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Antibiotic Resistance Trends among Enterococcus Species Isolated from Urinary Tract Infections in a Tertiary Healthcare Facility

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ABSTRACT

INTRODUCTION: Enterococcus was first identified by Thiercelin in 1899. These organisms normally inhabit the gastrointestinal tract of humans and animals. Initially, enterococci were classified under the genus Streptococcus as Group D streptococci; however, they are now recognized as a distinct genus. Among the various species, Enterococcus faecalis and Enterococcus faecium are the most clinically significant in human infections. Enterococci are well known for their ability to develop resistance to multiple classes of antimicrobial agents. Urinary tract infection represents the most frequent clinical manifestation of enterococcal disease, accounting for nearly 10% of all UTIs.

OBJECTIVE: To identify Enterococcus species, analyze their antibiotic susceptibility including high-level aminoglycoside resistance, and evaluate phenotypic and molecular methods for detecting vancomycin-resistant Enterococcus (VRE).

MATERIAL AND METHOD: This study was carried out in the Department of Microbiology, Integral Institute of Medical Sciences and Research, Lucknow, from March 2023 to 2025. A total of 856 clinical specimens were processed for the isolation of Enterococcus species using standard microbiological techniques. Identification was based on colony characteristics, Gram staining, and a series of biochemical tests, followed by species-level differentiation using carbohydrate fermentation reactions. Antimicrobial susceptibility testing was performed by the Kirby–Bauer disk diffusion method in accordance with CLSI guidelines, including screening for high-level gentamicin and streptomycin resistance. Vancomycin resistance was confirmed by agar dilution to determine minimum inhibitory concentrations. Molecular detection of vancomycin resistance genes (vanA and vanB) was carried out using polymerase chain reaction, with further confirmation by sequence analysis.

RESULT: Out of 856 suspected UTI cases, Enterococcus species were isolated from 150 (17.5%), predominantly from female patients and individuals aged 21–50 years. E. faecalis was the most common species (85.3%), followed by E. faecium (14.7%). High-level gentamicin and streptomycin resistance was observed in 56.7% and 42.7% of isolates, respectively. Vancomycin-resistant enterococci (VRE) accounted for 5% of isolates, mainly among elderly patients, with all VRE exhibiting the VanA genotype. All VRE isolates remained susceptible to linezolid.

CONCLUSION: Enterococcus spp. are emerging uropathogens, especially in females and hospitalized patients. Inpatient predominance and seasonal trends suggest healthcare-associated transmission. High-level aminoglycoside resistance and VanA-type VRE present major treatment challenges. Linezolid remains uniformly effective against VRE. Ongoing surveillance, infection control, and antibiotic stewardship are crucial to curb resistance.

KEYWORDS: UTI, VanA, VanB, AST, Enterococcus, Vancomycin.

