Determination of Glycopeptide Resistance Genes and Virulence Factors in Vancomycin-Resistant Enterococci Isolates and the Relationship Between Glycopeptide Resistance Genes and Endogenous/Exogenous Flora

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ABSTRACT

Objective: This study aimed to identify glycopeptide resistance genes and virulence factors in vancomycin-resistant *Enterococcus* species and to investigate the influence of the microbiota and hospital environment on glycopeptide resistance.

Materials and Methods: A total of 107 enterococcal isolates were collected from patients' rectal swab cultures and environmental samples taken for surveillance purposes. Multiplex Polymerase Chain Reaction (PCR) analysis was conducted to investigate specific virulence genes (*esp, hyl, asa1, cyl,* and *galE*) and glycopeptide resistance genes (*vanA, vanB, van C1-C2, van D, vanE,* and *vanG*). Additionally, perirectal swab cultures were obtained from patients without vancomycin-resistant enterococcal colonization to investigate the presence of glycopeptide resistance genes in their microbiota.

Results: Seven isolates (6.5%) were identified as infectious agents. The most common vancomycin resistance genes were *vanA* (23.3%), followed by *vanA + vanB* (14%) and *vanB + vanD* (14%), respectively. The Random Amplified Polymorphic DNA Polymerase Chain Reaction (RAPD-PCR) method showed that patient surveillance and environmental isolates were clonally related. Moreover, microbiota analysis of patients without vancomycin-resistant enterococcal colonization revealed *Clostridium* spp. in two patients and *Lactobacillus* spp. in one patient, with the *vanG* gene found in the microbiota of only one (2.5%) patient.

Conclusion: The detection of genes responsible for dissemination indicates that colonized isolates also have the potential for infection, and the hospital environment plays a primary role in the acquisition of vancomycin-resistant enterococci.

Keywords: Vancomycin-resistant *Enterococcus*, multiplex PCR, glycopeptide, virulence, gene.

INTRODUCTION

Enterococci cause endogenous infections in humans, both in hospital and out-of-hospital environments, primarily because they are found in the gastrointestinal flora and are resistant to environmental conditions. Generally, most infections with enterococci are thought to be caused by the patient's own flora.[1] However, it is known that enterococcal infections can develop in hospitalized patients and those undergoing peritoneal or hemodialysis treatment. It is thought that the causative agent in such infections is often exogenous.[1]

Patients colonized with vancomycin-resistant enterococci (VRE) are asymptomatic and, therefore, might go undetected. The gastrointestinal tract is the main reservoir of *Enterococcus faecium* (*E. faecium*), which is the predominant species in the hospital environment. The intensive use of antibiotics in hospitalized patients with drug-resistant enterococci cause gastrointestinal colonization, leading to changes in the intestinal microbiota.[1] Therefore, in controlling VRE infections and preventing their spread, it is crucial to identify patients with VRE colonization in a timely manner, clarify their relationship with outbreaks of nosocomial infections, and determine virulence factors for colonization and infection.

Antibiotic-resistant strains of enterococci have been isolated from animal shelters, plants, soil, water, and human-produced products, including fermented foods and dairy products.[2] Antibiotic resistance determinants are found on mobile genetic elements like conjugative transposons and plasmids.[1,3] Recently, vancomycin resistance has also been found in intestinal flora bacteria such as the *Clostridium* genus, *Lactobacilli*, *Arcanobacterium haemolyticum*, *Corynebacterium* genus, and *Eggerthella lenta*, suggesting that the glycopeptide resistance gene may have been acquired from anaerobic bacteria.[4] Other neighboring species may have acquired these genes or developed new mechanisms to protect themselves from the protective effects of antibiotics to which they are exposed. In support of this theory, there is homology between antibiotic-producing bacteria and resistance determinants (such as *vanA* and *vanB*) found in other bacteria.

In the investigation of the hospital epidemiology of enterococci, classical phenotypic typing methods can be employed. However, these methods often fail to distinguish between different types of enterococci and have limited utility for epidemiological studies. Molecular typing is a more effective method in the investigation, prevention, and control of resistant enterococci.[5]

The aim of our study is to determine the virulence factors and resistance of enterococci to glycopeptide antibiotics, and to investigate the clonal relationship between VRE isolates using genotypic tests.

MATERIALS AND METHODS

Ethical Approval

The study received approval from the University's Ethics Committee in the session dated December 6, 2017, under Decision No: 16/2017-20. Informed consent was obtained from all participants.

Study Design and Data Collection

The study was conducted in Kahramanmaraş, located in the Mediterranean Region of southeastern Türkiye. Enterococcal isolates identified as VRE, isolated from samples routinely sent to a tertiary hospital's microbiology laboratory from various clinics between May 1, 2018, and December 31, 2019, were included in the study. Isolates not resistant to glycopeptides were excluded. The BD Phoenix 100 automated system was used for antibiotic resistance testing and species-level identification of isolates. The isolates were categorized into two groups: 7 as infectious agents and 100 as colonization.

