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Phenotypic Characterization and Antimicrobial Susceptibility Profile of Vancomycin-Resistant Enterococci Isolated from Clinical Specimens in a Tertiary Care Hospital

Rounak Chahal[1] Dr. Kailash Jatav[2]

[1]PhD Scholar, Department of Microbiology, Index Medical College, Hospital & Research Centre, Indore, (M.P) India (Malwanchal University)
[2]Associate Professor, Department of Microbiology, Index Medical College, Hospital & Research Centre, Indore (M.P)India. (Malwanchal University)

Abstract

Background: Vancomycin-resistant enterococci (VRE) are important healthcare-associated pathogens because of their limited therapeutic options and potential for multidrug resistance. Phenotypic characterization and antimicrobial susceptibility testing are essential for timely detection and infection-control surveillance.

Aim: To phenotypically characterize *Enterococcus* isolates recovered from clinical specimens and determine the antimicrobial susceptibility profile of VRE in a tertiary care hospital.

Methods: An observational cross-sectional study was conducted in the Department of Medical Microbiology, Index Medical College Hospital and Research Centre, Indore, from January 2023 to July 2026. A total of 400 clinically significant clinical specimens suspected of *Enterococcus* infection were evaluated. Pure, clinically significant *Enterococcus* isolates were identified by conventional phenotypic methods. Antimicrobial susceptibility testing was performed by the Kirby–Bauer disc diffusion method according to applicable CLSI guidelines. Vancomycin resistance was screened using vancomycin screen agar containing 6 µg/mL vancomycin.

Results: Fifty-eight *Enterococcus* isolates were recovered, predominantly from urine (34; 58.6%), followed by blood (9; 15.5%). *E. faecalis* was the predominant species (31; 53.4%), followed by *E. faecium* (25; 43.1%). Seven (12.1%) isolates were identified as VRE. VRE positivity was higher among *E. faecium* (20.0%) than *E. faecalis* (6.5%). Among VRE isolates, resistance was highest to erythromycin and vancomycin (100% each), followed by ampicillin and high-level gentamicin (85.7% each). Teicoplanin resistance was observed in 42.9%, whereas all isolates remained susceptible to linezolid.

Conclusion: VRE constituted a significant proportion of clinical *Enterococcus* isolates, with greater resistance observed among *E. faecium*. Continuous surveillance, antimicrobial stewardship, and appropriate infection-control measures are essential for limiting VRE transmission.

Keywords: *Enterococcus*; VRE; vancomycin resistance; antimicrobial susceptibility; *E. faecium*; *E. faecalis*; multidrug resistance; tertiary care hospital.

Introduction

Enterococci are important Gram-positive opportunistic pathogens and major causes of healthcare-associated infections, particularly urinary tract, and bloodstream and wound infections. The most clinically important species are *Enterococcus faecalis* and *Enterococcus faecium*. Their ability to acquire antimicrobial resistance has made enterococcal infections an important therapeutic and infection-control challenge [1,2].

Vancomycin-resistant enterococci (VRE) are of particular concern because treatment options for serious infections are limited. A systematic review of Indian studies reported a pooled VRE prevalence of 12.4%, with *E. faecium* being the predominant VRE species [3,4]. Indian studies have also demonstrated resistance to multiple commonly used antibiotics, including ampicillin, erythromycin, ciprofloxacin and high-level gentamicin [5]. Phenotypic characterization includes species identification, vancomycin screening, antimicrobial susceptibility testing and determination of vancomycin minimum inhibitory concentration (MIC) [6]. Accurate detection is essential because VRE may show multidrug resistance and can contribute to hospital transmission. Therefore, the present study was undertaken to characterize VRE phenotypically and determine their antimicrobial susceptibility profile among clinical isolates from a tertiary care hospital [7,8].

