PUBLICAÇÃO OFICIAL DO NÚCLEO HOSPITALAR DE EPIDEMIOLOGIA DO HOSPITAL SANTA CRUZ E PROGRAMA
DE PÓS GRADUAÇÃO EM PROMOÇÃO DA SAÚDE - DEPARTAMENTO DE BIOLOGIA E FARMÁCIA DA UNISC

Revista de Epidemiologia e Controle de Infecção

Hospital Santa Cruz

ORIGINAL ARTICLE

Characterization of vancomycin resistance mechanisms in *Enterococcus faecium* isolates from a Brazilian tertiary hospital

Caracterização dos mecanismos de resistência à vancomicina em isolados de Enterococcus faecium de um hospital terciário brasileiro

Caracterización de los mecanismos de resistencia a la vancomicina en aislamientos de Enterococcus faecium de un hospital terciario brasileño

https://doi.org/10.17058/reci.v14i2.18967

Received: *11/28/2023*
Accepted: *04/12/2024*
Available online: *5/21/2024*

Corresponding Author:
Igor Vasconcelos Rocha
igor.rocha@fiocruz.br

Address: Instituto Aggeu Magalhães, Fundação Oswaldo Cruz - Fiocruz - Pernambuco, Recife, Pernambuco, Brasil.

Igor Vasconcelos Rocha[1];
Carlos Alberto das Neves de Andrade[2];
Antônio Marcos Saraiva[2];
Erika Danielle Gameiro da Fonsêca[3];
Danilo Elias Xavier[1];
Danielle Patrícia Cerqueira Macêdo[3];

[1] *Instituto Aggeu Magalhães, Fundação Oswaldo Cruz - Fiocruz-PE, Recife, PE, Brasil.*
[2] *Hospital das Clínicas da Universidade Federal de Pernambuco - HC-UFPE, Recife, PE, Brasil.*
[3] *Universidade Federal de Pernambuco (UFPE), Recife, PE, Brasil.*

ABSTRACT

Background and Objectives: Vancomycin-resistant *Enterococcus faecium* (VREf) is an opportunistic pathogen responsible for hospital infections, characterized by increasing prevalence and a lack of comprehensive epidemiological studies. We aimed to assess the occurrence of VREf and vancomycin resistance genetic elements *vanA* and *vanB* in strains isolated from clinical samples of patients treated at a tertiary hospital in Brazil. **Methods:** The isolates were obtained from convenience sampling according to routine medical requests for nine months. *Enterococcus faecium* strains were identified by routine biochemical tests, BD Phoenix® Automated Microbiology System and confirmed by MALDI-TOF Mass Spectrometry. The antimicrobial sensitivity profile was determined by disk-diffusion method and BD Phoenix® Automated Microbiology System. Vancomycin resistance was specially assessed and confirmed by the conventional microdilution technique. Molecular detection of *vanA* and *vanB* resistance genes was investigated by polymerase chain reaction (PCR) and confirmed by Sanger DNA sequencing. **Results:** A total of 8,376 cultures was performed, of which 19 (0.22%) were identified as *Enterococcus* sp. and nine (47%) as vancomycin-resistant *E. faecium*. The antimicrobial susceptibility testing analysis of *E. faecium* showed high resistance to antimicrobial agents. The analysis to determine the genetic profile of *E. faecium* isolates by PCR showed that all of them carried the *vanA* gene associated with vancomycin resistance. **Conclusion:** During the study period, there was low occurrence of *Enterococcus* species observed. However, all VREf isolates carried the *vanA* gene associated with vancomycin resistance and showed resistance to commonly used antimicrobial agents, highlighting concerns about the effectiveness of available antimicrobial treatments for infections caused by these isolates.