For this study, perirectal swab samples were collected from a total of 40 patients hospitalized in the Pediatric Intensive Care Unit (PICU) and the Internal Medicine Intensive Care Unit (IMCU), who were scheduled to receive antibiotic treatment. Perirectal swab samples were taken simultaneously from these 40 patients, placed in 500 µl of deionized sterile distilled water, and stored at -20 ºC. Following deoxyribonucleic acid (DNA) extraction from these samples, glycopeptide resistance genes were investigated.

Perirectal swab samples were also obtained from three patients hospitalized in the PICU, known to be colonized/infected with VRE. Additionally, swab samples from the bedsheet, headboard, infusion pump, shelving, and curtain in the patients' room were collected. The clinical samples were transported to the microbiology laboratory in Stuart media. Samples from the hospital environment and those from intensive care patients were collected under the supervision of an infection control nurse and transported to the laboratory at +4 ºC promptly for analysis.

Isolation and Identification

For enterococcal isolation, swabs were inoculated onto 5% sheep blood agar (BD, USA) and chromogenic VRE agar (OR-BAK, Türkiye). The inoculated media were incubated for 24–48 hours at 37 °C under aerobic conditions. Furthermore, 40 rectal swab samples from patients in intensive care units were analyzed for the presence of *Clostridium* spp. and *Lactobacillus* spp., which are Gram-positive bacilli. These samples were inoculated on CDC Schaedler agar (Sanotema, Türkiye) and incubated under anaerobic conditions at 37 °C for 48–72 hours. After the anaerobic incubation period, positive colonies

were subcultured to perform aerotolerance testing to confirm whether the isolates were strictly anaerobic or facultatively aerobic bacteria. Colony morphology, Gram staining, oxidase, catalase tests, and other features were examined at the end of the incubation period.

The BD Phoenix 100 automated identification system (BD Diagnostic, USA) was used for the identification of *Enterococcus* spp. and antibiotic susceptibility testing. For the identification of anaerobic microorganisms, the BD BBL Crystal Anaerobic identification kit (BD, USA) was utilized. Isolates identified with both systems were determined at the species level. *Enterococcus faecalis* ATCC 29212 served as the quality control strain in the study.

Confirmation of Species Identification and Detection of Glycopeptide Resistance Genes by Molecular Method

The multiplex Polymerase Chain Reaction (mPCR) method, as described by Depardieu et al.,[6] was employed to confirm the enterococcal isolates identified by phenotypic methods and to detect glycopeptide resistance genes in these isolates. DNA extraction was performed using the single-cell lysing buffer method, as previously detailed by Neyaz et al.[7]

For glycopeptide resistance gene analysis, the PCR mixture was prepared as follows: 0.5 μL of 10 mM deoxynucleotide triphosphates (dNTP) mixture, 5 μl of 10X buffer (including 20 mM $MgCl_2$), 1 μL of each 10 μM primers for glycopeptide resistance genes (*vanA, vanB, vanC, vanD, vanE, vanG*), primers for the identification of *E. faecalis* and *E. faecium* (*ddl*), primers for the *S. aureus* thermonuclease gene (*nuc*), and primers for glycopeptide-sensitive *S. epidermidis* BM4577 (Se705-1 and Se705-2), 0.4 μL Taq DNA polymerase (DreamTaq DNA polymerase, 5U/μl, Thermo Fisher Scientific, Israel), and 5 μL of DNA, in a total volume of 50 μL. The DNA thermocycler (GeneAmp PCR System 9700, PE Applied Biosystems, USA) was programmed as follows: 3 minutes at 95 °C; 40 cycles of 30 seconds at 95 °C, 30 seconds at 54 °C, and 1 minute at 72 °C; followed by a final extension of 7 minutes at 72 °C. The amplified products were analyzed by gel electrophoresis in 2% agarose.

Analysis of Virulence Genes by Molecular Method

Molecular analysis of virulence genes was performed as previously described by Vankerckhoven et al.[8] A PCR mixture was prepared containing; 1 μL of 10 mM dNTP mix, 0.5 μL of *asa1*, *gelE*, and *hyl* primers at a 10 μM concentration, 1 μl of 10 μM *cylA* and *esp* primers, 5 μl of 10x buffer (including 20 mM $MgCl_2$), 0.25 μL Taq DNA polymerase (DreamTaq DNA Polymerase, 5U/μl, Thermo Fisher Scientific, Israel), and 5 μL DNA in a total volume of 50 μL. A DNA thermocycler

(GeneAmp PCR System 9700, PE Applied Biosystems, USA) was set as follows: 3 minutes at 95 °C; 40 cycles of 30 seconds at 95 °C, 30 seconds at 56 °C, and 1 minute at 72 °C; and a final extension of 7 minutes at 72 °C. Amplified products were evaluated by gel electrophoresis containing 2% agarose.