Phenotypic characterization of VRE is therefore essential for early laboratory detection and infection-control surveillance. Conventional identification includes colony morphology, Gram staining, catalase testing, bile-esculin reaction, PYR testing, and species-level biochemical identification [9]. Antimicrobial susceptibility testing can be performed using standardized methods such as disk diffusion and minimum inhibitory concentration (MIC) determination. Current CLSI recommendations provide standardized antimicrobial susceptibility breakpoints and quality-control procedures to improve the reliability of susceptibility reporting [10].

Material and method

An observational cross-sectional study was conducted in the Department of Medical Microbiology, Index Medical College Hospital and Research Centre, Malwanchal University, Indore, Madhya Pradesh, from January 2023 to July 2026. A total of 58 (14%) clinically significant *Enterococcus* isolates recovered from 400 clinically suspected specimens**,** were isolated from **,**urine, blood, pus/wound swabs, cerebrospinal fluid, body fluids, and other clinical specimens were included. Only the first pure, clinically significant isolate from each patient was considered. Duplicate isolates, contaminated specimens, mixed cultures and environmental isolates were excluded.

Clinical specimens were collected aseptically, transported promptly, and cultured on appropriate media. Urine samples were inoculated on CLED agar using a calibrated loop, while other specimens were cultured on 5% sheep blood agar, MacConkey agar, and Bile Esculin Azide agar. Blood samples were inoculated into Brain Heart Infusion broth and incubated at 37°C for up to 5 days, with subculturing when growth was detected. Plates were incubated at 37°C for 18–48 hours. *Enterococcus* spp. were identified by colony morphology, Gram staining, catalase test, bile esculin hydrolysis, growth in 6.5% NaCl, and PYR test.

Antimicrobial susceptibility testing was performed using the Kirby–Bauer disc diffusion method on Mueller–Hinton agar with a 0.5 McFarland bacterial suspension, in accordance with applicable CLSI M100 guidelines. The isolates were tested against ampicillin, erythromycin, high-level gentamicin, teicoplanin, linezolid, and vancomycin. Inhibition zones were measured and interpreted according to the recommended CLSI breakpoints. Appropriate quality-control strains were included.

Vancomycin resistance was screened using vancomycin screen agar containing 6 µg/mL vancomycin. Isolates showing growth on the screening agar or a resistant phenotype to vancomycin were considered phenotypically suspected vancomycin-resistant *Enterococcus* (VRE).

Results

A total of 58 Enterococcus isolates were recovered from clinical specimens. Urine was the most common source, accounting for 34 (58.6%) isolates, followed by blood 9 (15.5%), pus/wound swabs 8 (13.8%), body fluids 5 (8.6%), and CSF 2 (3.4%). Among the isolates, *E. faecalis* was predominant (31, 53.4%), followed by *E. faecium* (25, 43.1%), *E. gallinarum* (1, 1.7%), and *E. casseliflavus* (1, 1.7%). Overall, 7 (12.1%) isolates were identified as VRE. VRE were more frequent among *E. faecium* (5/25, 20.0%) than *E. faecalis* (2/31, 6.5%). Specimen-wise, VRE were most frequently detected in blood (2/9, 22.2%), followed by body fluids (1/5, 20.0%), pus/wound swabs (1/8, 12.5%), and urine (3/34, 8.8%). No VRE were detected in CSF. VRE positivity was comparable between males (4/34, 11.8%) and females (3/24, 12.5%). The highest VRE positivity was observed among patients aged >60 years (3/13, 23.1%), followed by those aged 51–60 years (2/10, 20.0%). Among the 7 VRE isolates, resistance was highest to erythromycin and vancomycin (7, 100.0% each), followed by ampicillin and high-level gentamicin (6, 85.7% each). Teicoplanin resistance was observed in 3 (42.9%) isolates, while all VRE isolates remained susceptible to linezolid.