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INTRODUCTION

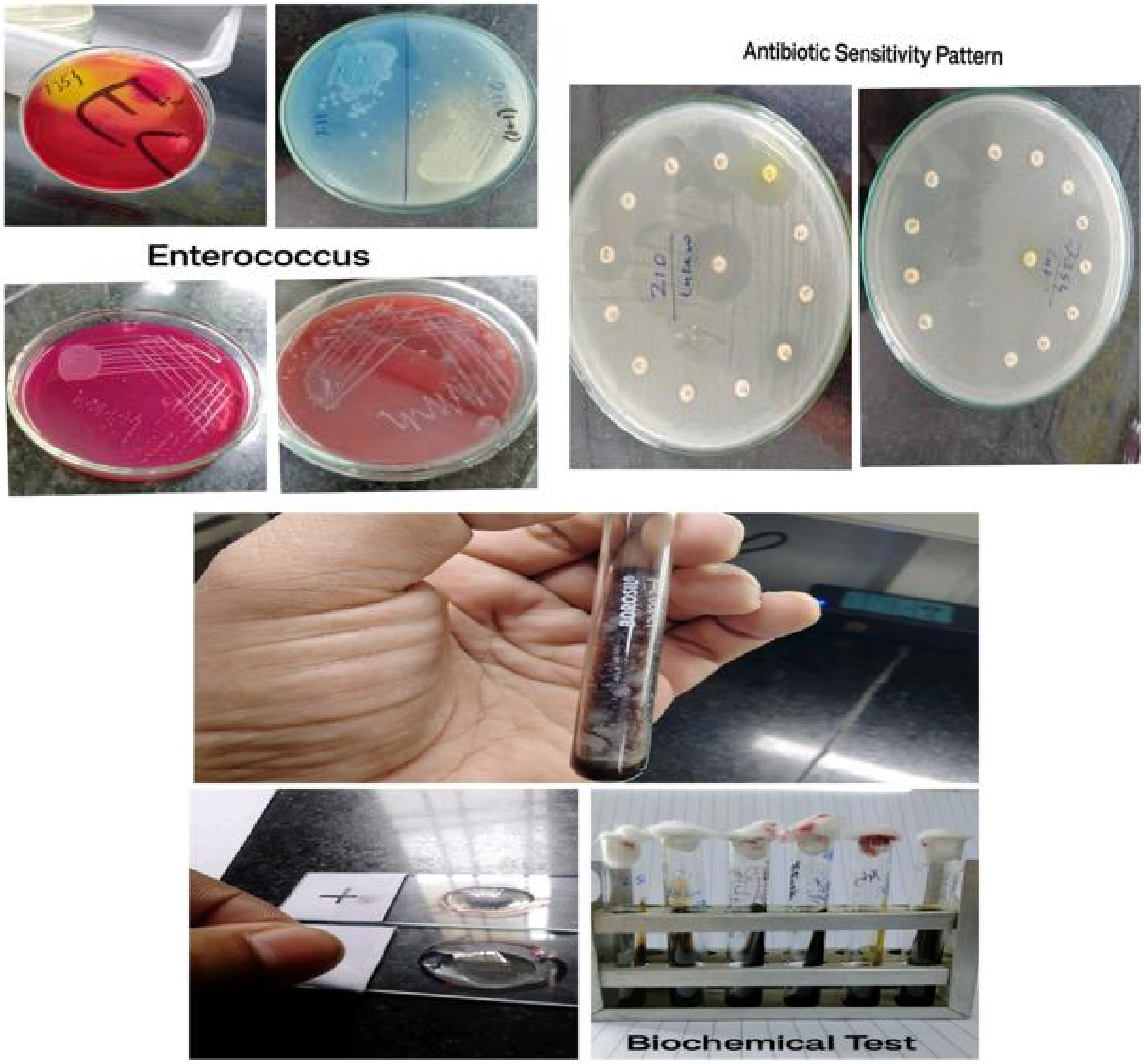
The rapid rise of antimicrobial resistance among commonly encountered pathogens has become a serious worldwide public health concern, especially in healthcare-associated environments. Among these organisms, Enterococcus species have emerged as significant contributors to hospital-acquired infections, including urinary tract infections, surgical site infections, and bloodstream infections (1-2). Unlike Streptococcus, Enterococcus species possess inherent resistance to several commonly used antibiotics, including penicillins, cephalosporins, low-level aminoglycosides, clindamycin, and trimethoprim–sulfamethoxazole, thereby creating substantial challenges in clinical management (3). The ability of vancomycin-resistant Enterococcus (VRE) to transfer resistance genes to Staphylococcus aureus is particularly alarming, as it significantly limits therapeutic options and worsens the management of multidrug-resistant infections (4-5).

Enterococcus species are implicated in a broad spectrum of infections ranging from urinary tract infections to bloodstream infections. E. faecalis is more commonly linked with community-acquired infections, whereas E. faecium is predominantly associated with healthcare-associated infections. Urinary tract infection remains the most frequent clinical presentation of enterococcal disease. Lower UTIs are commonly observed in elderly men, while Enterococcus is an infrequent cause of uncomplicated cystitis in young women. Upper UTIs may progress to bacteremia and are seen mainly in older male patients. Overall, enterococci account for approximately 10% of urinary tract infections (6-7).