Keywords*: Enterococcus faecium. Epidemiological surveillance. Drug Resistance. Vancomycin Resistance.*

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RESUMO

Justificativa e Objetivos: *Enterococcus faecium* resistente à vancomicina (VREf) é um patógeno oportunista responsável por infecções hospitalares, caracterizado por crescente prevalência e falta de estudos epidemiológicos abrangentes. O objetivo foi avaliar a ocorrência de VREf e dos elementos genéticos de resistência à vancomicina *vanA* e *vanB* em cepas isoladas de pacientes atendidos em um hospital terciário no Brasil. **Métodos:** Os isolados foram obtidos por amostragem de conveniência de acordo com as solicitações médicas de rotina durante nove meses. As cepas foram identificadas por testes bioquímicos, Sistema de Microbiologia Automatizada BD Phoenix®, e confirmadas por MALDI-TOF. O perfil de sensibilidade aos antimicrobianos foi determinado por difusão em disco e pelo BD Phoenix®. A resistência à vancomicina foi avaliada e confirmada pela técnica de microdiluição. A detecção molecular dos genes de resistência *vanA* e *vanB* foi investigada por reação em cadeia da polimerase (PCR) e sequenciamento de DNA. **Resultados:** Um total de 8.376 culturas foi realizado, sendo 19 (0.22%) identificadas como *Enterococcus* sp., e nove (47%), como *Enterococcus faecium* resistente à vancomicina. A análise do teste de sensibilidade aos antimicrobianos do *E. faecium* mostrou alta resistência aos antimicrobianos. A análise para determinar o perfil genético dos isolados de *E. faecium* por PCR mostrou que todos eles carregavam o gene vanA associado à resistência à vancomicina. **Conclusão:** Durante o período de estudo, observou-se baixa ocorrência de espécies de *Enterococcus*. No entanto, todos os isolados de VREf apresentaram o gene *vanA* associado à resistência à vancomicina e mostraram resistência aos antimicrobianos comumente utilizados, alertando sobre a eficácia dos tratamentos antimicrobianos disponíveis para infecções causadas por esses isolados.

Descritores: *Enterococcus faecium. Vigilância Epidemiológica. Resistência a Medicamentos. Resistência à Vancomicina.*

RESUMEN

Justificación y Objetivos: *Enterococcus faecium* resistente a vancomicina (VREf) es un patógeno oportunista responsable de infecciones hospitalarias, caracterizado por su creciente prevalencia y la falta de estudios epidemiológicos exhaustivos. El objetivo fue evaluar la ocurrencia de VREf y los elementos genéticos de resistencia a vancomicina *vanA* y *vanB* en cepas aisladas de muestras clínicas de pacientes tratados en un hospital terciario en Brasil. **Métodos:** Los aislamientos se obtuvieron mediante muestreo de conveniencia según las solicitudes médicas de rutina durante nueve meses. Las cepas fueron identificadas mediante pruebas bioquímicas, utilizando el BD Phoenix® y MALDI-TOF. El perfil de sensibilidad a los antimicrobianos se determinó mediante difusión en disco y el BD Phoenix®. La resistencia a vancomicina se evaluó mediante microdilución. La detección molecular de los genes de resistencia *vanA* y *vanB* se investigó mediante reacción en cadena de la polimerasa (PCR) y secuenciación de ADN. **Resultados:** Se realizaron un total de 8,376 cultivos, identificándose 19 (0.22%) como *Enterococcus* sp., de las cuales 9 (47%) fueron VREf. El análisis de la sensibilidad a los antimicrobianos mostró una alta resistencia. El análisis para determinar el perfil genético de los aislados de *E. faecium* mediante PCR mostró que todos portaban el gen *vanA* asociado a la resistencia a la vancomicina. **Conclusión:** Durante el período de estudio, se observó una baja incidencia de especies de *Enterococcus*. Sin embargo, todos los aislamientos de VREf presentaron el gen *vanA* asociado con resistencia a la vancomicina y mostraron resistencia a los antimicrobianos comúnmente utilizados, lo cual alerta sobre la eficacia de los tratamientos antimicrobianos disponibles para infecciones causadas por VREf.