Table 1. Distribution of Enterococcus Isolates According to Clinical Specimen

Clinical specimen	Number of *Enterococcus* isolates (n)	Percentage (%)
Urine	34	58.6
Blood	9	15.5
Pus/Wound swab	8	13.8
Cerebrospinal fluid	2	3.4
Body fluids	5	8.6
Total	**58**	**100.0**

Table 2. Species-wise Distribution of Enterococcus Isolates

Enterococcus species	Number (n)	Percentage (%)
Enterococcus faecalis	31	53.4
Enterococcus faecium	25	43.1
Enterococcus gallinarum	1	1.7
Enterococcus casseliflavus	1	1.7

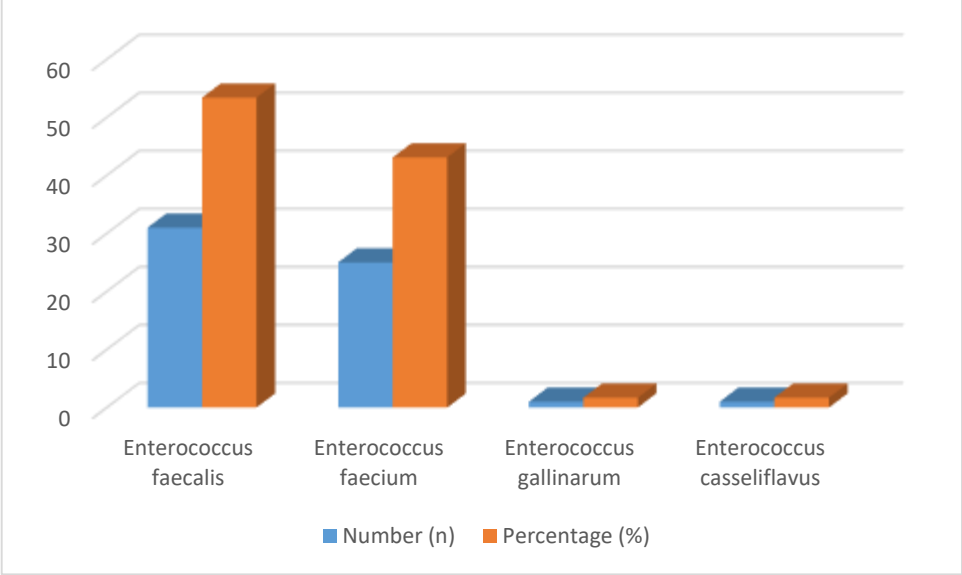

Figure 1- Species-wise Distribution of Enterococcus Isolates

Table 3. Species-wise Distribution of VRE

Enterococcus species	Total isolates	VRE positive n (%)	VRE negative n (%)
E. faecalis	31	2 (6.5)	29 (93.5)
E. faecium	25	5 (20.0)	20 (80.0)
E. gallinarum	1	0 (0.0)	1 (100.0)
E. casseliflavus	1	0 (0.0)	1 (100.0)
Total	**58**	**7 (12.1)**	**51 (87.9)**

Table 4. Specimen-wise Distribution of VRE

Clinical specimen	Enterococcus isolates	VRE positive n (%)	VRE negative n (%)
Urine	34	3 (8.8)	31 (91.2)
Blood	9	2 (22.2)	7 (77.8)
Pus/Wound swab	8	1 (12.5)	7 (87.5)
Cerebrospinal fluid	2	0 (0.0)	2 (100.0)
Body fluids	5	1 (20.0)	4 (80.0)
Total	**58**	**7 (12.1)**	**51 (87.9)**

Table 5. Sex-wise Distribution of VRE

Sex	Enterococcus isolates	VRE positive n (%)	VRE negative n (%)
Male	34	4 (11.8)	30 (88.2)
Female	24	3 (12.5)	21 (87.5)
Total	**58**	**7 (12.1)**	**51 (87.9)**

Table 6. Age-wise Distribution of VRE

Age group	Enterococcus isolates	VRE positive n (%)	VRE negative n (%)
≤18 years	5	0 (0.0)	5 (100.0)