Enterococcus isolated from urine often represents colonization and usually does not require treatment, except in high-risk groups such as pregnant women, patients undergoing urological procedures, or those with significant comorbidities; in catheterized patients, catheter removal alone is often sufficient (8). Enterococcal infections are mainly healthcare-associated, particularly in intensive care units, where they contribute substantially to multidrug-resistant UTIs, though community-acquired cases are increasingly reported. These organisms can also cause serious infections such as bacteremia and endocarditis, especially in elderly or immunocompromised patients and those with invasive devices. Transmission commonly occurs via healthcare workers and contaminated hospital equipment, facilitating persistence and spread of resistant strains, including vancomycin-resistant enterococci (10-11). Owing to extensive intrinsic and acquired antimicrobial resistance and limited therapeutic options, this study focuses on the prevalence, species distribution, and resistance patterns of enterococci to aid effective treatment and infection control.

MATERIALS AND METHODS

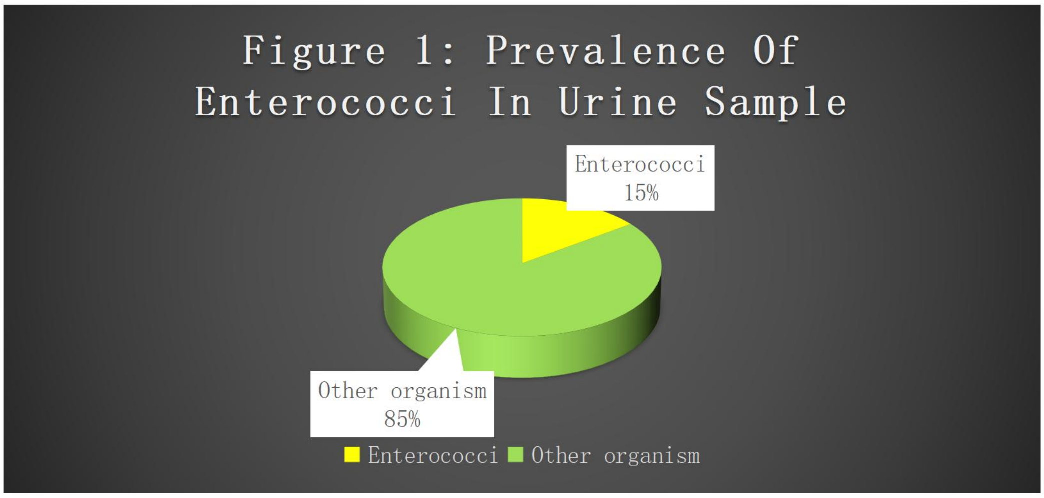
The study was conducted in the Department of Microbiology, Integral Institute of Medical Sciences and Research, Lucknow, over a two-year period from March 2023 to 2025. A total of 856 clinical specimens were processed following standard microbiological protocols for the isolation and identification of Enterococcus species. Samples were inoculated onto MacConkey agar, nutrient agar, blood agar, and CLED agar, and preliminary identification was based on colony morphology, lactose fermentation, hemolytic pattern, and pigment production. Gram staining was performed, and isolates appearing as Gram-positive cocci in pairs or short chains were subjected to confirmatory biochemical tests including catalase test, bile esculin hydrolysis, and growth in 6.5% sodium chloride. Species differentiation was carried out using motility testing and a panel of carbohydrate fermentation reactions, namely mannitol, sorbitol, sorbose, arginine, arabinose, raffinose, sucrose, pyruvate, and lactose, which enabled identification of clinically relevant Enterococcus species such as E. faecalis and E. faecium. Antimicrobial susceptibility testing was performed on Mueller–Hinton agar by the Kirby–Bauer disc diffusion technique as per CLSI guidelines, including screening for high-level aminoglycoside resistance using high-level gentamicin and streptomycin discs, with Enterococcus faecalis ATCC 29212 employed as the quality control strain. Vancomycin susceptibility was further evaluated by determining the minimum inhibitory concentration using the agar dilution method, and isolates exhibiting an MIC of ≥32 μg/mL were classified as vancomycin-resistant enterococci. Detection of vancomycin resistance determinants (vanA and vanB) was carried out by polymerase chain reaction following genomic DNA extraction, and the amplified products were visualized by agarose gel electrophoresis using appropriate controls. Confirmation of resistance genes was completed by sequence analysis, and all laboratory and clinical data were systematically recorded and analyzed using Microsoft Excel 2021.