Palabras Clave: *Enterococcus faecium. Monitoreo Epidemiológico. Farmacorresistencia Bacteriana. Resistencia a la Vancomicina.*

INTRODUCTION

Enterococci, characterized as non-sporulated Gram-positive cocci, typically appear in short chains. They are facultative anaerobes, lack catalase activity, and exhibit dimensions spanning from 0.6 to 2.5 µm. These microorganisms are comprehensive components of the gastrointestinal microbiota in mammals and can also be encountered in the genitourinary tract and on the skin.[1] In the environment, *E. faecium* can be found in soil, water, and food, and it has the ability to survive on inanimate surfaces for long periods.[1]

Enterococci's remarkable capacity to swiftly acquire virulence traits, either through mutational events or by incorporating genetic material from other bacteria via plasmid and transposon transfer, is augmented by their intricate virulence mechanisms. These mechanisms encompass toxin secretion, stress response proteins, transport systems, and specific gene regulators. Collectively, these attributes enhance the microorganism's efficacy in causing infections, establishing colonization and persisting in both biotic and abiotic environments. This adaptability confers a selective advantage that aids in withstanding adverse conditions.[1]

The initial accounts of antimicrobial resistance among *Enterococci* surfaced during the 1970s, primarily involving isolates displaying high-level resistance to gentamicin.[2] Subsequently, strains exhibiting resistance traits associated with modifications of penicillin-binding proteins (PBPs) were reported.[1,2] In a pivotal development, in

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1986, the first instances of vancomycin-resistant strains were documented, marking a significant shift towards the prevalent isolation of *Enterococci* resistant to ampicillin, aminoglycosides, and vancomycin in hospital-acquired infections. [1,3] Notably, in Brazil, the inaugural case of vancomycin-resistant *Enterococci* (VRE) was identified in 1996, specifically in Curitiba, Paraná.[3]

Vancomycin (C66H75Cl2N9O24) is a tricyclic glycopeptide antibiotic developed in 1956 that inhibits peptidoglycan biosynthesis, altering cytoplasmic membrane permeability and RNA synthesis. Its mechanism of action occurs through the binding to D-Alanyl-D-Alanine of peptidoglycan pentapeptide, resulting in a destabilized cell wall caused by interference of transpeptidation and transglycosylation steps.[2,4,5]

Vancomycin resistance in *E. faecium* is associated with nine resistance genes, classified according to its gene sequence and organization, as follows: i) D-Ala-D-lac, including *vanA*, *vanB*, *vanD* and *vanM* genes; and ii) D-Ala-D-Ser, including *vanC*, *vanE*, *vanG*, *vanL* and *vanN* genes.[2,5]

Globally, the *vanA* and *vanB* genes hold paramount clinical significance, distinguished by their varying levels of resistance to vancomycin, transferability between organisms, and capacity to trigger resistance in the presence of an antimicrobial agent. [2,5,6]

Vancomycin-resistant *Enterococcus faecium* (VREf) is categorized as opportunistic pathogens and represents a significant etiology of healthcare-associated infections (HAI).[1] These infections predominantly originate from patients' indigenous microbiota or through direct or indirect contact.[1] Common clinical manifestations of VREf infections encompass urinary tract infections (UTI), wound infections, meningitis, infections associated with catheters and other implanted medical devices, bacteremia, and endocarditis. These infections primarily afflict hospitalized patients who are grappling with severe underlying conditions, including cancer, hematological disorders, chronic renal insufficiency, transplant recipients, and individuals with compromised immune systems.[7]

Given the escalating prevalence of VREf and the notable dearth of comprehensive epidemiological investigations pertaining to this microorganism, the primary aim of this study was to assess the frequency of VREf and the presence of genetic components associated with vancomycin resistance, specifically *vanA* and *vanB* genes, within strains obtained from clinical specimens of patients undergoing treatment at a tertiary healthcare facility in Brazil. The objective was to assess the occurrence of VREf and the presence of *vanA* and *vanB* genetic elements associated with vancomycin resistance in strains isolated from clinical samples of patients treated at a tertiary hospital in Brazil.