19–30 years	8	0 (0.0)	8 (100.0)
31–40 years	10	1 (10.0)	9 (90.0)
41–50 years	12	1 (8.3)	11 (91.7)
51–60 years	10	2 (20.0)	8 (80.0)
>60 years	13	3 (23.1)	10 (76.9)
Total	**58**	**7 (12.1)**	**51 (87.9)**

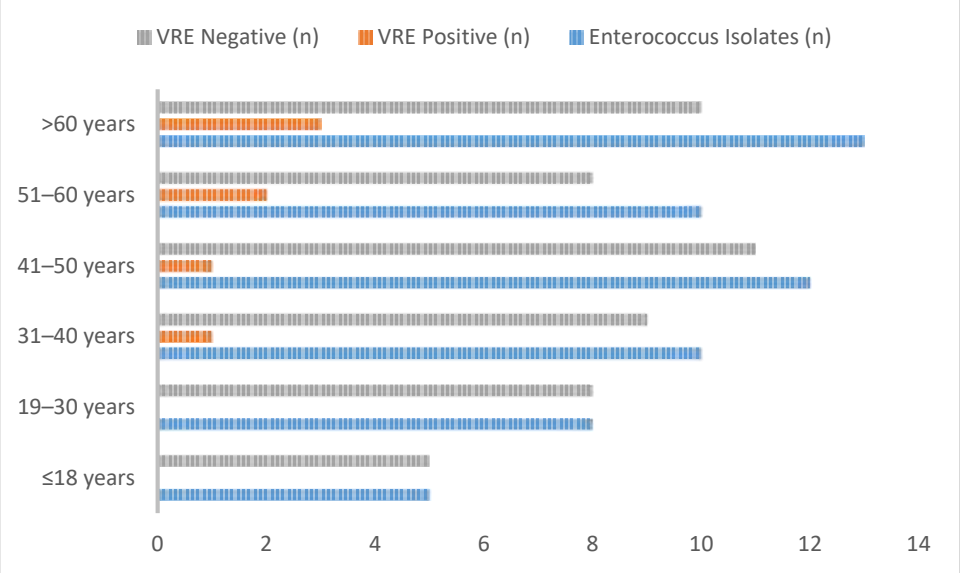

Figure 2- Age-wise Distribution of VRE

Table 7. Antimicrobial Resistance Pattern among VRE Isolates

Antimicrobial agent	Resistant VRE isolates n (%)
Ampicillin	6 (85.7)
Erythromycin	7 (100.0)
High-level Gentamicin	6 (85.7)
Teicoplanin	3 (42.9)
Linezolid	0 (0.0)
Vancomycin	7 (100.0)

