Journal of Cardiovascular Disease Research

ISSN: 0975-3583,0976-2833 VOL 16, ISSUE 7, 2025

PREVALENCE OF VANCOMYCIN-RESISTANT ENTEROCOCCUS AND HIGH-LEVEL GENTAMICIN RESISTANCE AMONG ENTEROCOCCUS ISOLATES IN TERTIARY CARE HOSPITAL

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Abstract

Enterococcus faecalis and *Enterococcus faecium* are key nosocomial pathogens, increasingly associated with vancomycin resistance and high-level gentamicin resistance (HLGR). This study investigated the prevalence and molecular characteristics of VRE and HLGR among clinical isolates. A prospective study was conducted from January to December 2024, including 320 Enterococcus isolates from various clinical samples. Samples were processed using standard microbiological techniques. Species identification was done by various biochemical tests. Antibiotic susceptibility was assessed via the Kirby-Bauer disc diffusion method per CLSI M100 (2024). HLGR was screened using gentamicin (120 µg); MIC ≥500 µg/mL confirmed resistance. VRE screening used vancomycin disc diffusion and CHROMagar; vanA/vanB genes were detected via RT-PCR. The results showed that out of 320 Enterococcus isolates, *E. faecalis* accounted for 266 (83.1%) and *E. faecium* for 54 (16.9%). Vancomycin-resistant enterococci (VRE) were identified in 54 isolates (16.8%), comprising 43 *E. faecalis* and 11 *E. faecium*. High-level gentamicin resistance (HLGR) was observed in 153 isolates (47.8%). Molecular

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characterization revealed the presence of the vanA gene in 94.4% and vanB in 5.6% of VRE isolates. A high level of resistance was noted against ampicillin, ciprofloxacin, erythromycin, and nitrofurantoin, while all isolates remained susceptible to linezolid. The majority of VRE and HLGR cases were found in patients aged 41–60 years, with a female preponderance and higher occurrence in urban populations. Urine was the most common specimen source for both VRE (53.7%) and HLGR (49%) isolates. The dominance of *vanA* and high HLGR prevalence underscores the need for molecular surveillance and antimicrobial stewardship.

Key words: VRE; HLGR; *Enterococcus faecalis*; *Enterococcus faecium*; Antimicrobial Resistance

Introduction

Enterococcus spp. is Gram-positive cocci that are part of the normal gastrointestinal flora but have become important opportunistic pathogens, particularly in hospitalized and immunocompromised patients [1]. These organisms are responsible for a range of clinical infections including urinary tract infections (UTIs), bacteremia, endocarditis, intra-abdominal and pelvic infections, wound infections, and meningitis. In recent years, Enterococci have gained increasing clinical attention due to their ability to develop resistance to multiple antibiotics, significantly limiting treatment options [2].

Particular concern is the emergence of vancomycin-resistant Enterococci (VRE), now recognized globally as one of the leading causes of nosocomial infections. The World Health Organization (WHO) has classified VRE among the ESKAPE pathogens, which are responsible for the majority of hospital-acquired, multidrug-resistant infections [3]. Vancomycin resistance is primarily mediated by the acquisition of *vanA* and *vanB* genes. These genes alter the D-Ala-D-

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Ala terminus in the bacterial cell wall, thereby reducing vancomycin binding and rendering it ineffective. While *vanA* confers high-level resistance to both vancomycin and teicoplanin, *vanB* usually results in variable resistance and retains susceptibility to teicoplanin [4].

High-level gentamicin resistance (HLGR) is another critical resistance mechanism, primarily caused by the *aac(6')-Ie-aph(2")-Ia* gene, which encodes a bifunctional aminoglycoside-modifying enzyme [5]. HLGR abolishes the synergistic effect of gentamicin with beta-lactam antibiotics, significantly complicating treatment regimens [6].

The prevalence of VRE and HLGR varies geographically and is higher in settings such as intensive care units, oncology wards, and among patients with long hospital stays or prior antibiotic exposure [7]. This study aims to determine the prevalence of VRE and HLGR among clinical Enterococcus isolates in a tertiary care hospital in Northern India. It also focuses on the molecular characterization of resistance genes and antimicrobial susceptibility patterns to support infection control and antibiotic stewardship strategies.

Materials and Methods

This prospective observational study was conducted over one year, from January 1, 2024 to December 31, 2024, in the Department of Microbiology, Government Medical College, Patiala, a tertiary care hospital in Northern India. The study included all clinically significant, non-repetitive Enterococcus isolates obtained from patients of all age groups and both sexes, admitted or attending various outpatient departments.

Urine, blood, pus, wound swabs, catheter tip, body fluids, and endotracheal aspirates samples were collected and processed by using standard microbiological techniques [8]. Preliminary

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identification included colony morphology on blood agar and Macconkeys agar, Gram staining, catalase test (negative), bile esculin hydrolysis, growth in 6.5% NaCl broth, and sugar fermentation profiles.Species identification was based on conventional biochemical tests such as arginine dihydrolase activity, mannitol and sorbitol fermentation, pyruvate utilization, and pigment production [8].