RESULT

A total of 856 clinically suspected cases of urinary tract infection (UTI) were screened during the study period. Enterococcus species were isolated from 150 cases, accounting for an overall prevalence of 17.5% (Figure 1).

Out of 150 enterococci, 70.7% were isolated from female patients and 29.3% were isolated from males. Most of the enterococci, 102/150 (68%) were isolated from sexually reproductive age group (21-50 years). 8.7% were isolated from 51-60 years age group, and 10% were isolated from 61-70 years age group. 5.3% were isolated from 71-80 years age group, 2% from >80 years, 2.7% from 1-10 years, 2% from < 1 year and 1.3% were isolated from 11-20 years age group (Table 1).



AGE GROUP	NUMBER (%)	MALE	FEMALE
<1 year	3 (2%)	1	2
1-10 years	4 (2.6%)	2	2
11-20 years	2 (1.3%)	1	1
21-30 years	35 (23.3%)	5	30
31-40 years	48 (32%)	9	39
41-50 years	19 (12.6%)	13	6
51-60 years	13 (8.6%)	5	8
61-70 years	15 (10%)	5	10
71-80 years	8 (5.3%)	2	6
>80 years	3 (2%)	1	2
Total	150(100%)	44	106

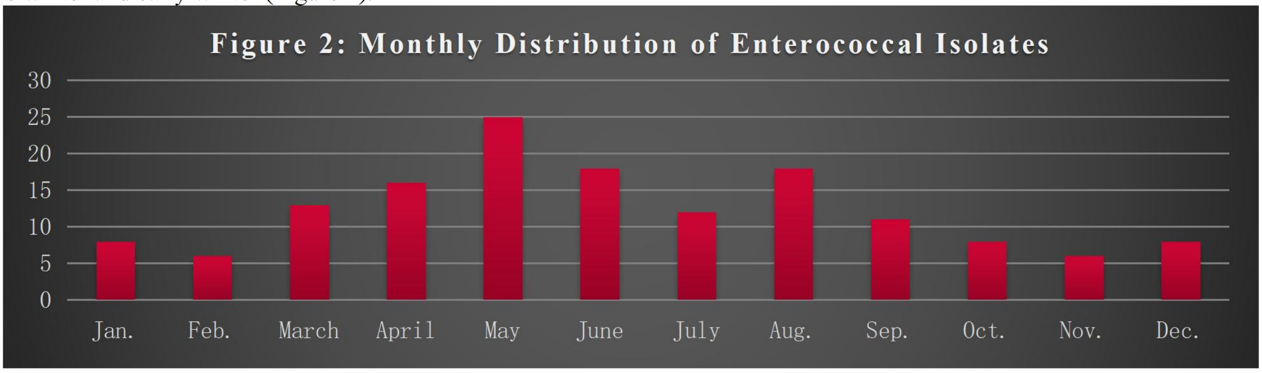
Table No. 1 Age Wise Distribution of Enterococci

Mostly, enterococci were isolated from inpatients (72.1%), and 27.9% were isolated from outpatient department. Most enterococcal isolates originated from the surgery ward (34.7%), with substantial contributions from the medicine ward (23.3%) and the medicine OPD (22.7%). The ICU (7.3%) and gynecology ward (5.3%) accounted for fewer isolates, while the rest were distributed among other departments (Table 2).

