATTCAC-3'	Buffer DFS Taq DNA polymerase MgCl2 dNTP	1X 1/25 Unit 2 mM 0/2 mM	Initial denaturation: 94°C 2 min, 30 cycles: 94°C 1 min, 52°C 1 min, 72°C 3 min, Final extension: 72°C 10 min
	Eric-2	5'-AAGTAAGTGACTGGGGTGAGCG-3'	Forward Primer (Eric-1) Reverse Primer (Eric-2) DNA Distilled water	0/5 mM 0/5 mM up to 50 ng up to 25µL	

Among 121 GPB, which were assessed for nosocomial infection, 64 isolates (52.9%) had the HAI criteria. In addition, the HAI criteria were met by all patients who utilized a CV line, and 89% of patients who used a catheter. There was also one child who used a ventilator, due to having the criteria of nosocomial infection.

The prevalence of Gram-positive organisms isolated was: 76 (55.1%) *S. epidermidis*, 27 (19.5%) *S. aureus*, 12 (8.7%) *E. faecium*, 9 (6.5%) *S. viridans*, 7 (5.1%) *S. haemolyticus*, 4 (2.9%) *S. pneumoniae*, 2 (1.4%) *Streptococcus* group B, and 1 (0.7%) diphtheroid. The frequency of HAI among isolated bacteria is shown in Table 2. Obviously, *S. epidermidis* was reported as the most frequent bacteria having nosocomial infection (64.2%), followed by *S. aureus* (17.9%).

Most of the GPB were isolated from the Pediatric Intensive Care Unit, which was responsible for 20.3% of isolates, followed by the emergency unit (18.1%). The most isolated bacteria separated from these units were *S. epidermidis* (75% and 68%, respectively).

As shown in Table 3, *S. aureus* and *S. epidermidis* were determined as the most resistant to penicillin (96.3% and 98.6%, respectively), and all were sensitive to vancomycin. Among *S. aureus* strains, 26% MRSA strains were reported. The highest resistance rate among *S. viridans* strains was observed to be against erythromycin, while none was

Table 2 Frequency Of Nosocomial Infections Among Isolated Bacteria

Isolated Bacteria	No	Yes	Total
	***N* (%)**	***N* (%)**	***N* (%)**
Staphylococcus aureus	15 (55.5)	12 (44.5)	27 (22.3)
Staphylococcus epidermidis	33 (43.4)	43 (56.6)	76 (62.8)
Staphylococcus hemolyticus	ND	ND	ND
*Streptococcus*alpha hemolytic (viridans group)	0 (0)	1 (100)	1 (0.82)
Streptococcus pneumoniae	3 (75)	1 (25)	4 (3.03)
Enterococcus faecium	2 (16.7)	10 (83.3)	12 (9.9)
Streptococcus beta hemolytic strep (Group B)	ND	ND	ND
Diphtheroids	1 (100)	0 (0)	1 (0.82)
Total	54 (44.6)	67 (55.4)	121 (100)

Abbreviation: ND, not determined.

Table 3 Antibiotic Resistance Rates Of Evaluated Gram-Positive Bacteria

Bacteria	Vancomycin	Erythromycin	Clindamycin	Penicillin	Methicillin	Trimethoprim/ Sulfamethoxazole	Ampicillin	Ciprofloxacin	Linezolid	Ampicillin Sulbactam	Gentamycin	Cefazolin
	N(%)	*N* (%)	*N* (%)	*N* (%)	*N* (%)	*N* (%)	*N*(%)	*N* (%)	*N* (%)	*N* (%)	*N* (%)	*N*(%)
S. aureus	0/27 (0)	12/27 (44.4)	12/27 (44.4)	26/27 (96.3)	7/27 (26)	1/27 (3.7)	–	–	–	–	–	–
S. epidermidis	0/76 (0)	69/76 (90.7)	62/76 (81.5)	75/76 (98.6)	68/76 (89.4)	51/76 (67.1)	–	–	–	–	–	–
S. haemolyticus	0/7 (0)	5/6 (83.3)	5/6 (83.3)	7/7 (100)	6/7 (85.7)	4/7 (57.1)	–	–	–	–	–	–
S. viridans	0/9 (0)	7/9 (77.7)	0/9 (0)	2/9 (22.2)	–	0/4 (0)	3/9 (33.3)	–	–	–	–	–
S. pneumoniae	0/4 (0)	4/4 (100)	2/4 (50)	1/3 (33.3)	–	¼ (25)	0/4 (0)	–	–	–	–	–
*Enterococcus*spp.	9/12 (75)	–	–	10/12 (83.3)	–	–	10/12 (83.3)	10/10 (100)	0/10 (0)	7/8 (87.5)	9/10 (90)	–
Diphtheroids	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)	–	0/1 (0)	–	–	–	–	–	–
GBS*	0/1 (0)	–	–	0/2 (0)	–	–	0/1 (0)	–	–	–	–	0/2 (0)