Antibiotic susceptibility testing was done using the Kirby-Bauer disc diffusion method according to CLSI M100 guidelines (2024) [9]. Antibiotics tested included ampicillin (10 µg), ciprofloxacin (5 µg), erythromycin (15 µg), nitrofurantoin (300 µg), norfloxacin (10 µg), teicoplanin (30 µg), vancomycin (30 µg), linezolid (30 µg), and fosfomycin (200 µg).High-Level Gentamicin Resistance (HLGR) was screened using gentamicin (120 µg) discs; isolates showing zones <6 mm or no zone was considered HLGR-positive. MIC ≥500 µg/mL confirmed HLGR. Vancomycin resistance was screened with disc diffusion, vancomycin screening agar.To detect vancomycin resistance genes, specifically *vanA* and *vanB*, real-time PCR (RT-PCR) was performed using the HiMedia Hi-PCR® VAN Gene (Multiplex) Probe RT-PCR Kit. Statistical analysis was done using SPSS, with $p<0.05$ considered significant.

Results

This study analyzed 320 *Enterococcus* isolates collected from clinical specimens at Government Medical College & Rajindra Hospital, Patiala, from January 24 to December 2024. The samples were processed using standard microbiological techniques, and molecular detection of *vanA* and *vanB* genes was performed via RT-PCR.

Demographic Profile: The study population ranged from 7 days to 82 years, with a median age of 49 years and a mean of 50.46 ± 15.8 years. The majority of isolates were from patients aged

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41–60 years (39.7%), followed by 21–40 years (29.4%) and 61-80 years; 90 (28.1%) (fig. 1). Most patients were female 207 (64.6%) and 113 (35.4%) were male patients. Out of the 320 cases studied, 225 cases (70.4%) belonged to the urban background, and 95 cases (29.6%) belonged to the rural background.

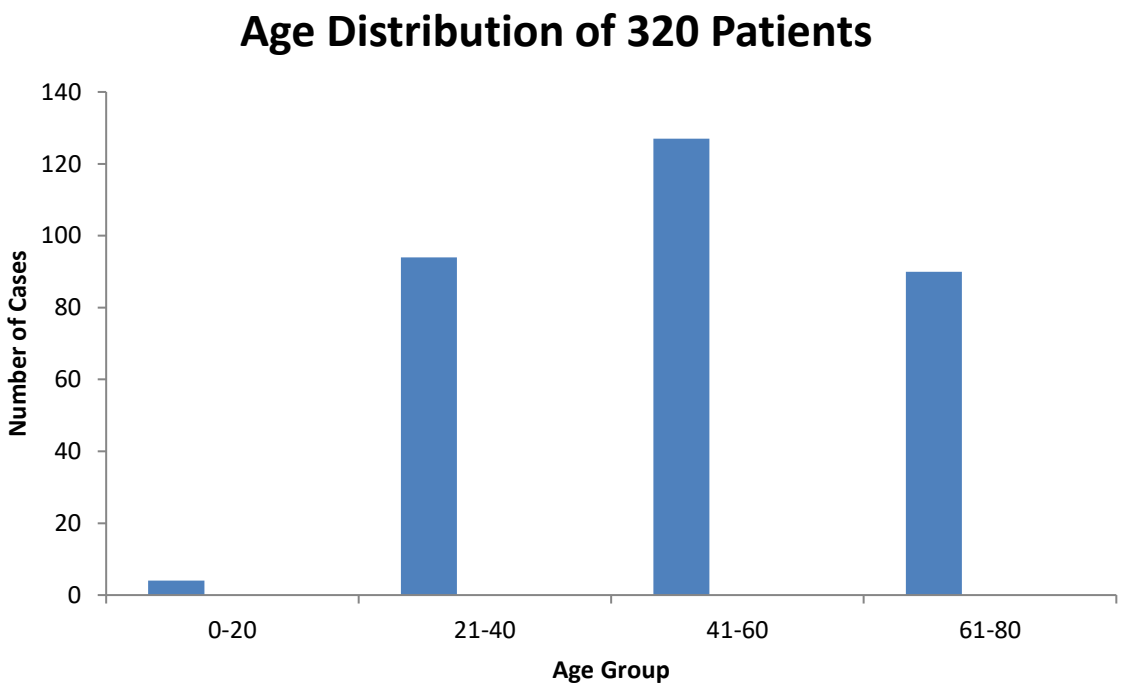

Fig. 1: **Age-Wise Distribution of 320 Cases**

Species Distribution: Table 1 showed that of the total 320 isolates, 280 (87.5%) were from *E. faecalis* and 40 (12.5%) from *E. faecium*. Urine was the most common sample source (53.8%), followed by blood (25.7%) and pus (14%), while body fluids accounted for 15 isolates (4.7%). The remaining 6 isolates (1.8%) were from other miscellaneous specimens.