16 S Ribosomal Ribonucleic Acid (rRNA) Sequence Analysis

16 S rRNA sequence analysis was utilized for some isolates whose species could not be identified in mPCR analysis. BAK2 and BAK11 primers were used for both amplification and sequence analysis Bosshard et al.[9] Briefly, the PCR mixture consisted of five microliters of template DNA, 1.25 U of Taq DNA polymerase (DreamTaq DNA Polymerase, 5U/μl, Thermo Fisher Scientific, Israel), 10 mM of primers BAK11 (5'-AGTTTGATCMTGGCTCAG) and BAK2 (5'-GGACTACHAGGGTATCTAAT), and 10x buffer (including 20 mM $MgCl_2$). Forty cycles of 95 °C for 1 minute for denaturation, 48 °C for 1 minute for annealing, and 72 °C for 1 minute for extension, followed by a final extension at 72 °C for 10 minutes, were performed in the thermocycler (GeneAmp PCR System 9700, PE Applied Biosystems, USA). Nucleotide sequences were determined by the Sanger method and identified with the BLAST program (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch).

Molecular Typing

Randomly Amplified Polymorphic DNA Polymerase Chain Reaction (RAPD-PCR) and Enterobacterial Repetitive Intergenic Consensus Polymerase Chain Reaction (ERIC-PCR) methods were used for molecular typing of isolates obtained from various clinics, intensive care units, and environmental samples.[10,11] The RAPD-PCR method was utilized for genotyping *E. faecium* isolates obtained from the hospital environment and surveillance samples.

Clonal affinities of *E. faecium* and *E. faecalis* species were investigated using the ERIC-PCR method as previously described by Versalovic et al.[11]

Statistical Analysis

Categorical variables were presented as frequencies (n) and percentages (%). This study was conducted on independent groups. IBM Statistical Package for the Social Sciences version 22 (IBM SPSS for Windows, version 22, IBM Corporation, Armonk, NY, USA) was utilized for data evaluation.

RESULTS

Out of the 107 isolates used in the study, 90 (84.1%) were identified as *E. faecium*, 16 (14.9%) as *E. faecalis*, and 1 (0.9%) as *Enterococcus gallinarum* (*E. gallinarum*).

Table 1. Glycopeptide genes detected by multiplex PCR

Enterococcus spp.	Glycopeptide resistance gene							
	vanA	*vanB*	*vanD*	*vanA+vanB*	*vanA+vanD*	*vanB+vanD*	**Negative**	**Total**
E. faecium, n (%)	20 (22.2)	3 (3.33)	2 (2.22)	11 (12.22)	7 (7.77)	15 (16.66)	32 (35.55)	90 (100.00)
E. faecalis, n (%)	5 (31.25)	0 (0.00)	0 (0.00)	4 (25.00)	0 (0.00)	0 (0.00)	7 (43.75)	16 (100.00)
E. gallinarum, n (%)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (100.00)	1 (100.00)
Gene positivity	25 (23.33)	3 (3.33)	2 (2.22)	15 (14.02)	7 (6.50)	15 (14.02)	40 (37.30)	107 (100.00)
Infection (n=7)								
E. faecium, n (%)	3 (50.00)	0 (0.00)	0 (0.00)	1 (16.66)	0 (0.00)	2 (33.34)	0 (0.00)	6 (100.00)
E. faecalis, n (%)	0 (0.00)	0 (0.00)	0 (0.00)	1 (100.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (100.00)
E. gallinarum, n (%)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Colonization (n=100)								
E. faecium, n (%)	17[a] (18.40)	3 (3.26)	2 (2.17)	10 (10.86)	7 (7.60)	13 (14.13)	40 (43.47)	92 (100.00)
E. faecalis, n (%)	5 (62.50)	0 (0.00)	0 (0.00)	3 (37.50)	0 (0.00)	0 (0.00)	0 (0.00)	8 (100.00)
E. gallinarum, n (%)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)

PCR: Polymerase chain reaction; a: Environmental culture isolate.

From the culture of perirectal swab samples of 40 patients without VRE colonization, *Staphylococcus aureus* was isolated from one specimen. *Clostridium* spp. was isolated from two samples, and *Lactobacillus* sp. from one sample.

In the results of the cultural examination, *E. faecium* was isolated from all rectal swabs belonging to three patients hospitalized in the PICU. *E. faecium* was isolated from environmental swab samples (curtain, bedding, and bedside) of only one patient, while the other two patients' samples were found to be negative.