GBS* Group B *Streptococcus*

resistant to vancomycin, clindamycin, and trimethoprim/sulfamethoxazole. The whole *Enterococcus*spp. isolates were resistant to ciprofloxacin, and along with that, sensitive to linezolid.

The genotyping of *S. aureus* causing hospital-acquired infection showed the presence of all strains in one cluster with more than 80% genetic similarity (Figure 1). In addition, the dendrogram, based on analysis of *S. epidermidis* strains indicated the presence of two clones with 80% genetic similarity. Twenty-six strains were located in one cluster and 17 other strains were in another (Figure 2). Genotyping of *Enterococcus*spp. strains demonstrated the presence of three clusters, of which 50% of strains was placed in one of them (Figure 3).

Discussion

In the current investigation, we assessed the antimicrobial resistance and genotyping of GPB isolated from patients who were referred to Children's Medical Center over a period of 6 months from July 2017 to January 2018.

In our current study, the most frequent Gram-positive organisms were *S. epidermidis* (*n*= 76, 55.1%) and *S. aureus* (*n*= 27, 19.5%). In the earlier study by Mamishi et al.,[20] coagulase-negative *Staphylococcus*(48.4%) and *S. aureus* (16.7%) were reported as the most frequent bacteria. In addition to the mentioned organisms, *Enterococcus*spp. were also recognized as the most frequent GPB in the study performed by Bagherzadeh et al.[21]

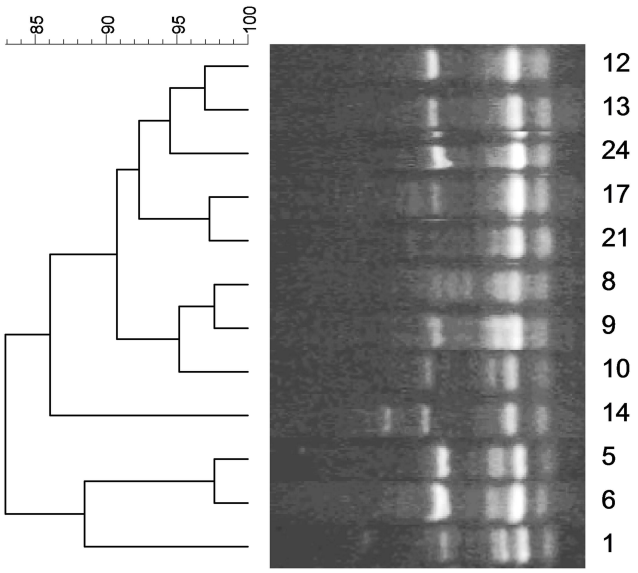

Figure 1 Genotyping of *S. aureus* strains with HAI at the Children's Medical Center.