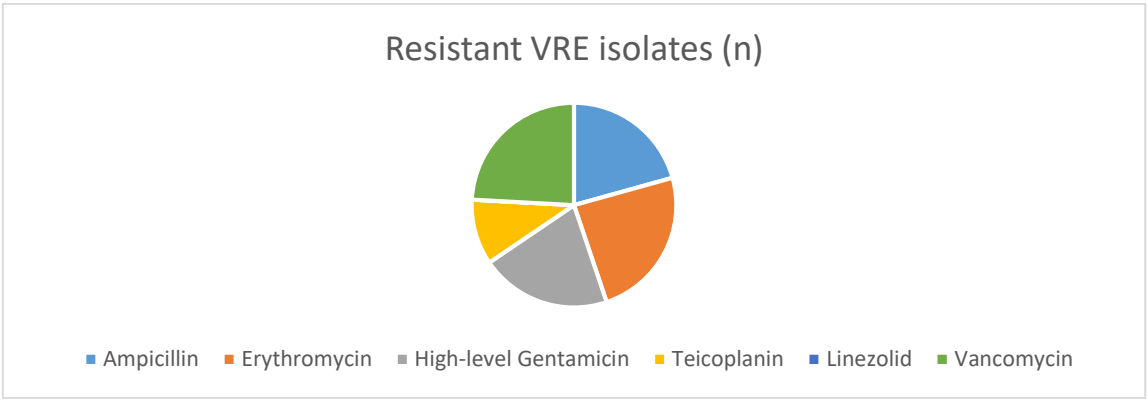

Figure 3. Antimicrobial Resistance Pattern among VRE Isolates

Discussion

In the present study, 58 Enterococcus isolates were recovered from clinical specimens, with urine being the predominant source (58.6%). This finding is consistent with Meena et al. (2017) [11], who studied urinary Enterococcus isolates and reported a substantial occurrence of VRE among patients with urinary tract infections. Similarly, Sreeja et al. (2012) [12] reported Enterococcus from urine, blood, pus and other clinical specimens, supporting the broad clinical distribution observed in the present study. Purohit et al. (2017) [13] also recovered VRE from blood, pus and urine specimens among hospitalized patients.

In the present study, *E. faecalis* was the predominant species (53.4%), followed by *E. faecium* (43.1%). A similar predominance of *E. faecalis* was reported by Sreeja et al. (2012) [12] Shinde et al. (2012) [14] also reported *E. faecalis* as the predominant species among their clinical Enterococcus isolates. However, *E. faecium* showed greater vancomycin resistance in the present study, which is consistent with the recognized association of *E. faecium* with acquired glycopeptide resistance.

The overall VRE prevalence in the present study was 12.1%. This was comparable to the pooled Indian prevalence of 12.4% reported by Smout et al. (2023) [16]. Considerable variation has been reported in individual Indian studies. Praharaj et al. (2013) [17] reported VRE in 8.72% of Enterococcus isolates, while Purohit et al. (2017) [13] reported a higher prevalence of 22.8%.Meena et al. (2017) [11] reported an even higher prevalence of 37.14% among urinary isolates. In contrast, Sami et al. (2020) [18] reported a lower prevalence of 3.06%.These differences may be explained by variations in study population, specimen type, antimicrobial exposure, hospital setting, infection-control practices and laboratory methods.

In the present study, *E. faecium* showed a higher VRE positivity rate (20.0%) compared with *E. faecalis* (6.5%). This finding is consistent with the observations of Purohit et al. (2017)[13] who demonstrated an important role of *E. faecium* in VRE among hospitalized patients. Phukan et al. (2016) [19] also demonstrated considerable vancomycin resistance among clinical enterococci, although their distribution of resistant isolates differed from the present study.Such differences may reflect local epidemiological variations.

Urine was the most common source of VRE in the present study. Meena et al. (2017) [11] similarly reported VRE predominantly among urinary isolates. Taneja et al. (2004) [20] also emphasized the importance of VRE in urinary clinical specimens. In the present study, blood showed the highest specimen-specific proportion of VRE, although the number of blood isolates was relatively small. These findings indicate that both urinary and bloodstream infections require attention during VRE surveillance.

VRE positivity was comparable between males and females in the present study. The higher positivity observed among patients aged over 60 years may be related to increased healthcare exposure, comorbid conditions, previous antimicrobial use and prolonged hospitalization. Purohit et al. (2017) [13] similarly highlighted hospitalized and high-risk patients as important groups for VRE acquisition and colonization.

Teicoplanin resistance was observed in 42.9% of VRE isolates. Praharaj et al. (2013) [17] reported variation in vancomycin and teicoplanin susceptibility among VRE isolates and emphasized the importance of determining the glycopeptide resistance phenotype. Gupta et al. (2015) [23] also demonstrated different glycopeptide resistance phenotypes among VRE isolates, including isolates showing resistance to both vancomycin and teicoplanin. Therefore, testing of both glycopeptide agents is important for appropriate phenotypic characterization.

Conclusion

The study demonstrates a significant burden of vancomycin-resistant enterococci with greater resistance among *E. faecium* and considerable multidrug resistance. Linezolid remained an effective therapeutic

option. Continuous surveillance, antimicrobial stewardship, and strict infection-control practices are essential to control VRE transmission.

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