Confirmation of Species Identification and Detection of Glycopeptide Resistance Genes by Molecular Method

Glycopeptide resistance genes were detected in 67 (62.6%) of the 107 enterococcal isolates tested in this study, while 40 (37.3%) of the isolates were found to be negative for these genes. The Multiplex PCR method detected more than one glycopeptide resistance gene in some isolates. The glycopeptide resistance genes detected in enterococci, in order of frequency, were *vanA* (23.3%), *vanA* + *vanB* (14%), *vanB* + *vanD* (14%), *vanA* + *vanD* (6.5%), *vanB* (3.3%), and *vanD* (2.2%). The frequency of these genes in *E. faecium* isolates was 22.2% for *vanA*, 3.3% for *vanB*, 2.2% for *vanD*, 12.2% for *vanA* + *vanB*, 7.7% for *vanA* + *vanD*, 16.6% for *vanB* + *vanD*. In *E. faecalis* isolates, the frequencies were 31.25% for *vanA* and 25% for *vanA* + *vanB* (Table 1, Fig. 1).

The *vanG* gene (941 kb), isolated from one of the 40 patients without VRE colonization and analyzed in the perirectal swab, was negative for the glycopeptide resistance genes examined in the other samples (Fig. 2).
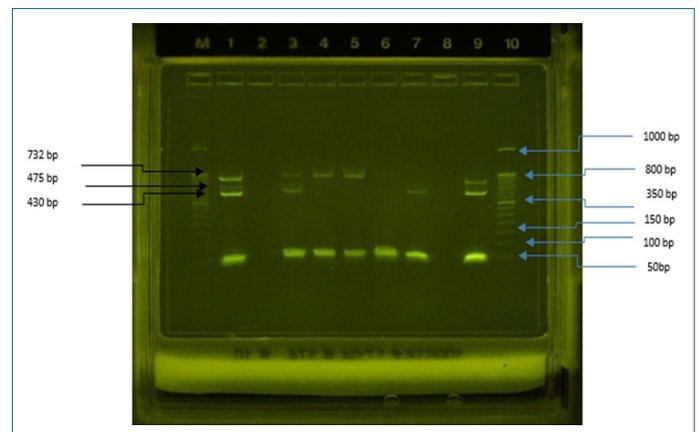

Figure 1. 2% agarose gel image displaying amplification products of glycopeptide gene fragments in VRE isolates. M: marker (Invitrogen 50 bp DNA ladder), 1: *E. faecalis* (475 bp), *vanA* (732 bp), and *vanE* (430 bp), 2: negative control, 3: *E. faecalis* with *vanA*, 4: *vanA*, 5: *vanA*, 6: negative control, 7: *E. faecalis*, 8: negative control, 9: *E. faecalis* with *vanA*, 10: marker.

Analysis of Virulence Genes by Molecular Method

The virulence genes investigated in this study were detected only in *E. faecium* isolates from the infection group, while in the colonization group, they were detected in both *E. faecium* and *E. faecalis* isolates. Among the *E. faecium* isolates from the infection group, 4 (66.6%) were found to have the *hyl* gene, 1 (16.6%) had *hyl* + *esp*, and 1 (16.6%) had *hyl* + *cylA* virulence genes. In the colonization group of *E. faecium* isolates, *hyl* was detected in 19

Table 2. Distribution of virulence genes detected in the infection and colonization groups

Gene	Enterococcus spp. (n=107)		Gene positivity (n=107)	Infection (n=7)		Colonization (n=100)**	
	E. faecium	E. faecalis		E. faecium	E. faecalis	E. faecium	E. faecalis
hyl, n (%)	23 (25.55)	3 (18.75)	26 (24.53)	4 (66.67)*	0 (0.00)	19 (22.62)	3 (20.00)
esp, n (%)	11 (12.22)	0 (0.00)	11 (10.38)	0 (0.00)	0 (0.00)	11 (13.09)	0 (0.00)
cylA, n (%)	2 (2.22)	1 (6.25)	3 (2.83)	0 (0.00)	0 (0.00)	2 (2.38)	1 (6.67)
asa1, n (%)	1 (1.11)	0 (0.00)	1 (0.94)	0 (0.00)	0 (0.00)	1 (1.19)	0 (0.00)
gelE, n (%)	1 (1.11)	0 (0.00)	1 (0.94)	0 (0.00)	0 (0.00)	1 (1.19)	0 (0.00)
hyl+esp, n (%)	13 (14.44)	3 (18.75)	16 (15.09)	1 (16.66)	0 (0.00)	12 (14.29)*	0 (0.00)
hyl+asa1, n (%)	4 (4.44)	1 (6.25)	5 (4.72)	0 (0.00)	0 (0.00)	4 (4.76)	1 (6.67)
hyl+cylA, n (%)	3 (3.33)	0 (0.00)	3 (2.83)	1 (16.66)	0 (0.00)	2 (2.38)	0 (0.00)
asa1+cylA, n (%)	2 (2.22)	2 (12.50)	4 (3.77)	0 (0.00)	0 (0.00)	2 (2.38)	2 (13.33)
asa1+hyl+esp, n (%)	1 (1.11)	2 (12.50)	3 (2.83)	0 (0.00)	0 (0.00)	1 (1.19)	2 (13.33)
asa1+hyl+cylA, n (%)	1 (1.11)	0 (0.00)	1 (0.94)	0 (0.00)	0 (0.00)	1 (1.19)	0 (0.00)
Negative, n (%)	28 (31.11)	4 (25.00)	32 (30.19)	0 (0.00)	1 (100.00)	28 (33.33)	6 (40.00)
Total	90 (100.00)	16 (100.00)	106 (100.00)	6 (100.00)	1 (100.00)	84 (100.00)	15 (100.00)

