

ORIGINAL RESEARCH

A Study of Microbial Characterization of Enterococci with special Emphasis on VRE at Tertiary care centre

¹Dr. Kuldeep Singh, ²Dr. Saurabh G Agarwal, ³Dr. Sanyogita Jain, ⁴Mr. Veerendra Sen, ⁵Dr. Rajdeep Paul, ⁶Dr. Akshaya Khattri, ⁷Dr. Arpita Soni

^{1,5}Assistant Professor, ^{2,3}Professor, ⁴Tutor, ^{6,7}Junior Resident, Department of Microbiology, Chirayu Medical College & Hospital, Bhopal, M.P., India

Corresponding Author

Dr. Rajdeep Paul

Assistant Professor, Department of Microbiology, Chirayu Medical College & Hospital, Bhopal, M.P., India

Email: rimo.micro@gmail.com

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ABSTRACT

Introduction: Vancomycin-resistant Enterococci (VRE) pose a significant challenge in healthcare settings, necessitating urgent attention due to their role in healthcare-associated infections. The emergence of VRE underscores the evolving nature of microbial pathogens and highlights the need for vigilant surveillance and intervention. Enterococci, once considered harmless inhabitants of the gastrointestinal tract, have become prominent causes of nosocomial infections. The increasing global incidence of nosocomial infections, including urinary tract infections (UTIs) and other device-associated infections, underscores the virulence and adaptability of Enterococci. Traditionally, vancomycin has been the mainstay of therapy, but the emergence of vancomycin-resistant strains has necessitated a shift in treatment paradigms. **Objective:** This study aims to evaluate and characterize different Enterococcus species isolated from various clinical samples, with a particular emphasis on screening for vancomycin resistance, considering the scarcity of information on VRE in India. **Materials & Methods:** The study employed a prospective laboratory-based approach with a cross-sectional design, conducted at the Department of Microbiology, Chirayu Medical College & Hospital, Bhopal, over one year. Samples include those from hospitalized patients and outpatient department attendees, excluding respiratory, stool, and vaginal sources. Samples were subjected to bacteriological processing using standard methods, with a focus on Enterococcus isolation and identification. Data analysis includes detailed sample characteristics, microbiological findings, and antimicrobial susceptibility profiles. **Results:** Among 16,993 samples analysed, urinary isolates were most common (27.33% positivity), with 17.63% yielding Enterococcus spp. Blood samples showed 22.58% positivity, with 6.31% yielding Enterococcus spp. Female patients accounted for 57.68% of isolates, and positivity varied across age groups. VRE prevalence was 7.77% in urine and 6.25% in blood samples. Sensitivity to antibiotics varied, with Vancomycin, Teicoplanin, and Linezolid showing high Sensitivity. **Conclusion:** This study provides insights into Enterococcus spp. prevalence and VRE at a tertiary care center, emphasizing the need for surveillance and tailored interventions to address the rising threat of VRE. Understanding distribution, resistance patterns, and antibiotic sensitivities is crucial for optimizing treatment and infection control measures. Continued research is essential to combat Enterococcus infections and antimicrobial resistance effectively.

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INTRODUCTION

Vancomycin-Resistant Enterococci (VRE) represent a formidable challenge in contemporary healthcare settings, significantly augmenting the burden of healthcare-associated infections. Recognized as a pressing public health concern by the World Health Organization, VRE necessitates urgent attention and the development of novel therapeutic strategies [1]. Enterococci, once regarded as benign commensals of the gastrointestinal tract (GIT), have undergone a remarkable transformation, emerging as

prominent culprits in nosocomial infections over recent decades. This shift underscores the evolving nature of microbial pathogens and highlights the imperative for vigilant surveillance and intervention. The escalating global incidence of nosocomial infections, including bacteraemia, endocarditis, urinary tract infections (UTIs), surgical site infections (SSIs), and other device-associated infections, underscores the heightened virulence and adaptability of Enterococci [2]. Notably, Enterococci have risen to become the

second most common etiological agent of nosocomial infections, with UTIs representing a predominant clinical manifestation. Furthermore, intra-abdominal and intra-pelvic abscesses, along with post-surgical wound infections, constitute significant sources of Enterococcal morbidity.