Table 1: Species Distribution of Enterococcus Isolates

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Sample Type	E. faecalis	E. faecium	Total Cases	Percentage
Urine	142	30	172	53.8%
Blood	79	3	82	25.7%
Pus	40	5	45	14%
Body Fluids	13	2	15	4.7%
Others	6	0	6	1.8%
Total	280	40	320	100%

The most frequent comorbidity was diabetes mellitus (60.6%). This was followed by end-stage renal disease in 42 patients (13.2%), malignancy in 36 patients (11.3%), chronic obstructive pulmonary disease (COPD) in 22 patients (6.8%), liver cirrhosis in 15 patients (4.7%), and 11 patients (3.4%) were undergoing immunosuppressive therapy (fig. 2).

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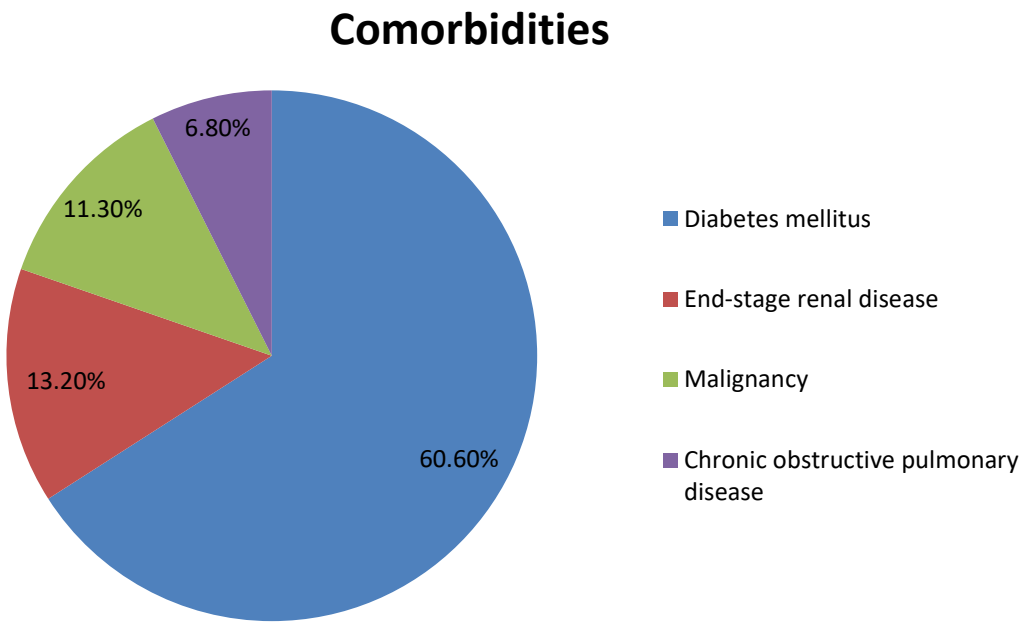

Fig. 2: **Distribution of 320 Cases According to Co-Morbidity Conditions**

Antimicrobial Resistance: High-level gentamicin resistance (HLGR) was observed in 153 (47.8%) isolates, with a higher prevalence in 123 (80.39%) *E. faecium* followed by 30 (19.60%) *E. faecalis*.

Among VRE isolates, 59.3% were from patients aged 41–60 years and 64.8% were from females. Among 320 Enterococcus isolates, 172 (53.7%) were from urine—*E. faecalis* (142) and *E. faecium* (30). *E. faecalis* showed high resistance to norfloxacin (84.5%), ciprofloxacin (78.8%), and HLG (38.7%), with no linezolid resistance. *E. faecium* showed 66.6% resistance to ampicillin, HLG, and ciprofloxacin; 30%-40% towards norfloxacin (40%), nitrofurantoin (33.3%), fosfomycin (33.3%), and vancomycin (30%). All were linezolid-sensitive. Among 148 non-urine isolates, *E. faecalis* (93.2%) had highest resistance to erythromycin (90.5%), followed by ampicillin (65.2%), ciprofloxacin (57.9%) and HLG (49.2%). *E. faecium* (6.8%) showed

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100% HLG resistance, 50% Ampicillin, 50% ciprofloxacin and 30% vancomycin. No isolates from either species showed resistance to linezolid in non-urine samples (Table 2).

Table 2: Antimicrobial Resistance Pattern of *Enterococcus* spp.

Antibiotic	*E.faecalis* (Urine, n=142)	*E. faecalis* (Non-Urine, n=138)	*E.faecium* (Urine, n=30)	*E.faecium* (Non-Urine, n=10)
Ampicillin	52 (36.6%)	90 (65.2%)	20 (66.6%)	5 (50%)
HLG	55 (38.7%)	68 (49.2%)	20 (66.6%)	10 (100%)
Ciprofloxacin	112 (78.8%)	80 (57.9%)	20 (66.6%)	4 (40%)
Norfloxacin	120 (84.5%)	-	12 (40%)	-
Nitrofurantoin	43 (30.2%)	-	10 (33.3%)	-
Fosfomycin	17 (11.9%)	-	10 (33.3%)	-
Erythromycin	-	125 (90.5%)	-	5 (50%)
Vancomycin	26 (18.3%)	22 (15.9%)	9 (30%)	3 (30%)
Teicoplanin	10 (7.0%)	13 (9.4%)	0 (0%)	0 (0%)
Linezolid	0 (0%)	0 (0%)	0 (0%)	0 (0%)

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Co-resistance Patterns: Among the 54 VRE isolates, 39 (72.2%) also exhibited HLGR. These isolates demonstrated high rates of co-resistance to other antimicrobials: erythromycin (87%), ciprofloxacin (82%), ampicillin (78%), nitrofurantoin (67%), and teicoplanin (24%). All isolates remained sensitive to linezolid.