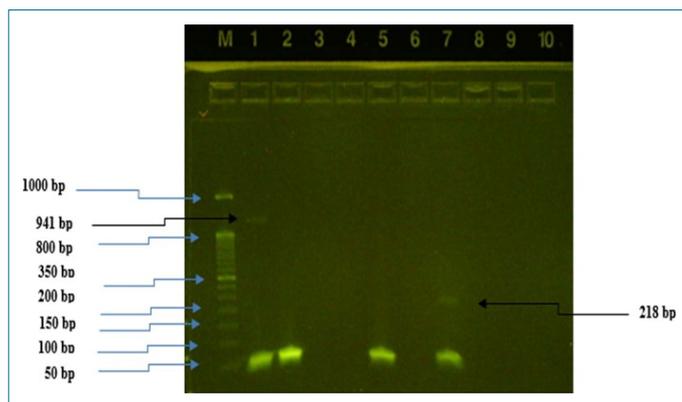*: Environmental culture isolate; **: Negative for E. gallinarum virulence gene.

Figure 2. Samples from patients without VRE colonization. M: marker (Invitrogen 50 bp DNA ladder) 1: vanG (941 bp), 7: S. aureus (218 bp).

(22.6%), hyl + esp in 12 (14.2), esp in 11 (13.0%), hyl + asa1 in 4 (4.7%), hyl + cylA in 2 (2.3%), and asa1 + cylA in 2 (2.3%).

In the colonization group of E. faecalis isolates, hyl was found in 3 (20%), asa1 + cylA in 2 (13.3%), asa1 + hyl + esp in 2 (13.3%), cylA in 1 (1%), and hyl + asa1 in 1 (6.6%) (Table 2).

According to this, the prevalence of hyl and hyl + esp virulence genes was found to be notably high in isolates carrying the vanA gene (Table 3).

It was observed that 3 surveillance culture isolates, isolated from the same clinic using the RAPD method, and the environment culture isolates were clonally close (Fig. 3). Clonal affinities were investigated in 82 E. faecium isolates and 14 E. faecalis isolates.

As a result of the ERIC-PCR analysis, the similarity matrix obtained by Unweighted Pair Group Method with Arithmetic Mean (UPGMA) analysis in the GelCompar-II program and the dendrogram analysis based on this revealed that E. faecalis and E. faecium isolates essentially formed two large groups. E. faecium isolates displayed a heterogeneous distribution with 3 different clusters and 34 different band profiles, while E. faecalis isolates showed a heterogeneous distribution with 4 different clusters and 9 different band profiles (Fig. 4, 5).

DISCUSSION

Despite guidelines aimed at preventing contamination and the spread of antibiotic resistance in hospitals, the rise in the incidence of nosocomial infections, especially those caused by E. faecium, has not been halted.[1] In this study, 90 of the 107 VRE isolates were identified as E. faecium by both phenotypic and molecular methods, aligning with the literature. In a study conducted in Türkiye, VRE was isolated in 76 (36.1%) of 210 intensive care patients.[12] VRE isolates, which exhibit both intrinsic and acquired resistance to many antibiotics, present limited treatment options. They can easily transfer resistance, especially to glycopeptide group antibiotics, to other bacteria

Table 3. Comparison of glycopeptide resistance genes and virulence genes of *Enterococcus* spp. isolates