Traditionally, vancomycin stood as the cornerstone of therapy against Enterococcal infections.

However, the emergence of vancomycin-resistant strains has become increasingly prevalent, challenging conventional treatment paradigms and necessitating a paradigm shift in antimicrobial stewardship. Consequently, the identification and characterization of VRE strains assume paramount importance, serving as a linchpin for the implementation of stringent infection control measures and the formulation of evidence-based antibiotic policies.

This Research endeavours to elucidate the Microbial landscape of Enterococci, with a particular focus on VRE, within the confines of a tertiary care center. By delineating the epidemiological patterns and antimicrobial susceptibility profiles of Enterococci, this study aims to furnish invaluable insights for healthcare practitioners. Specifically, the identification of VRE strains will facilitate the initiation of rigorous contact isolation protocols, curbing the dissemination of resistant pathogens within healthcare facilities. Moreover, the compilation of local antibiograms will inform the development and refinement of antibiotics policies, empowering clinicians in the judicious selection of empirical antimicrobial therapy.

In essence, this study embodies a proactive stance towards antimicrobial stewardship, championing the optimization of therapeutic interventions and the preservation of antimicrobial efficacy. By bolstering our understanding of Enterococcal dynamics and resistance mechanisms, this research endeavours to fortify the arsenal of tools available for combatting nosocomial infections and safeguarding public health.

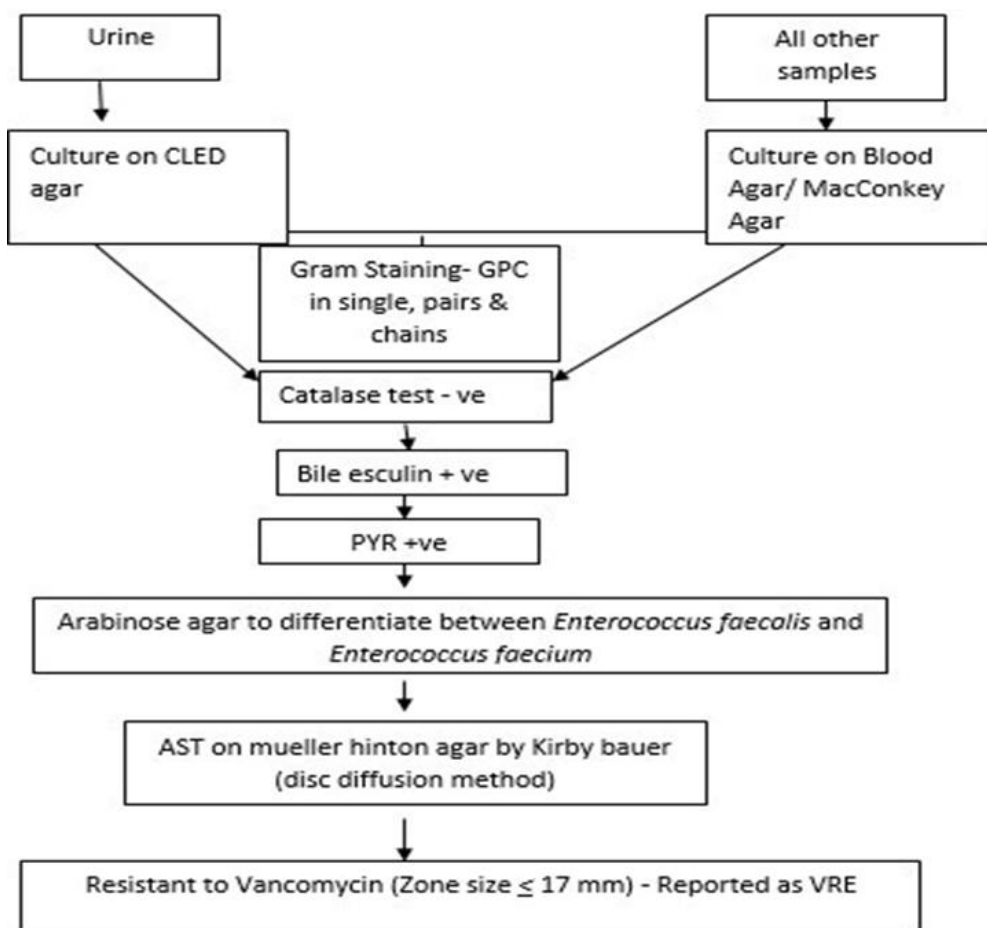
The aim of this study is to evaluate and characterize different *Enterococcus* species isolated from various clinical samples.