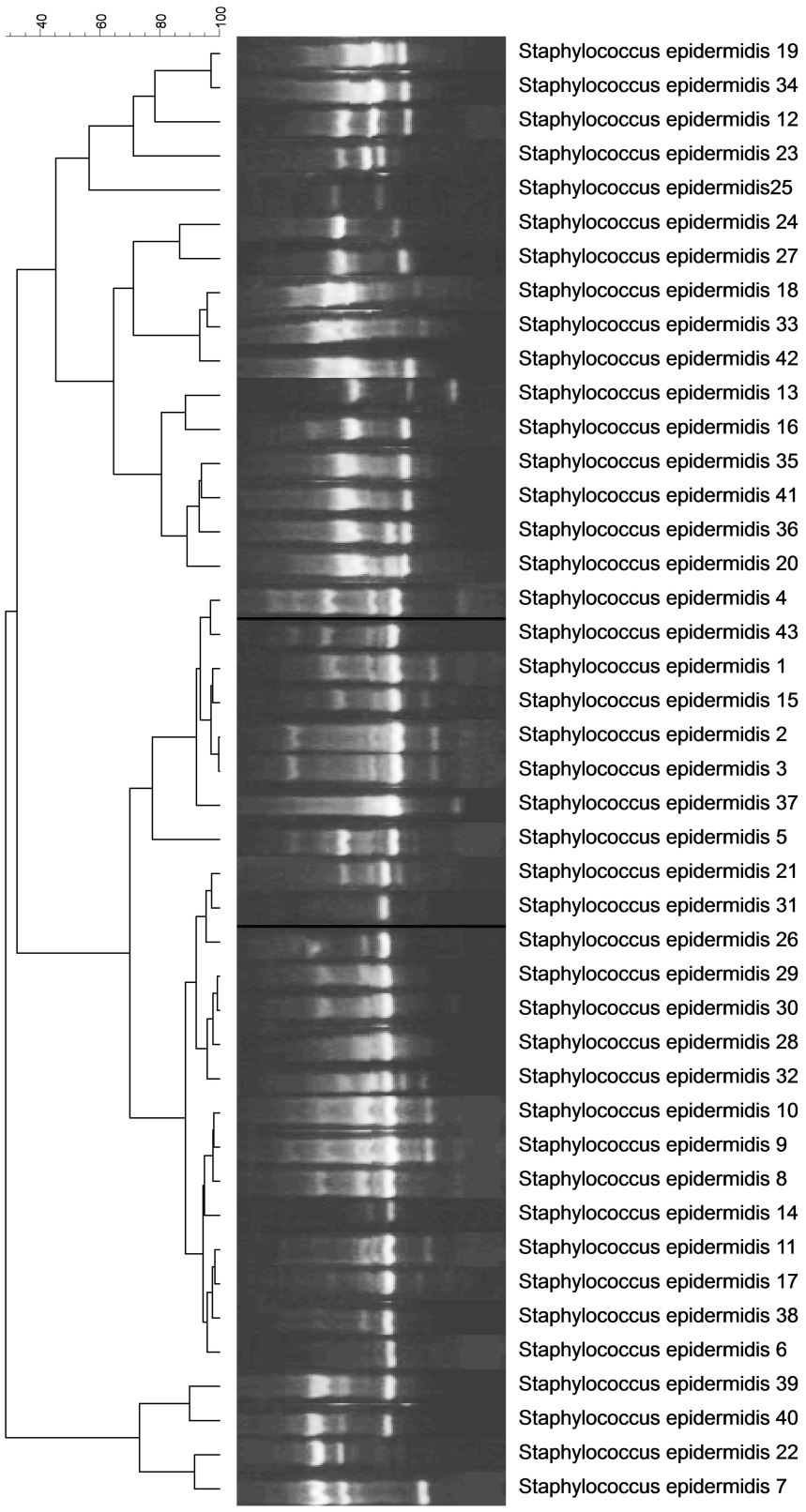

Figure 2 Genotyping of *S. epidermidis* strains with HAI at the Children's Medical Center.