Virulence gene	Glycopeptide resistance gene							
	vanA	*vanB*	*vanA+vanB*	*vanD*	*vanA+vanD*	*vanB+vanD*	Negative	Total
asa1, n (%)	0 (0.00)	1 (33.30)	0 (0.00)	0 (0.00)	1 (12.50)	0 (0.00)	0 (0.00)	1 (1.00)
cylA, n (%)	1 (4.00)	0 (0.00)	1 (7.10)	0 (0.00)	1 (12.50)	0 (0.00)	0 (0.00)	3 (3.00)
esp, n (%)	4 (16.00)	1 (33.30)	1 (7.10)	0 (0.00)	0 (0.00)	3 (18.75)	2 (5.70)	11 (14.80)
gelE, n (%)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (12.50)	0 (0.00)	0 (0.00)	1 (1.00)
hyl, n (%)	6 (24.00)	1 (33.30)	6 (40.00)	0 (0.00)	2 (25.00)	4 (25.00)	8 (22.90)	24 (32.40)
asa1+hyl+esp, n (%)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (2.70)	3 (4.00)
asa1+hyl, n (%)	0 (0.00)	0 (0.00)	1 (6.60)	0 (0.00)	0 (0.00)	3 (18.75)	1 (2.70)	5 (6.70)
asa1+cylA, n (%)	1 (4.00)	0 (0.00)	0 (0.00)	1 (50.00)	0 (0.00)	0 (0.00)	1 (2.70)	3 (4.00)
hyl+esp, n (%)	6 (25.00)	0 (0.00)	4 (28.60)	0 (0.00)	1 (12.50)	2 (12.50)	3 (8.60)	16 (15.80)
hyl+asa1+cylA, n (%)	1 (4.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (1.00)
hyl+cylA, n (%)	1 (4.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (12.50)	1 (6.25)	0 (0.00)	3 (3.00)
Negative, n (%)	5 (20.0)	0 (0.00)	1 (7.10)	1 (50.00)	1 (12.50)	3 (18.75)	19 (54.30)	30 (29.70)
Total, n (%)	25 (100.00)	3 (100.00)	14 (100.00)	2 (100.00)	8 (100.00)	16 (100.00)	35 (100.00)	101 (100.00)

through mobile genetic elements and exhibit asymptomatic, persistent colonization in the microbiota, leading to endogenous infections.[3] In a study conducted by Bhatt et al.,[13] glycopeptide resistance genes in VRE isolates were investigated by mPCR, and the *vanA* gene was detected in all 14 VRE isolates examined. In our study, the presence of glycopeptide resistance genes in isolates defined as VRE was found to be 63.5% (67/107).

Transferable genetic elements and chromosomal mutations have been largely identified as mechanisms responsible for resistance to glycopeptide group antibiotics seen in enterococci.[3] It was found for the first time that approximately 25% of the genome of the *E. faecalis* V583 strain, exhibiting the *vanB* phenotype, consists of DNA sequences acquired as mobile or exogenous genes.[14] Indeed, Ballard et al.[15] reported isolating the *vanB* operon from flora bacteria such as *Clostridium* spp. and *Ruminococcus*. Additionally, the *vanG* gene has been identified in *Clostridium argentinense*.[16] Similarly, a study conducted by Lu et al.[17] failed to detect resistance genes by mPCR in vancomycin-resistant *Leuconostoc* and *Pediococcus* isolates.

In our study, anaerobic flora bacteria were isolated at a rate of 7.5% (3/40). *Clostridium* spp. was isolated from two patients, *Lactobacillus* spp. from one patient, and the *vanG* gene was detected in only one patient's sample. The failure to detect resistance genes in gastrointestinal samples with dense microbial flora may be attributed to the absence of risk factors that predispose to resistance gene expression, such as the presence of inhibitors or the use of antibiotics. In a study conducted in two different hospitals with high vancomycin
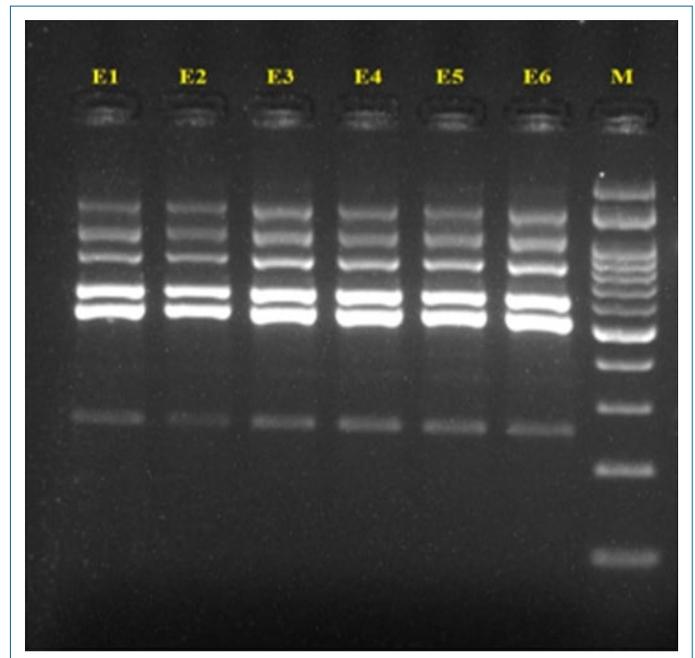

Figure 3. 1.5% agarose gel image of RAPD-PCR profiles of *E. faecium* isolates, amplified with Primer 1254. E1: Patient 1 (895504), E2: Patient 2 (5558724), E3: Patient 3 (37943), E4: Patient 3 (duvet cover), E5: Patient 3 (bedside), E6: Patient 3 (curtain), M: Marker, 100 bp (PLUS DNA Ladder).