Given the lack of information on vancomycin resistance in Enterococci from India, this study aims to screen Enterococci isolated from various specimens for vancomycin resistance.

MATERIALS & METHODS

This study employed a prospective laboratory-based approach with a cross-sectional design to examine the microbial characteristics of Enterococci, with particular attention to Vancomycin-Resistant Enterococci (VRE). Conducted at the Department of Microbiology, Chirayu Medical College & Hospital, Bhopal, for duration of one year, from March 2023 to February 2024. The study population encompasses all patients admitted to the hospital during this period, as well as individuals attending the outpatient department (OPD). Inclusion criteria encompass all clinically relevant samples from patients of all age groups, while excluding samples derived from respiratory, stool, and vaginal sources. Primary sample types for analysis include urine, blood, and sterile body fluids.

Sample collection adhere to established protocols outlined in the primary sample collection manual, with meticulous attention to aseptic techniques to minimize contamination. Following collection, samples are promptly transported to the microbiology laboratory for processing, ensuring maintenance of sample integrity during transit. In the laboratory, samples were subjected to bacteriological processing using standard microbiological methods, including culture-based techniques, biochemical assays, and antimicrobial susceptibility testing. Special emphasis was placed on the isolation and characterization of Enterococci, with a specific focus on identifying VRE strains. Samples were processed according to following algorithm provided for the identification of VRE-



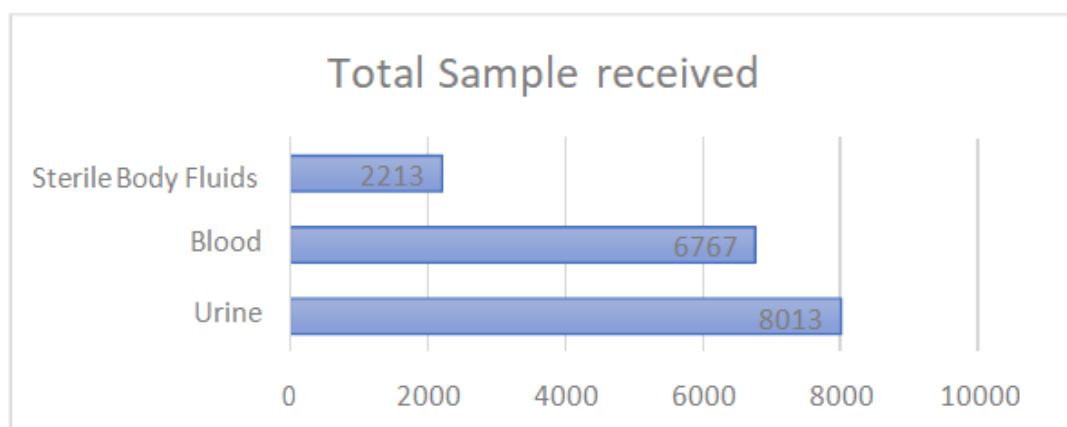
Data collection involves recording detailed information regarding sample characteristics, microbiological findings, and antimicrobial susceptibility profiles. Statistical analysis was conducted to discern patterns of microbial resistance

and to identify potential risk factors associated with VRE colonization or infection. All procedures involving human participants were conducted with appropriate ethical approvals and informed consent, while patient confidentiality was rigorously upheld.