Distribution of VRE isolates among *Enterococcus* species: Out of 320 isolates, 60 (18.75%) were vancomycin-resistant Enterococci (VRE), with *E. faecalis* accounting for 80% and *E. faecium* for 20%. In the present study, screening of 60 VRE isolates of Enterococcus spp. was performed on Chromogenic VRE medium as shown in Table 3 revealed that 54(90%) isolates were screened as positive and 6(10%) isolates were screened as negative.

Table 3: Comparison of Disc Diffusion Method and Chromogenic Agar for Detection of VRE

Growth on Chromogenic VRE Medium	Number of VRE by Chromogenic Media
Positive	54 (90%)
Negative	6 (10%)
Total	60 (100%)

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DISTRIBUTION OF VRE ISOLATES AND NON-VRE ISOLATES: Table 4 showed that among the 320 isolates of Enterococcus faecium and Enterococcus faecalis, 54 (16.8%) were vancomycin-resistant enterococci (VRE), while 266 (83.2%) were non-VRE. Statistical analysis comparing the distribution of VRE and non-VRE among E. faecium and E. faecalis yielded a non-significant result (p= 0.616).

Table 4: Distribution of VRE isolates and Non- VRE isolates

Total isolates	VRE ISOLATES		NON VRE ISOLATES		X2	p value
	Number	Percentage	Number	Percentage	0.251	0.616
320	54	16.8%	266	83.2%		

Molecular Characterization of VRE: The study found a significant association between Enterococcus species and vancomycin resistance genes, with *E. faecalis* predominantly carrying the **vanA** gene (97.7%) compared to *E. faecium* (81.8%) as shown in Table 5. RT-PCR confirmed that 94.4% of VRE isolates carried the *vanA* gene, while 5.6% had the *vanB* gene as shown in Table 5. Chi-square analysis confirmed this association as statistically significant (χ^2 = 4.197, p = 0.040).

Table 5: Distribution of vanA and vanB Genes among VRE Isolates

Species	Total Isolates	vanA	vanB	χ^2	p-value

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E. faecalis	43	42 (97.7%)	1 (2.3%)	4.197	0.040
E. faecium	11	9 (81.8%)	2 (18.2%)		
Total	54	51 (94.4%)	3 (5.6%)		

Collaborative Demographic and Clinical Characteristics for VRE and HLGR: The highest proportion of VRE (68.5%) and HLGR (57.6%) cases occurred in the 41–60 years age group, followed by the 21–40 and >60 age groups as shown in Table 6. A slight female predominance was observed in both VRE (64.8%) and HLGR (54.3%) cases. Urban residents formed the majority of both VRE (59.3%) and HLGR (53.6%) cases.

Table 6: Collaborative Demographic and Clinical Characteristics for VRE and HLGR:

Age	VRE Isolates	HLGR Isolates
0-20	0 (0%)	2 (1.5%)
21-40	10 (18.6%)	40 (26%)
41-60	37 (68.5%)	88 (57.6%)
61-80	5 (9.2%)	20 (13%)
>81	2 (3.7%)	3 (1.9%)
Mean ±SD	50.96±14.1	48.97±13.6

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Median	49.5	48.0
Range	58 (21-79)	70 (18-88)

Clinical Specimen distribution among VRE and HLGR: Urine was the most common clinical specimen source for both VRE (53.7%) and HLGR (49%) isolates as shown in Table 7 followed by blood (20.4%, 26.1%), Pus (16.7%, 16.3%) respectively. While wound swabs (5.5%, 4%) and body fluids (3.7%, 4.6%) in both VRE and HLGR.

Table 7: Specimen distribution of VRE isolate and HLGR isolate

Name of the sample	**VRE *E. faecalis***	**VRE *E. faecium***	**Total**	**HLGR *E. faecalis***	**HLGR *E. faecium***	**Total**
Urine	20	9	29 (53.7%)	55	20	75 (49%)
Blood	10	1	11 (20.4%)	35	5	40 (26.1%)
Pus	8	1	9 (16.7%)	22	3	25 (16.3%)
Wound Swab	3	0	3 (5.5%)	5	1	6 (4%)
Body Fluid	2	0	2 (3.7%)	6	1	7 (4.6%)

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Total	43	11	54 (100%)	123	30	153 (100%)

Similarly, co morbidities also showed the same pattern in VRE and HLGR (Table 8). It was found that the majority of the patients admitted to the ICU and confirmed to carry van genes have diabetes mellitus as the most common co morbidity (59.3%, 55.6%), followed by end-stage renal disease (24%, 15%), malignancy (11.1%, 17%), and COPD (5.6%, 6.5%) respectively in both VRE and HLGR. No cases were associated with congestive heart failure or cirrhosis among VRE while (3.3%) liver cirrhosis and (2.6%) heart failure was found in HLGR patients.