METHODS

Bacterial isolates and species identification

This is a descriptive cross-sectional study developed in a tertiary hospital from Recife-PE, Brazil. The isolates were obtained from convenience sampling according to routine medical requests sent to the Clinical Microbiology Laboratory during nine months (December 2020 – August 2021) and including samples from rectal swab (surveillance culture), urine and soft tissue from patients treated in the vascular, orthopedics and oncology clinics. For bacterial growth and isolation, samples from different anatomopathological sites were plated on Müeller-Hinton agar (Difco®), supplemented with 0.5% (v/v) defibrinated sheep blood and on CHROMagar™ (Becton, Dickinson and Company®). The plates were incubated at 35 ± 2° C for 18 to 24 hours, and the cultures were visually inspected to assess bacterial growth and identify the different morphologies of the colonies. At least one colony with characteristic morphology for *Enterococcus* sp. was selected for further identification by BD Phoenix® Automated Microbiology System. Additionally, the isolates identified were also submitted to species

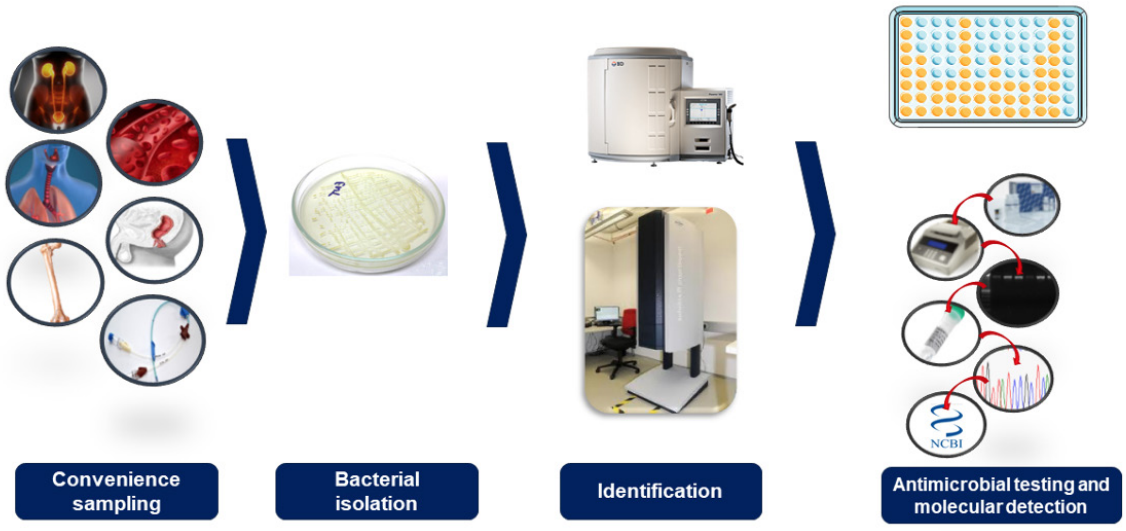

Figure 1. Schematic overview of methodology steps. Schematic representation detailing the sequential steps and procedures followed in the methodology, illustrating the key stages from sample collection to data analysis.

confirmation by the Matrix-Assisted Laser Desorption Ionization – Time of Flight (MALDI-TOF) mass spectrometry technique using the MALDI Biotyper system version 2.0 (Bruker Daltonics®). A summarized overview of the steps conducted during the methodology can be observed in a schematic diagram presented in Figure 1.