RESULT

Distribution of Samples

Sample	Total Sample received
Urine	8013
Blood	6767
Sterile Body Fluids	2213
Total	16993



A total of 16,993 samples were collected and analysed during the study period. Among these, urine samples constituted the highest proportion, with 8013 samples received. Blood samples followed closely behind, comprising 6767 samples. Additionally, sterile body fluids were collected and analyzed, totaling 2213 samples. This distribution

provide valuable insight into the types and quantities of samples processed at the tertiary care center, laying the groundwork for further investigation into the prevalence and characteristics of Enterococci, particularly focusing on Vancomycin-resistant strains.

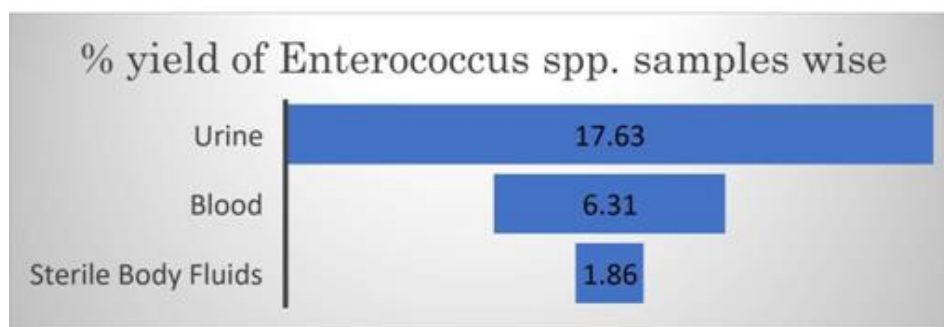
Percentage Positivity

Sample	n =	% Positivity
Urine	2190	27.33
Blood	1528	22.58
Sterile Body Fluids	459	5.73

Among the samples analysed, urine demonstrated the highest culture positivity rate, with 27.33% of urine samples testing positive. Blood samples exhibited a slightly lower positivity rate at 22.58%, while sterile body fluids showed the lowest percentage positivity at 5.73%.

% yield of Enterococcus spp. samples wise

Sample	n =	% Enterococcus spp. isolated
Urine	386	17.63
Blood	96	6.31
Sterile Body Fluids	9	1.86

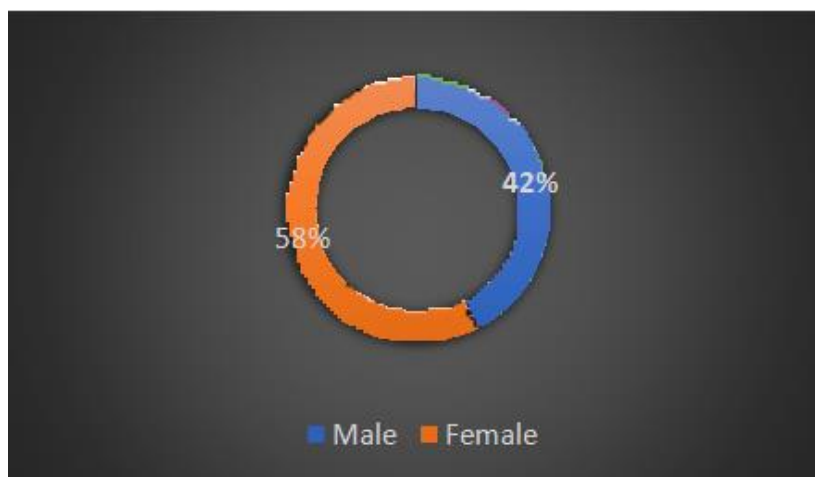


The percentage yield of Enterococcus spp. varied across sample types, with urine samples showing the highest isolation rate at 17.63%. Blood samples exhibited a lower percentage yield, with 6.31% of

samples yielding Enterococcus spp. Sterile body fluids displayed the lowest isolation rate, with only 1.86% of samples yielding Enterococcus spp.