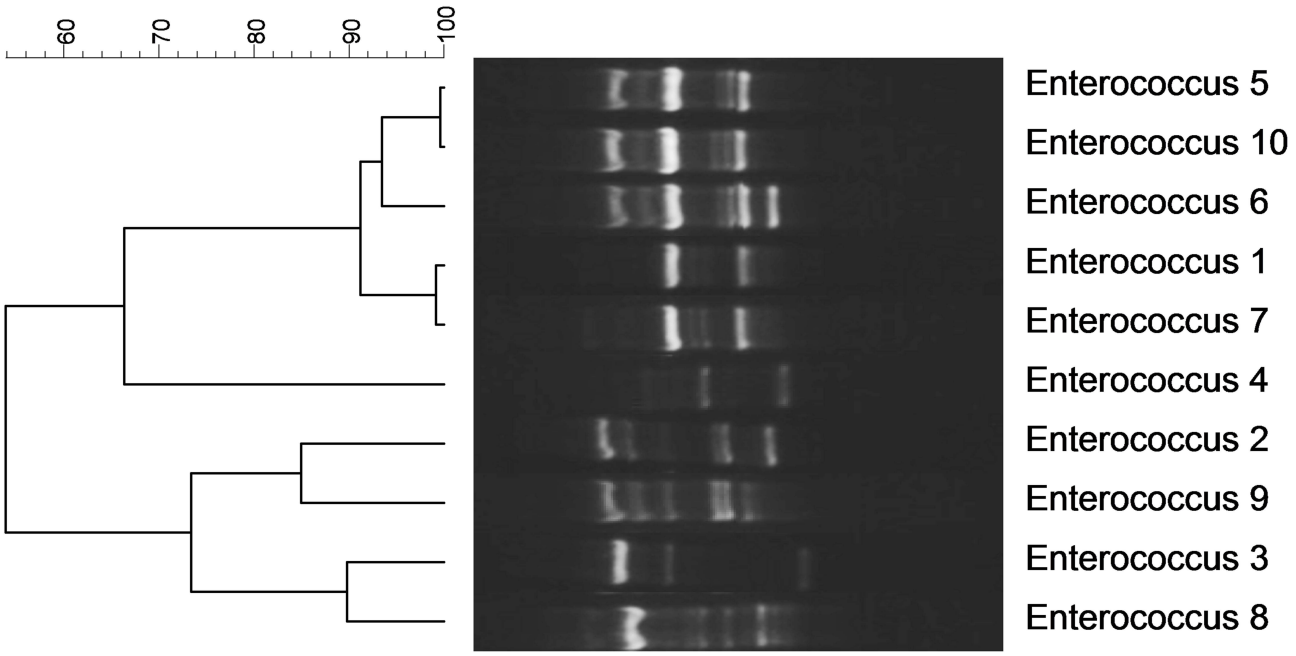

Figure 3 Genotyping of Enterococcus spp. strains with HAI at the Children's Medical Center.

In *S. aureus* strains, the highest antibiotic resistance rate was identified in penicillin (96%), while the highest sensitivity rate was observed in vancomycin (100%) and trimethoprim/sulfamethoxazole (96%). Also, 26% of isolates were MRSA, which was compatible with the two studies performed in Kuwait, and in Saudi Arabia, Brazil and Iran;[22–25] however, they were far lower than the figures reported by the previous studies in the Children's Medical Center.[20,26–28]

Enterococcus spp. strains indicated significantly high resistance to several used drugs (vancomycin, 67%; ciprofloxacin, 100%; gentamycin, 90%; ampicillin, 83%; ampicillin-sulbactam, 87.5%). In the study of Sattari-Maraji et al., *E. faecium* was found to have high resistance against ampicillin (92.5%), ciprofloxacin (96%), and vancomycin (70%).[29] In our study, all isolates were sensitive to linezolid, which is consistent with previous studies.[20,30–33]

Although the occurrence of VRE varies in different countries, a high frequency was described in Iran, Ethiopia and Turkey.[29,34–36]

The dendrogram of genotyping of *S. epidermidis* strains depicted the presence of two clones with more than 80% genetic similarity, suggesting the existence of an outbreak in the hospital. In addition, all *S. aureus* isolates belonged to one cluster with more than 80% genetic similarity. The analysis of genotyping of *Enterococcus* spp. demonstrated the presence of 50% of strains in one cluster with more than 80% genetic similarity. In the study of Banerjee et al. in India, RAPD typing of a multidrug-resistant *E. faecium* urinary isolate showed two major clusters, one of which had 10 strains of 100% similarity and were isolated from a common source.[37] According to their high resistance rate to vancomycin in the current study and the presence of half of the strains in one cluster, it might be considered as an alarm for transmission of these strains among different units and patients. In the study of Pourakbari et al.,[34] the results of RAPD-typing of *Enterococcus* strains demonstrated the presence of four distinct clusters, of which 100% of VREF isolates belonged to one cluster, indicating a nosocomial infection among units.

Vancomycin-resistant enterococci are thought to be spread mostly through cross-colonization.[38] To minimize this high pressure, health care workers (HCWs) hand washing and using gloves are crucial actions.[38] Besides, reducing the movement of HCWs between colonized and non-colonized patients may be attained by creating cohorts of either patients or nursing staff, and also by restricting the number of physicians entering patients' rooms during rounds.[38]

In conclusion, this study indicated frequent occurrence of antimicrobial resistance, especially in *Enterococcus*spp. isolates. Rapid spreads of MRSA and VREF from a clonal origin require implementing careful isolation and infection control measures. Therefore, environmental control by routine disinfection of patient area, in addition to screening high-risk patients and isolation of colonized patients, should be imposed to diminish the risk of acquiring nosocomial VRE.

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Disclosure

The authors report no conflicts of interest in this work.

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