Antimicrobial susceptibility testing

The antimicrobial susceptibility test was performed using the broth microdilution technique, according to the Brazilian Committee on Antimicrobial Susceptibility Testing (BrCAST, 2022) (http://brcast.org.br) and Clinical Laboratory Standard Institute (CLSI, 2020) (http://www.clsi.org/) standardization and recommendations. The resistance profile to teicoplanin, gentamicin, streptomycin, daptomycin, linezolid and ampicillin was assessed using data extracted from the identification process by BD Phoenix® Automated Microbiology System NMIC panel (Biomerieux). The vancomycin resistance profile was specially assessed by using 96-well microdilution plates containing serial dilutions of vancomycin (0.5 µg/mL to 256 µg/mL) in Cation-adjusted Müeller Hinton II Broth (Oxoid). As quality control, strains from the American Type Culture Collection (ATCC®) of *Enterococcus faecalis* ATCC® 29212 and *Escherichia coli* ATCC® 25922 were used.

Molecular detection of vancomycin-resistance associated genes

Genomic DNA extraction was performed using the DNeasy Blood & Tissue Kit (Qiagen), following the manufacturer's recommendations, from an aliquot of 1,500 µL of culture grown in Luria-Bertani (LB) broth (Himedia) at 35 ± 2°C for 18 hours. For this, an aliquot of each sample of interest was previously plated on BHI agar (Himedia), which was incubated at 35 ± 2 °C for 18 hours. The extracted genomic DNA was quantified in NanoDrop 2000c (Thermo Fisher Scientific Inc.) with verification of the parameters used to estimate the extraction (A260/280) purity and yield. DNA amplification reactions were performed by polymerase chain reaction (PCR) containing 50 ng of genomic DNA, 20 pmol of each *vanA* (5'- CATGAATAGAATAAAAGTTGCAATA -3'; 5'- CCCCTTTAACGCTAATACGATCAA -3') or *vanB* (5'- GTGACAAACCGGAGGCGAGGA -3'; 5'- CCOGCCATCCTCCTGCAAAAAA -3') oligonucleotide pair, 200 mM of each dNTP, 50 mM of Tris-HCl (pH 9.0), 50 mM of NaCl, 5 mM of MgCl2 and 1U of Taq DNA polymerase (Promega). Amplification reactions were thermocycling in GeneAmp PCR System 9700 (Applied Biosystems), and PCR amplicons were submitted to 1% agarose gel electrophoresis (m/v), being visualized and photographed under UV. The amplicons were purified by ExoSAP-IT PCR Product Cleanup kit (Affymetrix) and sequenced by the Sanger method in 3,500 xL Genetic Analyzer (Applied Biosystems). The chromatograms were analyzed using Chromas Lite 2.1.1 (Technelysium), and the contigs were assembled using BioEdit Sequence Alignment Editor (Tom Hall) software. The assembled contigs were compared with DNA sequences available in GenBank (http://www.ncbi.nlm.nih.gov/genbank) by Basic Local Alignment Search Tool (BLAST) to confirm amplification reaction specificity and gene identification (Figure 1).

Ethical approval

This study is part of the Program for Surveillance and Study of Bacterial Resistance to Antimicrobial agents (HC/UFPE), of the Department of Microbiology of IAM/Fiocruz-PE and of the *Hospital das Clínicas* Hospital Infection Control Service (SCIH/HC/UFPE), for studying local epidemiology of bacterial resistance among samples of clinically important species. This study was appreciated by the Research Ethics Committee of FIOCRUZ, with the title "Microbiological Collection for the Implementation of a Surveillance Program and Study of Bacterial Resistance to Antimicrobial agents: Genetic Study of the Determinants of Antimicrobial Resistance Among Bacterial Clinical Isolates", and its execution was approved by Certificate of Presentation for Ethical Consideration (CAAE - *Certificado de Apresentação para Apreciação Ética*) 45080915.0.0000.5190/REC 1.190.837. This study was conducted in accordance with the ethical standards required by Resolutions 466/2012, 510/2016 and 580/2018 of the Ministry of Health, Brazil.