resistance, of 248 rectal swab samples, *vanA* genes were detected in 23 (9.8%), *vanB* genes in 16 (6.5%), *vanD* genes
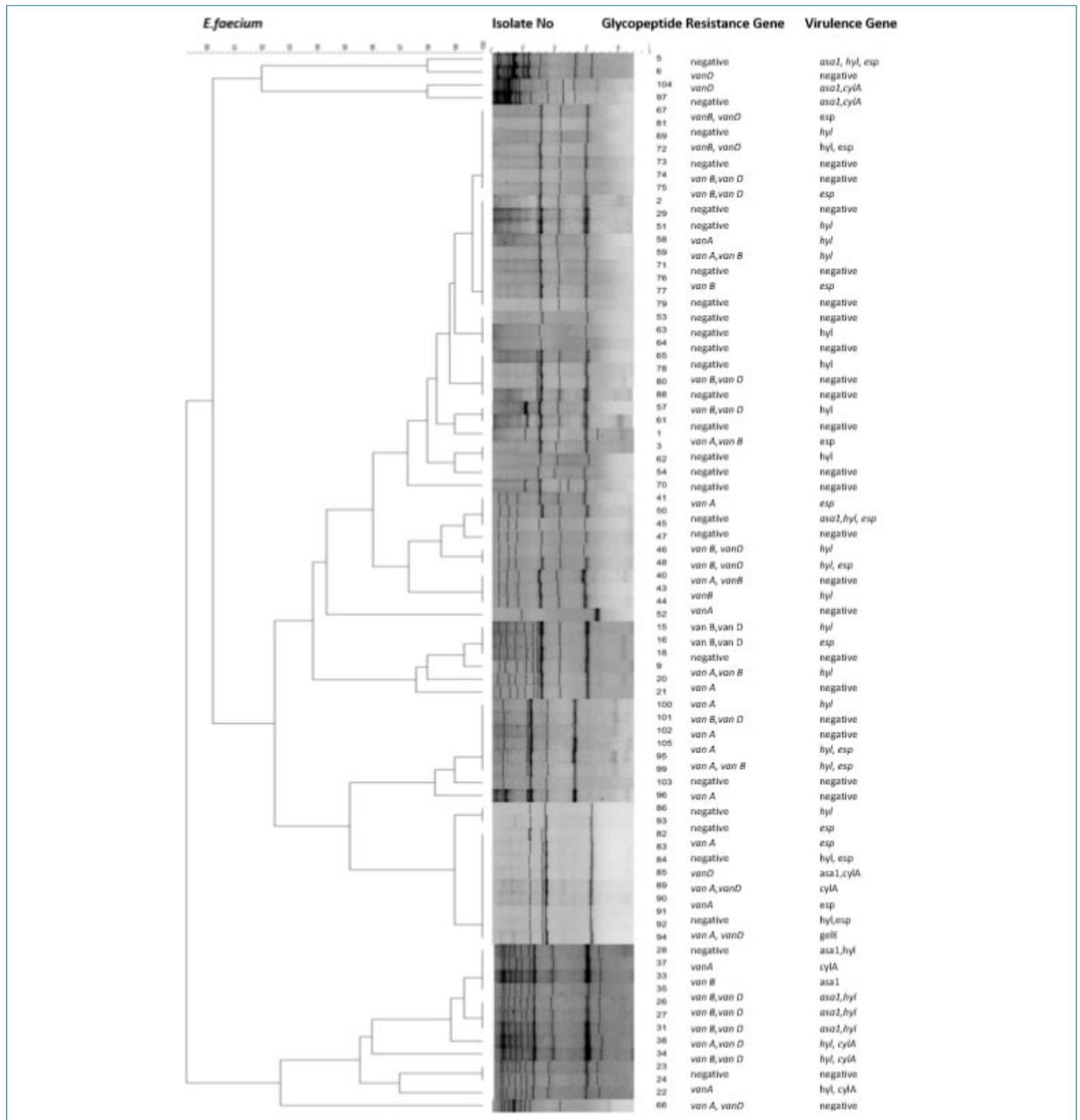
Figure 4. ERIC-PCR dendrogram of *E. faecium* isolates, highlighting the distribution of glycopeptide resistance genes and virulence genes. It was determined that *E. faecium* isolates formed 2 groups, 3 different clusters, and exhibited 34 different band profiles. According to the dendrogram analysis results, the isolates were observed to be at least 90% similar to each other. A total of 64 isolates were found to be 100% similar, grouped as follows: (67-81-69-72-73-74-75); (2-29-51-58-59-71-76-77-79); (53-63-64); (65-78-80-88); (57-61); (3-62); (41-50-45); (46-48); (40-43-44); (15-16-18); (100-101-102-105-95-99); (86-93); (82-83-84-85-89-90-91-92-94); (28-37-33-35); (26-27-31); and (23-24).
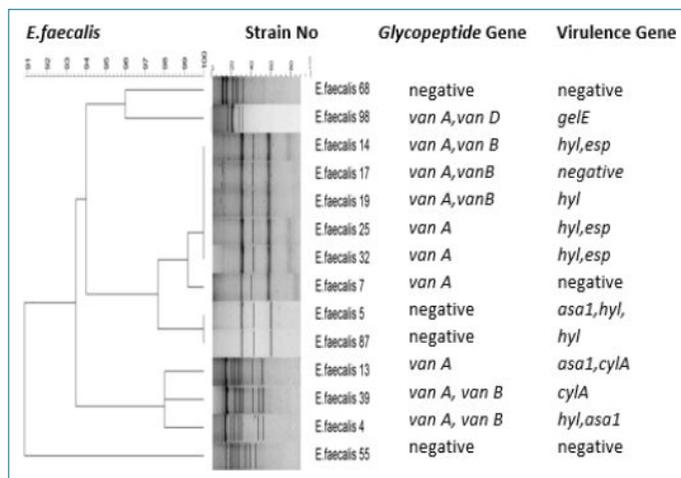
Figure 5. ERIC-PCR dendrogram of *E. faecalis* isolates. Among the *E. faecalis* isolates, 2 groups, 4 different clusters, and 9 different band profiles were observed. The dendrogram analysis results indicated that the isolates showed a minimum of 91% similarity to each other. Furthermore, 7 isolates (14-17-19-25-32 and 5-87) demonstrated 100% similarity.