Table 8: Co-morbid conditions among total number of VRE isolates (n=54) and HLGR isolates (n=153)

Co-morbidities	**VRE** ***E. faecalis***	**VRE** ***E. faecium***	**Total**	**HLGR** ***E. faecalis***	**HLGR** ***E. faecium***	**Total**
Diabetes	28	4	32(59.3%)	77	8	85(55.6%)
ESRD	10	3	13(24%)	14	9	23(15%)
Malignancy	2	4	6(11.1%)	20	6	26(17%)
COPD	3	0	3(5.6%)	8	2	10(6.5%)
Cirrhosis	0	0	0%	2	3	5(3.3%)

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CHF	0	0	0%	2	2	4(2.6%)

Collaborative Antimicrobial Resistance Patterns in VRE and HLGR: Table 9 showed that in urine samples, VRE E. faecalis isolates showed the highest resistance to norfloxacin (90%), followed by ampicillin (55%) and ciprofloxacin (40%), nitrofurantoin (30%), and both fosfomycin and high-level gentamicin (20% each). A single isolate (5%) showed resistance to teicoplanin while linezolid resistance was absent. VRE E. faecium also showed high resistance to norfloxacin (77.7%) and HLG (55.5%), followed by ampicillin (33.3%), nitrofurantoin (33.3%), fosfomycin (22.2%), and teicoplanin (11.1%) with complete sensitivity to linezolid.

Among 75 urine HLGR isolates, E. faecalis showed 100% resistance to HLG and high resistance to fosfomycin (80%) and ampicillin/ciprofloxacin (54.5%), while E. faecium isolates were 100% resistant to both HLG and nitrofurantoin, and highly resistant to ampicillin (80%) and norfloxacin (75%), with all remaining linezolid sensitive.

From other clinical specimens, all HLGR E. faecalis (n=60) and E. faecium (n=18) isolates were resistant to both HLG and erythromycin (100%), with notable resistance to ciprofloxacin, ampicillin, vancomycin, and teicoplanin, but again, no linezolid resistance was detected across any HLGR group.

Table 9: Collaborative Antimicrobial Resistance Patterns in VRE and HLGR

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Antibiotic Resistance (%)	VRE E. faecalis (Urine)	VRE E. faecium (Urine)	VRE E. faecalis (Other)	VRE E. faecium (Other)	HLGR E. faecalis (Urine)	HLGR E. faecium (Urine)	HLGR E. faecalis (Other)	HLGR E. faecium (Other)
Ampicillin	55%	33.3%	86.6%	80%	54.5%	80%	50%	80%
High-level Gentamicin	20%	55.5%	73.3%	70%	100%	100%	100%	100%
Ciprofloxacin	40%	33.3%	80%	60%	54.5%	70%	58.8%	70%
Norfloxacin	90%	77.7%	—	—	30.9%	75%	—	—
Nitrofurantoin	30%	33.3%	—	—	40%	100%	—	—
Fosfomycin	20%	22.2%	—	—	80%	55%	—	—
Erythromycin	—	—	86.6%	80%	—	—	100%	100%
Teicoplanin	5%	11.1%	6.6%	10%	20%	35%	26.4%	40%
Vancomycin	100%	100%	100%	100%	20%	50%	26.4%	50%
Linezolid	0%	0%	0%	0%	0%	0%	0%	0%

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Discussion

The current study provides a comprehensive analysis of 320 Enterococcus isolates with respect to their demographic distribution, clinical source, species identification, antimicrobial resistance patterns, and molecular detection of vancomycin resistance genes. Our findings offer significant insights into the epidemiology of vancomycin-resistant enterococci (VRE) and high-level gentamicin-resistant (HLGR) strains, especially in the context of North India.

The majority of Enterococcus isolates were derived from patients aged 41–60 years (39.7%), followed by those aged 21–40 years (29.4%) and 61–80 years (28.1%). Only a minimal number of isolates (1.4%) were observed in the 0–20 years age group. These results mirror those of Noohi et al. (2019) and Gupta et al. (2020), who also found peak incidences in the middle-aged groups [10,11]. This demographic trend may be attributed to a higher prevalence of comorbidities such as diabetes and chronic kidney disease in these age groups, contributing to increased susceptibility to hospital-acquired infections.

In our study, females accounted for 64.6% of cases, with males comprising 35.4%, indicating a female predominance. This finding aligns with Yilema et al. (2017) and Goel et al. (2016), though contrasting reports exist, such as Eltayeb et al. (2022) and Gupta et al. (2021), which noted male predominance [11-14]. The higher rate in females may be due to their increased risk of urinary tract infections, which are commonly caused by Enterococcus species.

Most isolates (70.4%) were from urban residents, consistent with Ashagrie et al. (2021), who attributed this to greater healthcare access and higher antimicrobial use in urban areas, potentially leading to increased selection pressure and resistance [15].