RESULTS

During the study period, a total of 8,376 cultures were conducted. Among the collected clinical samples, 19 were identified as *Enterococcus* species. Notably, within this group, nine isolates, designated as Ef_01 to Ef_09, displayed resistance to vancomycin, constituting 47% of the *Enterococcus* isolates. These VREf strains were then chosen for further comprehensive analysis, as detailed in Table 1.

Antimicrobial susceptibility testing analysis of *E. faecium* showed high resistant to antimicrobial agents commonly used in clinical practice and assessed in the present study. According to BD Phoenix® Automated Microbiology System data, all isolates showed resistance to teicoplanin, ampicillin and penicillin, exhibiting minimum inhibitory concentration (MIC) values of ≥ 16, 8 and 8 µg/mL, respectively. None showed resistance to linezolid and daptomycin (MIC ≤ 1 and ≤ 2 µg/mL for each antimicrobial, respectively). All isolates showed high resistance to vancomycin and to high concentrations of gentamicin (assessed by synergism test, 500 µg/mL). Only the Ef_04 isolate was not susceptible to streptomycin synergism test, exhibiting MIC of ≥ 1,000 µg/mL. Table 1 shows the MIC of each antimicrobial agent tested on different isolates.

The genetic profiling of *E. faecium* isolates via PCR analysis revealed that all of them harbored the vancomycin resistance-associated *vanA* gene. Furthermore, in silico analysis of the *vanA* gene and the predicted amino acid sequence of the *vanA* protein, conducted on the isolates from this study using Sanger DNA sequencing data, demonstrated complete coverage and identity, achieving 100% similarity with the d-alanine-d-lactate subfamily *vanA* sequence (GenBank accession number: WP_001079845.1). It is noteworthy that none of the isolates exhibited PCR amplification for the *vanB* gene.

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Table 1. Minimum inhibitory concentrations (MIC) of each antimicrobial agent tested to different *E. faecium* isolates.

Strain ID	VAN (µg/mL)	AMP (µg/mL)	PEN (µg/mL)	TEC (µg/mL)	LZD (µg/mL)	DAP (µg/mL)	GEN* (µg/mL)	STP* (µg/mL)
Ef_01	128	≥ 8	≥ 8	≥ 16	< 1	< 1	< 500	< 1000
Ef_02	128	≥ 8	≥ 8	≥ 16	< 1	< 1	< 500	< 1000
Ef_03	128	≥ 8	≥ 8	≥ 16	< 1	< 1	< 500	< 1000
Ef_04	≥ 256	≥ 8	≥ 8	≥ 16	< 1	< 1	< 500	> 1000
Ef_05	≥ 256	≥ 8	≥ 8	≥ 16	< 1	< 1	< 500	< 1000
Ef_06	≥ 256	≥ 8	≥ 8	≥ 16	< 1	< 1	< 500	< 1000
Ef_07	≥ 256	≥ 8	≥ 8	≥ 16	< 1	< 1	< 500	< 1000
Ef_08	≥ 256	≥ 32	≥ 8	≥ 16	< 1	< 1	< 500	< 1000
Ef_09	≥ 256	≥ 32	≥ 8	≥ 16	< 1	< 2	< 500	< 1000

*Caption: AMP – ampicillin; PEN – penicillin or benzylpenicillin; VAN – vancomycin; TEC – teicoplanin; LZD – linezolid; DAP – daptomycin; GEN – gentamicin; STP – streptomycin. The antimicrobial agents were tested using the corresponding MIC cut-offs (µg/mL) for classification into susceptible (S) or resistant (R) range: ampicillin, S≤4, R≥8; penicillin, S≤16, R≥16; vancomycin, S≤4, R≥4; teicoplanin, S≤2, R≥2; linezolid, S≤4, R≥4; daptomycin, S≤8, R≥8. *For gentamicin and streptomycin, synergism tests were performed. For gentamicin, the isolated were considered resistant when MIC was ≥ 128µg/mL and, for streptomycin, when MIC was ≥ 512µg/mL (EUCAST, 2020; BrCAST, 2021).*