Table No. 2 Distribution of Enterococcal Isolates by Source

Source	**Number**	**Male**	**Female**
Medicine Ward	35 (23.3%)	8	27
Surgery Ward	52(34.7%)	15	37
Medicine OPD	34(22.7%)	12	22
Surgery OPD	1(0.7%)	1	0
ICU	11(7.3%)	3	8
PICU	2(1.3%)	2	0
ENT OPD	1(0.7%)	1	0
OBS-Gynae Ward	8(5.3%)	0	8
Pediatric OPD	1(0.7%)	0	1
Pediatric Ward	2(1.3%)	1	1
Neurosurgery Ward	3(2%)	1	2
Total	**150(100%)**	**44**	**106**

The month-wise distribution of enterococcal isolates demonstrated seasonal variation.The highest isolation rate was observed in May (n = 26; 17.3%), followed by June and August (n = 18 each; ~12%). The lowest numbers were recorded in February and November (n = 6 each; ~4%). Moderate isolation rates (8–16 isolates/month) were noted during the remaining months. Overall, enterococcal isolates were detected throughout the year, with a distinct peak during the summer months and a decline during late winter and early winter (Figure 2).



AST of enterococci demonstrated resistance to HLG in 56.7% and to HLS in 42.7% of isolates. Vancomycin resistance was seen in 5.3%, with 2% showing intermediate susceptibility. Teicoplanin resistance was observed in 5.3% of isolates, while no resistance to linezolid was detected (Table 3)

Table No. 3 Antimicrobial Susceptibility Pattern of Enterococci

Name of Antibiotics	**Sensitive (%)**	**Intermediate (%)**	**Resistant (%)**
Ciprofloxacin	8(5.3%)	-	142(94.7%)
Penicillin	25(16.7%)	-	125(83.3%)
Erythromycin	52(34.7%)	-	98(65.3%)
Norfloxacin	45(30%)	-	105(70%)
High-Level Gentamicin	65(43.3%)	-	85(56.7%)
High-Level Streptomycin	86(57.3%)	-	64(42.7%)
Tetracycline	109(72.7%)	-	41(27.3%)
Nitrofurantoin	118(78.7%)	-	32(21.3%)
Vancomycin	139(92.7%)	3(2%)	8(5.3%)
Teicoplanin	142(94.7%)	-	8(5.3%)
Linezolid	150(100%)	-	-

The most commonly isolated species of enterococci was E. faecalis (128/150=85.3%), followed by E. faecium (22/150=14.7%) (Table 4).

Table No. 4 Distribution of Enterococcus Species

Species	**Number**	**Percent**
E. faecalis	128	85.3%
E. faecium	22	14.7%
Total	**150**	**100%**

Vancomycin resistance among enterococcal isolates was assessed using the agar dilution method. VRE accounted for 5% of isolates (8/150), while the majority were vancomycin-susceptible. Although the prevalence was low, the presence of VRE underscores the need for ongoing surveillance and precise detection to support effective therapy and infection control (Figure 3).