Gender wise distribution of Enterococcus spp. Isolates

Male	Female
(n= 208)	(n=283)
42.36%	57.64%



Among the isolates obtained, 42.31% were from male patients, while female patients accounted for the majority, with 57.68% of the isolates originating from them. These findings underscore the importance of considering demographic factors, such as gender, in understanding the epidemiology

and transmission patterns of *Enterococcus* spp. infections within the tertiary care center setting. Further analysis of gender-specific risk factors and interventions may be warranted to effectively mitigate the spread of *Enterococcus* spp. in healthcare settings.

Age wise distribution of *Enterococcus* spp. Isolates

Age group	n =	% Positive
0-1 years	42	8.55
1-12 years	67	13.65
12-18 years	51	10.38
18-40 years	141	28.72
40-60 years	101	20.57
60-70 years	53	10.79
>70 years	36	7.34



The results revealed variations in the percentage of positive isolates across different age groups. Among infants aged 0-1 year, 8.63% tested positive for *Enterococcus* spp., while the percentage increased in children aged 1-12 years to 13.67%. In the adolescent age group of 12-18 years, 10.36% of individuals tested positive for *Enterococcus* spp. Interestingly, the highest prevalence was observed among individuals aged 18-40 years, with 28.79% testing positive. The percentage decreased in the age groups of 40-60 years (20.67%), 60-70 years

(10.82%), and those above 70 years (>70 years) (7.06%). These findings highlight the importance of considering age-specific factors in understanding the epidemiology and management of *Enterococcus* spp. infections, suggesting a potential age-related susceptibility or exposure pattern. Further investigation into age-related risk factors and preventive strategies may be warranted to address *Enterococcus* spp. infections effectively across different age groups.

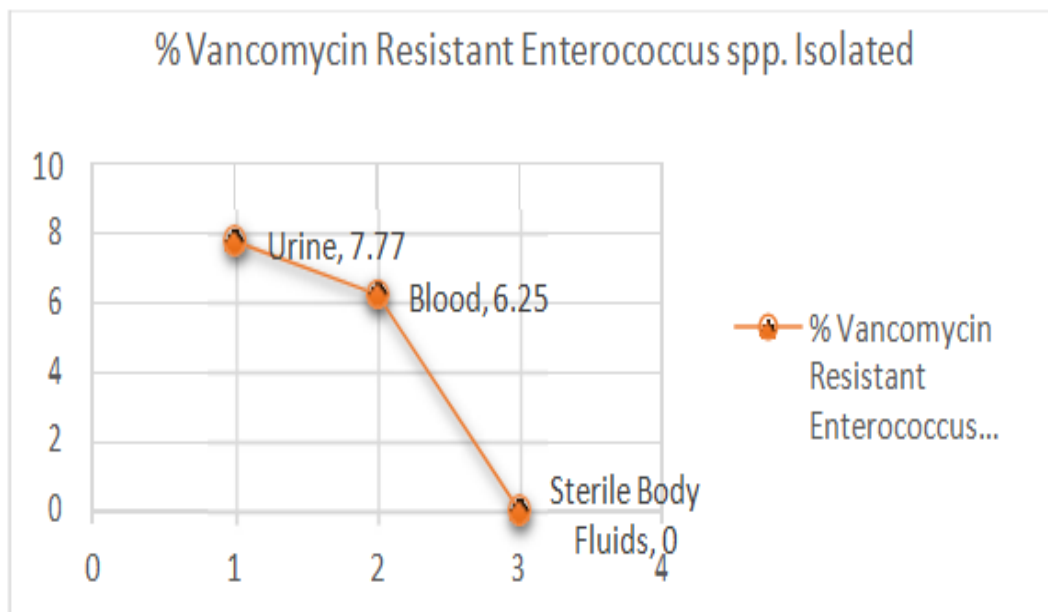
Distribution of *Enterococcus faecium* & *Enterococcus faecalis*