DISCUSSION

VRE is classified as one of the main pathogens causing HAIs in the United States.[8] This microorganism has intrinsic resistance to several antimicrobial agents and progressive resistance to ampicillin and aminoglycosides. Vancomycin resistance, however, has been reported more recently by beta-lactamases enzymes, probably due to indiscriminate use in antimicrobial therapy.[8]

In *E. faecium*, vancomycin resistance has changed its clinical relevance worldwide, being among the main causes of HAI.[8] In Brazil, the isolation of these pathogens is increasingly frequent, considering that many studies have reported the rapid emergence of VRE.[9]

During the study period, 19 strains of *E. faecium* were isolated, of which 47% (9) were resistant to vancomycin. These results are similar to those found by a multicenter study conducted in Europe.[10] In Europe, the occurrence rate of VREf in tertiary hospitals varies between the different hospitals and countries.[11,12] In 2021, resistance percentages below 1% were observed in six (14%) of the 44 countries reporting data on this microorganism. Conversely, percentages equal to or exceeding 25% were found in 17 (39%) countries. Alarmingly, vancomycin resistance percentages equal to or above 50% were reported by five (11%) countries.[11] Infections by VREf can result in prolonged hospital stays and additional antimicrobial therapy, comprising an important public health concern. This allows colonization and infection of patients, favoring HAI outbreaks, and promotes a significant increase in hospital expenses.

The emergence of VRE has coincided with the increase of the incidence of *E. faecium* in several countries, which also presents a high level of resistance to penicillin and aminoglycosides.[13] It represents a major threat to public health, since the combination of an aminoglycoside with a beta-lactam agent is a pharmacological synergism strategy used to "bypass" some bacterial resistance mechanisms, especially in the treatment of bacteremia and severe endocarditis. Thus, considering this scenario, it is also necessary to monitor aminoglycoside resistance. All the VREf in this study showed susceptibility to high levels of gentamicin (500 µg/mL). [11,13] These results are similar to other studies developed in a tertiary hospital in Brazil, whose sensitivity to gentamicin was observed in the majority of the assessed isolates.[14,15]

In the present study, all VRE isolates showed resistance to ampicillin (MIC≥08 µg/mL). Studies suggest that point mutations in penicillin-binding protein 5 (PBP 5) may change the bacteria's affinity for beta-lactams, leading to resistance to this class of antimicrobial agents.[16]

All the isolates were resistant to vancomycin and teicoplanin, having the *vanA* gene with identical sequences. Isolates with this resistance profile were previously reported in multicenter studies that assessed more than 1,200 VREf isolates from 26 hospitals in southwestern Brazil.[17] In silico analysis performed in the same study confirmed the presence of the *vanA* gene in all strains assessed. Considering that most of the *vanA* genes are associated with a Tn1546-type resistance transposon that can be transported by plasmids, the presence of VREf isolates in the hospital cannot be neglect, since *vanA* can be transferred by conjugation between different species.[15] Multifactor studies developed in North America observed that, in the first seven cases of vancomycin-resistant *S. aureus* (VRSA) in the United States, the *vanA* gene was transferred from *Enterococci*, in addition to identifying that patients with VRSA were also infected with VRE.[18]

In our study, all strains were sensitive to linezolid and daptomycin, used as last-line antimicrobial agents for the treatment of VREf infections. Linezolid and daptomycin resistance to *Enterococci* is still uncommon, although cases have already been reported. [19-22] Multicenter studies developed with VREf isolates from 66 different countries observed that those isolates with the *vanA* genotype were sensitive to these two antimicrobial agents (MIC < 2 µg/mL).