Figure 3: Prevalence Of VRE By Agar Dilution Method

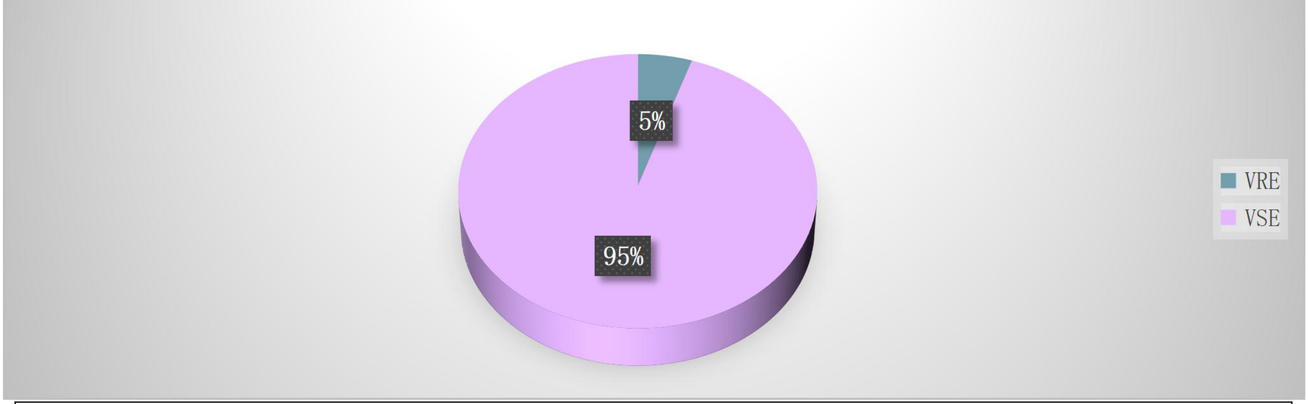
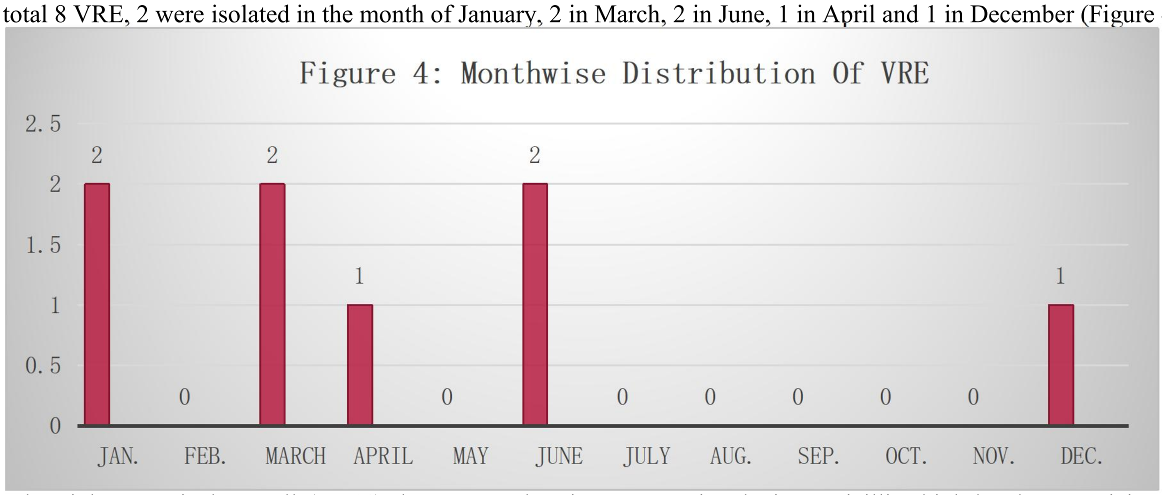