in 94 (37.9%), and *vanG* genes in 23 (9.3%). In this study, *Clostridium* spp. containing *vanB2* and *vanD1* were isolated from a rectal swab sample containing *vanB*. Additionally, both *vanA* and *vanB* genes were detected in 2 rectal swab samples, and *vanA* and *vanD* genes were detected together in 4 samples.[18] In our study, the genes found in enterococci isolated from nosocomial surveillance cultures were, in order of frequency, *vanA* (23.3%), *vanA* + *vanB* (14%), *vanB* + *vanD* (14%), *vanA* + *vanD* (6.5%), *vanB* (3%), and *vanD* (2.2%).

In studies investigating virulence factors in *E. faecium* isolates, *esp* and *hyl* are commonly identified. These genes were found to be significantly more prevalent in ampicillin-resistant clinical isolates compared to fecal samples.[8,19] In a study categorizing VRE as either related to infection or colonization, the *esp* gene emerged as the most frequently identified virulence gene. Furthermore, no significant correlation was observed between virulence genes and the infection/colonization groups.[20] It has been demonstrated that some virulence and resistance genes can spread among species within the gastrointestinal flora and hospital environments.[3,20] The identification of the gene responsible for this spread in both infection and colonization isolates indicates that colonizing isolates also possess the potential to cause infection. Among the studied virulence genes, *hyl* was the most frequently isolated gene in both categories. In our study, *hyl* was identified as the most prevalent virulence factor. Gök et al.[21] explored the relationship between antibiotic resistance and virulence factors in *E. faecalis* and *E. faecium* isolates, finding that the *efa* gene was the most common,

present in 92.7% of isolates from both species. The *hyl* gene was detected in 36.7% of *E. faecalis* species and 54.1% of *E. faecium* isolates. In our study, *E. faecium* isolates from the PICU were found to be molecularly similar. Likewise, a study conducted in Istanbul revealed that the *vanA* gene was present in all analyzed VRE isolates, with multiple clones identified using the RAPD-PCR method.[22] Moreover, virulence genes were identified in enterococci used as starters in milk and dairy products using the RAPD-PCR method, indicating their spread from clinical isolates through conjugation.[23] These findings suggest that there may be numerous sources for the transmission of antibiotic resistance genes among bacteria, particularly enterococci.

There are some limitations in this study. Due to the large number of cells containing the value "zero" in the cross-tables, hypothesis testing could not be performed using the chi-square test or Fisher's exact test. Consequently, the tables are presented descriptively, indicating rates (%) and frequencies (n).

CONCLUSION

In our study, *E. faecium* isolates were found to belong to two groups and three different clusters, while *E. faecalis* isolates were categorized into two groups and four different clusters. This classification underscores the presence of diverse origins within a hospital setting, as well as the occurrence of certain clinical sources sharing the same origins.

We deduced that colonizing isolates also possess the potential to cause infections, and the hospital flora plays a pivotal role in the acquisition of vancomycin-resistant enterococci. Furthermore, it was observed that patients without vancomycin-resistant enterococcal colonization may still harbor glycopeptide resistance genes within their gastrointestinal microbiota.

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