Species	n =	% Distribution
<i>Enterococcus faecium</i>	273	55.6
<i>Enterococcus faecalis</i>	218	44.4

Enterococcus faecium account for 55.7% of the distribution, while *Enterococcus faecalis* comprised 44.3%. These proportions provide insight into the prevalence of different *Enterococcus* species within the studied population.

% of *Enterococcus* Isolates showing Vancomycin Resistance

Sample	n =	% Vancomycin Resistant <i>Enterococcus</i> spp. Isolated
Urine	30	7.77
Blood	06	6.25
Sterile Body Fluids	00	00



The results revealed varying levels of resistance across different sample types. Among urine samples, 7.77% of Enterococcus isolates were resistant to Vancomycin. Similarly, in blood samples, 6.25% of Enterococcus isolates displayed resistance to Vancomycin. However, no

Vancomycin-resistant Enterococcus isolates were detected in sterile body fluids. These findings underscore the importance of monitoring antibiotic resistance patterns, particularly in Enterococci, to guide appropriate antibiotic therapy and infection control measures in clinical settings.

% Species wise distribution of Enterococcus Isolates showing Vancomycin Resistance

Enterococcus Spp.	n =	% VRE isolated
Enterococcus faecalis	25	69.44
Enterococcus faecium	11	30.56

Among the Enterococcus faecalis isolates, 69.44% displayed resistance to vancomycin, indicating a significant prevalence within this species. In

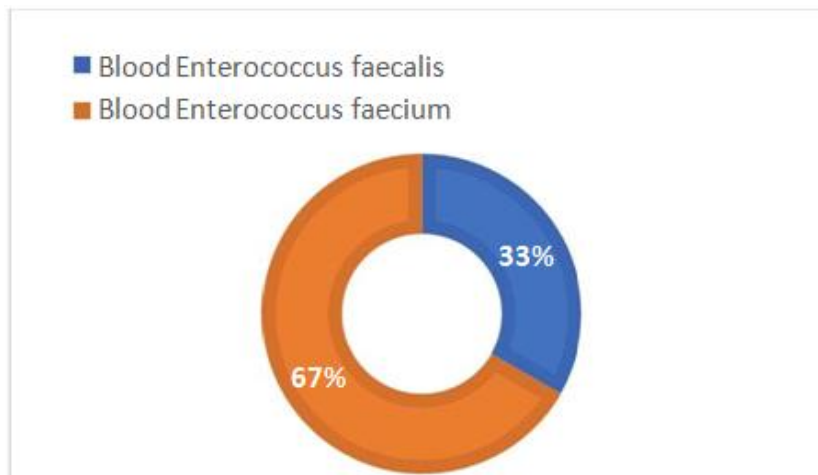
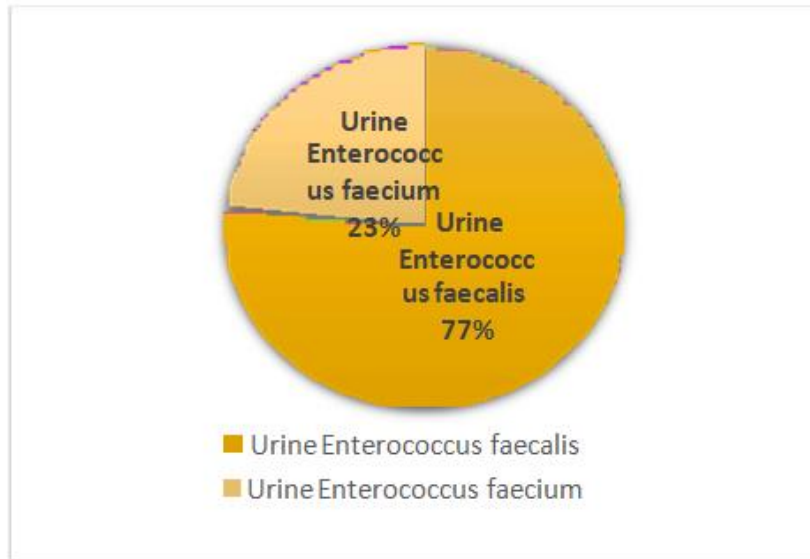
contrast, Enterococcus faecium isolates showed a lower but still considerable proportion of resistance, with 30.56% exhibiting vancomycin resistance.