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Urine was the predominant specimen type, accounting for 53.8% of isolates. This aligns with the study by Praharaj et al. (2013) and Yadav et al. (2017) reaffirming the role of Enterococcus as a major uropathogen [16,17]. Blood samples contributed 25.7% of isolates, significantly higher than earlier reports by Praharaj et al. (3.8%) and Yadav et al. (7%), possibly reflecting better diagnostic surveillance in tertiary care centers. Pus (14%) and body fluids (4.7%) were less common sources [16,17].

Notably, patients with diabetes mellitus constituted the majority (60.6%), followed by those with renal disease, malignancy, COPD, and liver disease. Similar comorbidity patterns have been reported by Moses et al. (2012). Diabetes compromises immune defenses and increases catheter usage, predisposing individuals to nosocomial infections [18].

Among urine isolates, Enterococcus faecalis was more prevalent than Enterococcus faecium. Resistance to ampicillin was higher in E. faecium (66.6%) compared to E. faecalis (36.6%). This pattern is corroborated by Goel et al. (2016) and Das et al. (2022) who also observed higher resistance in E. faecium. High-level gentamicin resistance (HLGR) was noted in 57.7% of E. faecalis and 66.6% of E. faecium isolates, consistent with national trends indicating growing resistance to aminoglycosides [13,19].

Fluoroquinolone resistance was substantial: ciprofloxacin (78.8% in E. faecalis, 66.6% in E. faecium) and norfloxacin (84.4% in E. faecalis, 40% in E. faecium). Similar high resistance levels have been reported by Goel et al. and Das et al. This may reflect the overuse of fluoroquinolones, particularly in urinary infections [13,19].

Nitrofurantoin resistance (30.2% in E. faecalis, 33.3% in E. faecium) was higher than in previous reports by Goel et al., highlighting its declining efficacy. Resistance to fosfomycin (11.9% in E.

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faecalis, 33.3% in E. faecium) also suggests emerging resistance, though levels remain relatively low [13]. Resistance to vancomycin (8.4% E. faecalis, 10% E. faecium) and teicoplanin (7% E. faecalis, 0% E. faecium) was comparatively low. Crucially, no resistance to linezolid was observed, indicating its continued utility.

In other clinical samples, resistance patterns were broadly similar, with higher erythromycin resistance in E. faecalis (90.5%) and lower in E. faecium (50%). HLGR rates were 22.4% in E. faecalis and 60% in E. faecium, while ciprofloxacin resistance was 57.9% and 40%, respectively. Teicoplanin resistance was low (9.4% in E. faecalis, 0% in E. faecium). Again, linezolid retained 100% sensitivity.

Out of 320 isolates, 54 (16.8%) were confirmed VRE. This aligns with Iris et al. (2013) (15%) and Shete et al. (2019) (12%), but exceeds rates reported by Yadav et al. (2017), and Nimmo et al. (2007) (0.8%–9.5%) [20,21,17,22]. The discrepancy may be due to differences in detection methods, regional resistance patterns, and hospital practices.

Of the 54 VRE isolates, 79.6% were E. faecalis and 20.3% E. faecium. This predominance of E. faecalis among VRE has been similarly observed by Pinholt et al. and Yadav et al., although studies by Iris et al. and Nimmo et al. found higher VRE rates in E. faecium [23,17,20,22]. This variation underscores geographic and institutional differences in Enterococcus species dynamics.

Multiplex PCR revealed that 94.4% of VRE isolates carried the vanA gene, and only 5.6% harbored vanB. vanA was significantly more prevalent in E. faecalis (97.7%) than in E. faecium (81.8%), as statistically confirmed ($p = 0.040$). This mirrors findings by Goel et al. and Yadav et al., reinforcing vanA's dominance in vancomycin resistance [13,17].

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Similar gene distributions have been reported across India and internationally, with Iris et al. observing higher vanB levels than our study [20]. These differences may result from environmental antibiotic pressures or genetic drift within clonal lineages.

Most VRE and HLGR cases occurred in the 41–60 years age group, consistent with Noohi et al. (2019) [10]. This may be attributed to increased hospital exposure and comorbidities in this age group. Females accounted for 64.8% of VRE and 54.3% of HLGR isolates, consistent with Ashagrie et al. and Toner et al. The female predominance may again reflect their higher susceptibility to UTIs, a common site for Enterococcus colonization and infection [24,25].

Urban residency was more common in both VRE (59.3%) and HLGR (53.6%) patients. Urban environments, often characterized by higher healthcare access and antibiotic misuse, may contribute to this pattern.

Among VRE isolates, urine samples were the most common (53.7%), followed by blood (16.6%) and pus (14.8%). Similar trends were observed for HLGR isolates, with urine (49%) being the leading source. These patterns are in agreement with Mittal et al., reinforcing the uropathogenic potential of VRE and HLGR Enterococcus species [26].

Among VRE and HLGR cases, diabetes mellitus was the most frequent comorbidity, followed by renal disease and malignancy. Yangzom et al. (2019) also found diabetes to be the leading risk factor [27]. These conditions compromise immunity and increase hospitalization, thus raising infection risks.