It is important to highlight that the present study was developed during the COVID-19 pandemic, in a

scenario where several hospitals had to adapt to receive a greater demand for medical and hospital care, in particular the lack/unequal distribution of healthcare professionals and medium/high complexity care infrastructure as well as limited capacity to produce and carry out diagnostic tests. Thus, all cases of VREf came from patients relocated from other hospitals of the Brazilian Health System (SUS - *Sistema Único de Saúde*) due to redistribution of beds. Transfer of patients between health units or even intra-hospital, associated with factors such as overcrowding, shortage of Intensive Care Unit beds, medical equipment and medicines, indiscriminate use of antimicrobial agents in COVID-19 treatment and improvisation of Intensive Care Unit beds in wards, contributes significantly to the worsening and dissemination of multidrug-resistant isolates in hospital settings.

It was observed that 50% of the isolates came from a surveillance culture sample. This fact has already been shown in Brazilian studies that VRE infection has a low prevalence; however, high rates of isolation are reported from intestinal colonization research by surveillance cultures of rectal swabs. Almost 67% of patients with positive cultures for VREf were hospitalized in the same ward, at different times, being exposed to the same healthcare professionals and medical devices. Previous studies have already highlighted the importance of healthcare settings and professionals as agents of cross-contamination, whether through transient or persistent colonization. In this scenario, non-colonized patients who share rooms or wards with patients colonized by VREf are exposed to contamination and possible infections.[23,24]

Studies indicates that the most common comorbidities among patients with VREf infections are associated with solid tumors (27%), cardiovascular diseases (31%), cerebrovascular diseases (12%) and joint diseases (23%), increasing the risk of mortality by up to 73% and hospitalization time by up to 124 days.[25] Additionally, non-observance of good hospital biosafety practices by healthcare professionals, such as proper hand hygiene, may represent a risk of contamination for outpatients belonging to these clinics, since healthcare professionals can be vectors for the dissemination of VREf in extra-hospital settings.[23,24] Thus, it is necessary to intensify the prevention and control measures of this microorganism in this health unit, such as contact precautions, decolonization, active surveillance culture and cleaning of settings.

During the study period, a low occurrence of *Enterococcus* species was observed.[8-9] However, all VREf isolates were found to harbor the *vanA* gene associated with vancomycin resistance and exhibited resistance to commonly used antimicrobial agents.

This study highlights the importance of epidemiological surveillance in the healthcare institution to identify, monitor and control the spread of VREf in hospital settings. The high incidence of VREf isolates carrying *vanA* genes represents an important risk factor for the emergence of hospital outbreaks, which may directly reflect the increase in mortality caused by *Enterococci* and other microbial infections, since this genetic element of vancomycin resistance can be easily transferred between the different bacterial species.

ACKNOWLEDGEMENTS

The expert assistance of the Department of Microbiology of *Hospital das Clínicas* laboratory staff, such as Maria do Carmo, Cristiane Vasconcelos and Lizie Franco, is gratefully acknowledged. We also would like to thank the *Centro de Tecnologias Estratégicas do Nordeste* (CETENE) for MALDI-TOF MS species identification and the *Núcleo de Plataformas Tecnológicas* (FIOCRUZ-PE) for all furtherance.

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AUTHORS' CONTRIBUTIONS

Igor Vasconcelos Rocha contributed to the conception of the study, data curation, analysis and interpretation of data, methodology, and drafting of the original manuscript. **Carlos Alberto das Neves de Andrade** contributed to the conception of the study, data curation, analysis and interpretation of data, and methodology. **Antônio Marcos Saraiva** contributed to data curation, analysis and interpretation of data, and methodology. **Erika Danielle Gameiro da Fonsêca** contributed to data curation, analysis and interpretation of data, methodology, and drafting of the manuscript. **Danilo Elias Xavier** contributed to project administration and writing. **Danielle Patrícia Cerqueira Macêdo** contributed to the conception of the study, project administration, and writing.

All authors approved the final version to be published and are responsible for all aspects of the work, including ensuring its accuracy and integrity.