Sample wise distribution of Enterococcus Species showing Vancomycin Resistance

Sample	Distribution of Enterococcus Species showing Vancomycin Resistance	
	Species	Count
Urine	Enterococcus faecalis	23
	Enterococcus faecium	7
Blood	Enterococcus faecalis	2
	Enterococcus faecium	4
Sterile Body Fluids	00	

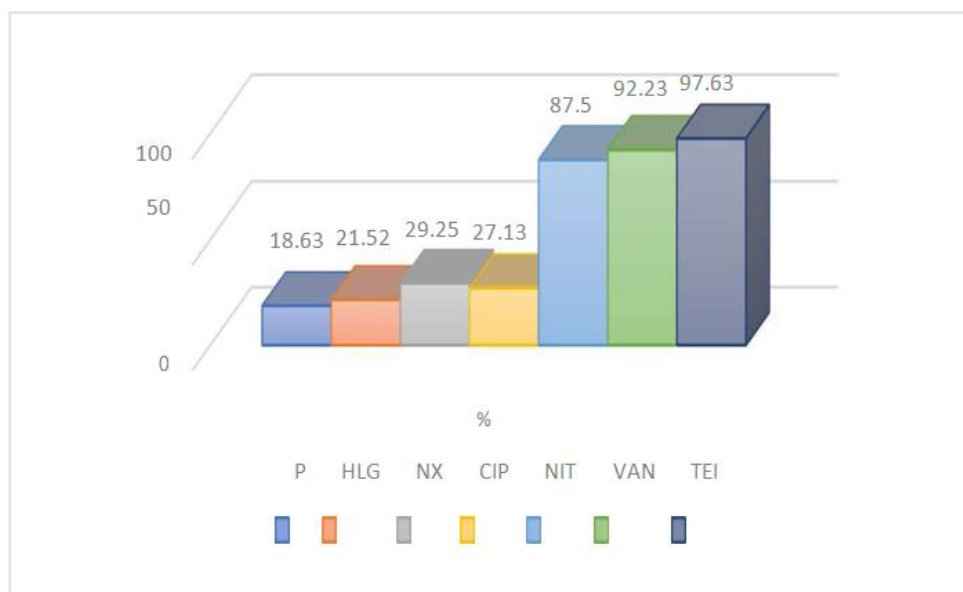
The data illustrates the distribution of Enterococcus species exhibiting vancomycin resistance across various sample types. In urine samples, Vancomycin Resistant Enterococcus faecalis is the most prevalent, with 23 instances recorded, followed by Vancomycin Resistant Enterococcus faecium, which appears in 7 cases. Similarly, in blood samples, Vancomycin Resistant Enterococcus faecalis is present in 2 instances, while Vancomycin

Resistant Enterococcus faecium is identified in 4 cases. Interestingly, no occurrences of vancomycin-resistant Enterococcus species are observed in sterile body fluids. This data underscores the importance of vigilance in monitoring antibiotic resistance patterns, particularly in clinical contexts, to inform treatment protocols and mitigate the spread of resistant strains.