Among 29 VRE urine isolates, E. faecalis displayed high resistance to norfloxacin (90%) and moderate resistance to fosfomycin (35%) and nitrofurantoin (31%). E. faecium showed complete

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fosfomycin resistance (100%) and high resistance to nitrofurantoin (88.8%) and norfloxacin (77.7%).

All VRE isolates remained sensitive to linezolid. This finding is crucial and parallels reports by Gupta et al. and Goel et al., supporting the continued use of linezolid in VRE infections [11,13].

In HLGR urine isolates, E. faecalis and E. faecium both exhibited 100% resistance to gentamicin. High resistance was observed to ciprofloxacin, ampicillin, and fosfomycin. E. faecium, in particular, demonstrated high-level multidrug resistance. Importantly, none of the HLGR isolates were resistant to linezolid.

HLGR isolates among other clinical samples, resistance to ampicillin, erythromycin, and ciprofloxacin was high, especially in E. faecium. Again, no linezolid resistance was seen, highlighting its role as a last-resort antimicrobial.The antimicrobial resistance trends noted in this study are generally in line with those of other Indian studies, albeit with some differences in individual drug resistance rates. The consistently low or absent resistance to linezolid across multiple studies confirms its preserved efficacy. However, emerging resistance to commonly used agents such as ampicillin, ciprofloxacin, and nitrofurantoin underscores the pressing need for antibiotic stewardship.

The predominance of the vanA gene across Enterococcus species, as well as its statistical correlation with E. faecalis, supports the role of genotypic testing in routine diagnostics. Confirmatory methods such as chromogenic agar and PCR remain essential tools in the accurate detection of VRE, preventing both under- and overestimation of resistance.

Conclusion

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Enterococci are important pathogens in both community and hospital settings, often exhibiting resistance to multiple antibiotic classes. For serious infections, treatment typically involves a combination of cell wall–active agents and high-level aminoglycosides. However, resistance to either agent compromises their synergistic effect, reducing therapeutic efficacy. Vancomycin remains a cornerstone for treating life-threatening enterococcal infections, but the increasing global prevalence of vancomycin-resistant enterococci (VRE) presents a serious public health threat. Their emergence poses significant challenges for hospital infection control, especially in resource-limited settings. Phenotypic methods such as disc diffusion are commonly used to detect VRE within 24 hours and are cost-effective. The use of CHROMagar can further improve sensitivity. Nevertheless, molecular techniques remain the gold standard for confirming VRE by detecting vancomycin resistance genes. Combining rapid, affordable phenotypic screening with molecular confirmation enhances early diagnosis, supports effective infection control, and strengthens antimicrobial stewardship.

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References

1. Krawczyk B, Wityk P, Gałęcka M, Michalik M. The many faces of Enterococcus spp.—commensal, probiotic and opportunistic pathogen. Microorganisms. 2021 Sep 7;9(9):1900.

2. Arias CA, Contreras GA, Murray BE. Management of multidrug-resistant enterococcal infections. Clinical microbiology and infection. 2010 Jun 1;16(6):555-62.

3. De Oliveira DM, Forde BM, Kidd TJ, Harris PN, Schembri MA, Beatson SA, Paterson DL, Walker MJ. Antimicrobial resistance in ESKAPE pathogens. Clinical microbiology reviews. 2020 Jun 17;33(3):10-128.

4. Stogios PJ, Savchenko A. Molecular mechanisms of vancomycin resistance. Protein Science. 2020 Mar;29(3):654-69.

5. Das AK, Dudeja M, Kohli S, Ray P, Nandy S. High-level gentamicin resistance mediated by Aac (6′)-Ie-aph (2”)-Ia gene in Enterococcus species isolated from clinical samples in Northern India. Indian Journal of Pharmacology. 2022 May 1;54(3):171-6.

6. Choukhachian M, Nahaei MR, Rezaee MA, Sadeghi J. High-level Gentamicin Resistance and detection of aac (6’) Ie-aph (2”) Ia gene in Enterococci isolated from Pediatric hospital in Northwest of Iran. Archives of Clinical Infectious Diseases. 2018 Jan 1;13(13).

7. Bhatti JM, Raza SA, Alam AF, Khan YN, Mala A, Batool I, Sameeullah FN. Antibiotic choices among healthcare professionals for enterococcal bacteremia with patterns of resistance and risk factors of mortality, in settings of poor antibiotic stewardship

program—a five-year retrospective cohort study. BMC Infectious Diseases. 2023 Aug 6;23(1):514.

8. , Karunaratne KU, Wijesuriya TH, Dassanayake MA, Nanayakkara KA. Laboratory manual in microbiology. The Sri Lanka College of Microbiologists. 2011.

9. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing. 33rd ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2023

10. Noohi N, Talebi M. Diversity and Genetic Clonality of Vancomycin and Gentamicin Resistant Enterococci Isolated from Healthy Humans in Iran. Journal of Medical Bacteriology. 2019 Feb 19;8(1-2):27-36.