Table No. 5 Distribution of VRE According to Age and Gender

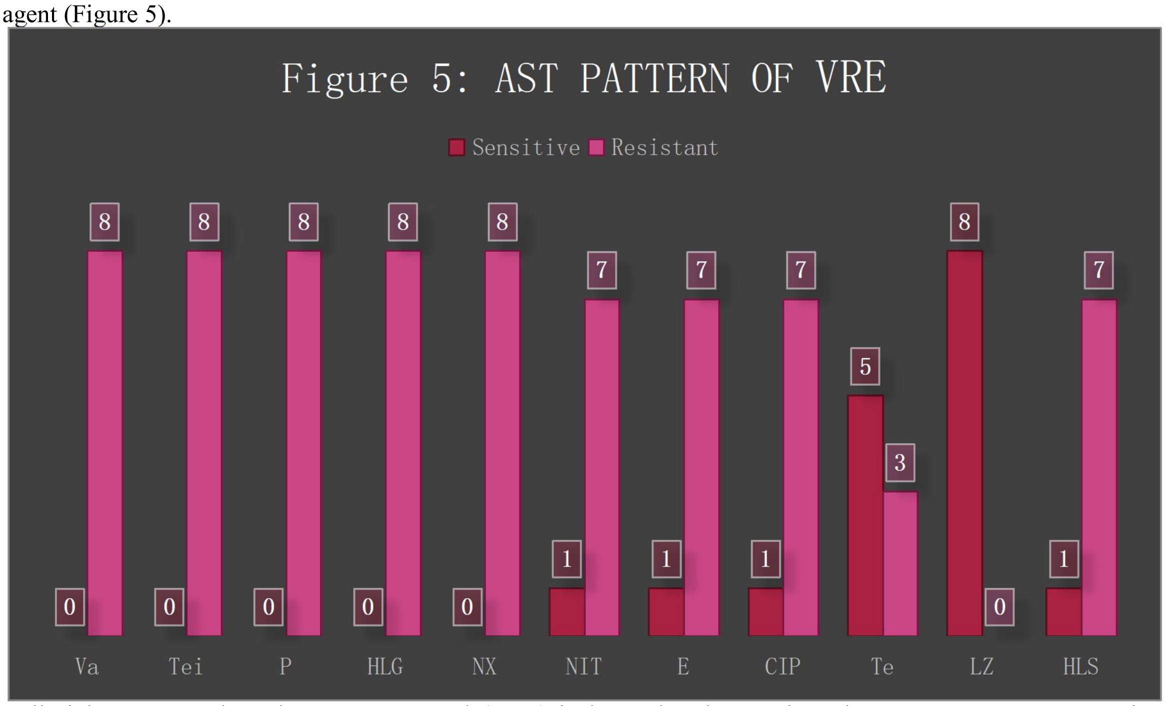
Age Group	Number (%)	Male	Female
31-40 years	1(12.5%)	-	1
61-70 years	2(25%)	-	2
71-80 years	4(50%)	1	3
>80 years	1(12.5%)	-	1
Total	**8(100%)**	**1**	**7**

Of the total VRE isolates, 75% were obtained from female patients, while 25% were from male patients. With respect to age distribution, the majority of VRE cases (87.5%) occurred in patients aged over 60 years, whereas 12.5% were observed in the 31–40-year age group (Table 5).

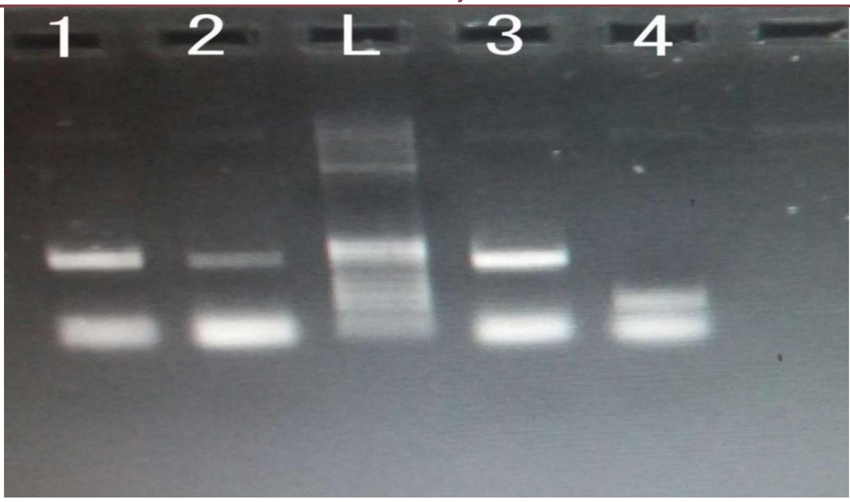
Out of total 8 VRE, 2 were isolated in the month of January, 2 in March, 2 in June, 1 in April and 1 in December (Figure 4).



Among the eight VRE isolates, all (100%) demonstrated resistance to teicoplanin, penicillin, high-level gentamicin (HLG), norfloxacin, and levofloxacin. A high level of resistance was also observed to ciprofloxacin, high-level streptomycin (HLS), and erythromycin, each seen in 87.5% of isolates. Resistance to nitrofurantoin was noted in 83.3% of isolates, while 37.5% showed resistance to tetracycline. None of the VRE isolates exhibited resistance to linezolid, indicating complete susceptibility to this agent (Figure 5).



Among all eight vancomycin-resistant enterococcal (VRE) isolates, the phenotypic resistance pattern was suggestive of the VanA genotype. All isolates exhibited resistance characteristics consistent with VanA-mediated vancomycin resistance, indicating that VanA was the predominant resistance phenotype among the VRE isolates identified in the present study.



The Figure 6 illustrates the detection of VanA and VanB resistance genes among vancomycin-resistant enterococci (VRE) by agarose gel electrophoresis, with the DNA ladder (L) loaded in the central lane. Lanes 1, 2, and 3 show amplification products corresponding to the VanA gene, indicating VanA-positive isolates, while lane 4 demonstrates amplification of the VanB gene, representing a VanB-positive isolate.

All patients infected with vancomycin-resistant enterococci (VRE) in the present study were successfully treated with linezolid, with favorable clinical outcomes observed in every case. This finding highlights the continued effectiveness of linezolid as a therapeutic option for the management of VRE infections and supports its role as a reliable treatment option in settings where resistance to other glycopeptide antibiotics is encountered.

DISCUSSION

The present study documented an enterococcal prevalence of 17.5%, which is higher than reports from Ethiopia (3.5%) and Nigeria (5.9%), but comparable to findings from Kanpur (13.9%). Such variability across studies may be attributed to differences in geographic location, patient populations, hospital settings, and laboratory methodologies. A clear female predominance was observed (70.7%), consistent with earlier studies, likely due to the higher incidence of urinary tract infections among females and anatomical predispositions that favor enterococcal colonization and infection (13-17).
Age-wise analysis revealed that the majority of isolates were obtained from patients aged 21–50 years (68%), in agreement with previous studies reporting higher isolation rates in this sexually and reproductively active age group. This may be related to increased healthcare utilization and a higher burden of UTIs, particularly among females. Lower isolation rates were observed at the extremes of age. Seasonal variation was also noted, with a peak in summer months, particularly May, June, and August, suggesting a possible association with increased hospital admissions, invasive procedures, and antibiotic exposure during this period (20).

Source-wise distribution showed that most isolates originated from the surgery ward, followed by the medicine OPD and medicine ward, findings comparable to earlier studies. Inpatients accounted for a significantly higher proportion of cases (72.1%), highlighting the role of hospital-associated risk factors such as prolonged hospitalization, invasive procedures, comorbidities, and exposure to broad-spectrum antibiotics in the epidemiology of enterococcal infections (18-19).

Antimicrobial resistance analysis revealed glycopeptide resistance in a small but clinically significant proportion of isolates. Using the agar dilution method, VRE prevalence was 5%, comparable to reports from North India but lower than those from some regions of South India. Enterococcus faecalis was the predominant species, followed by E. faecium. All VRE isolates were susceptible to linezolid, underscoring its importance as a therapeutic option; however, reliance on last-resort agents emphasizes the need for continuous surveillance, antimicrobial stewardship, and strict infection control practices (18,20).

CONCLUSION

The present study highlights the increasing clinical importance of enterococci as uropathogens, with a notable prevalence in urinary tract infections, particularly among females, sexually active age groups, and hospitalized patients. The predominance of infections in inpatient settings, along with seasonal variation, underscores the role of healthcare-associated and environmental factors in enterococcal infections. The emergence of high-level aminoglycoside resistance and the detection of vancomycin-resistant enterococci, predominantly Enterococcus faecium with a VanA resistance phenotype, pose significant therapeutic challenges. Although linezolid remains an effective treatment option, the reliance on last-resort antimicrobials emphasizes the need for judicious antibiotic use. Continuous antimicrobial surveillance, strict infection control measures, and robust antibiotic stewardship programs are essential to limit the spread of multidrug-resistant enterococci and to ensure effective management of enterococcal infections in hospital settings.

Conflicts of Interest
None.

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