11. Gupta S, Srivastava P, Yadav S, Tayade SN. Vancomycin-resistant enterococci causing bacteriuria in hospitalized patients from Northwest India. Journal of Datta Meghe Institute of Medical Sciences University. 2020 Jul 1;15(3):421-5.

12. Yilema A, Moges F, Tadele S, Endris M, Kassu A, Abebe W, Ayalew G. Isolation of enterococci, their antimicrobial susceptibility patterns and associated factors among patients attending at the University of Gondar Teaching Hospital. BMC infectious diseases. 2017 Apr 17;17(1):276.

13. Goel V, Kumar D, Kumar R, Mathur P, Singh S. Community acquired enterococcal urinary tract infections and antibiotic resistance profile in North India. Journal of laboratory physicians. 2016 Jan;8(01):050-4.

14. Eltayeb MM, Al-Naam YA, Al-Maqati T, Al-Saadi M, Al-Dosary M, Al- Malki A. Prevalence and molecular characterization of vancomycin-resistant enterococci among clinical isolates at Prince Sattam Bin Abdulaziz University, Saudi Arabia. Saudi J Biol Sci. 2022;29(7):103268.

15. Ashagrie D, Genet C, Abera B. Vancomycin-resistant enterococci and coagulase-negative staphylococci prevalence among patients attending at Felege Hiwot Comprehensive Specialized Hospital, Bahir Dar, Ethiopia. Plos one. 2021 Apr 8;16(4):e0249823.

16. Praharaj I, Sujatha S, Parija SC. Phenotypic & genotypic characterization of vancomycin resistant Enterococcus isolates from clinical specimens. Indian Journal of Medical Research. 2013 Oct 1;138(4):549-56.

17. Yadav G, Thakuria B, Madan M, Agwan V, Pandey A. Linezolid and vancomycin resistant enterococci: a therapeutic problem. Journal of clinical and diagnostic research: JCDR. 2017 Aug 1;11(8):GC07.

18. Moses V, Jerobin J, Nair A, Sathyendara S, Balaji V, George IA, Peter JV. Enterococcal bacteremia is associated with prolonged stay in the medical intensive care unit. Journal of Global Infectious Diseases. 2012 Jan 1;4(1):26-30.

19. Das AK, Dudeja M, Kohli S, Ray P. Genotypic characterization of vancomycin-resistant Enterococcus causing urinary tract infection in northern India. Indian Journal of Medical Research. 2022 Mar 1;155(3&4):423-31.

ISSN: 0975-3583,0976-2833 VOL 16, ISSUE 7, 2025

20. Iris NE, Sayıner H, Yıldırmak T, Simsek F, Arat ME. Vancomycin-resistant Enterococcus carrier status in the reanimation units and related risk factors. American journal of infection control. 2013 Mar 1;41(3):261-2.

21. Shete VC, Grover N, Kumar M, Bhatt P. Phenotypic detection and molecular characterization of vancomycin-resistant enterococci. J Nat Sc Biol Med 2019;10:34-7

22. Nimmo GR, Pearson JC, Collignon PJ, Christiansen K, Coombs GW, Bell JM, McLaws M, Australian Group on Antimicrobial Resistance. Prevalence of MRSA among Staphylococcus aureus isolated from hospital inpatients, 2005: report from the Australian Group for Antimicrobial Resistance. Communicable diseases intelligence. 2007;31(3):288-96.

23. Pinholt M, Østergaard C, Arpi M, Bruun NE, Schønheyder HC, Gradel KO, Søgaard M, Knudsen JD, Danish Collaborative Bacteraemia Network (DACOBAN). Incidence, clinical characteristics and 30-day mortality of enterococcal bacteraemia in D enmark 2006–2009: a population-based cohort study. Clinical Microbiology and Infection. 2014 Feb;20(2):145-51.

24. Ashagrie D, Genet C, Abera B. Vancomycin-resistant enterococci and coagulase-negative staphylococci prevalence among patients attending at Felege Hiwot Comprehensive Specialized Hospital, Bahir Dar, Ethiopia. Plos one. 2021 Apr 8;16(4):e0249823.

25. Toner L, Papa N, Aliyu SH, Dev H, Lawrentschuk N, Al-Hayek S. Vancomycin resistant enterococci in urine cultures: Antibiotic susceptibility trends over a decade at a tertiary

ISSN: 0975-3583,0976-2833 VOL 16, ISSUE 7, 2025

hospital in the United Kingdom. Investigative and clinical urology. 2016 Mar;57(2):129-34.

26. Mittal S, Singla P, Deep A, Bala K, Sikka R, Garg M, Chaudhary U. Vancomycin and high level aminoglycoside resistance in Enterococcus spp. in a tertiary health care centre: a therapeutic concern. Journal of pathogens. 2016;2016(1):8262561.

27. Yangzom T, Singh TS. Study of vancomycin and high-level aminoglycoside-resistant Enterococcus species and evaluation of a rapid spot test for enterococci from Central Referral Hospital, Sikkim, India. Journal of Laboratory Physicians. 2019 Jul;11(03):192-9.