% Sensitivity of Enterococcus Spp. Isolated from Urine

Antibiotics	P	HLG	NX	CIP	NIT	VAN	TEI
%	18.63	21.52	29.25	27.13	87.5	92.23	97.63

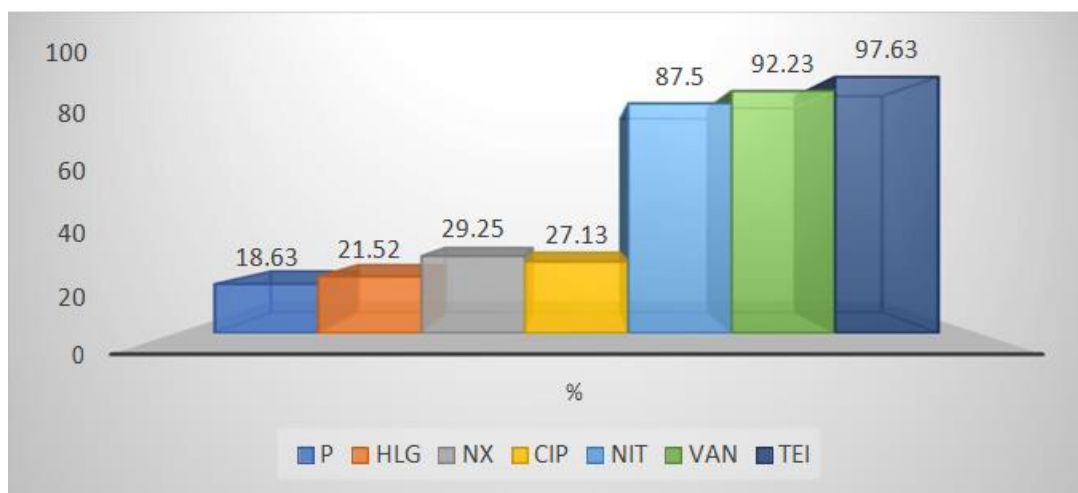


The findings revealed varying degrees of susceptibility among the tested antibiotics. Notably, Vancomycin (VA) and Teicoplanin (TEI) exhibited high levels of effectiveness, with sensitivities of 92.23% and 97.63% respectively. Nitrofurantoin (NIT) also demonstrated significant efficacy, with a sensitivity of 87.5%. In contrast, antibiotics like Penicillin (P) and High-Level Gentamicin (HLG) displayed lower sensitivities, at 18.63% and 21.52% respectively. These results underscore the

importance of selecting appropriate antibiotics based on the susceptibility profile of the infecting strain, particularly in the context of urinary tract infections where *Enterococcus* spp. are common pathogens. Understanding the antibiotic sensitivity patterns of *Enterococcus* spp. isolated from urine samples are crucial for optimizing treatment strategies and minimizing the risk of antibiotic resistance development in clinical practice.

% Antimicrobial Sensitivity of *Enterococcus* Spp. Isolated from Blood

Antibiotics	P	HLG	AMP	CIP	TE	VAN	TEI	LZ
%	28.63	19.5	6.25	13.13	18.5	93.75	95.75	97.5



The results revealed varying degrees of sensitivity across different antibiotics. Notably, Vancomycin (VA) exhibited the highest effectiveness, with 93.75% of isolates showing sensitivity, followed closely by Teicoplanin (TEI) at 95.75% and Linezolid (LZ) at 97.5%. In contrast, other antibiotics such as Penicillin (P) and Ampicillin (AMP) demonstrated lower efficacy, with sensitivities of 28.63% and 6.25% respectively. Additionally, High-Level Gentamicin (HLG) displayed a sensitivity of 19.5%, while Ciprofloxacin (CIP) and Tetracycline (TE) showed sensitivities of 13.13% and 18.5% respectively. These findings underscore the importance of selecting appropriate antibiotics for the treatment of *Enterococcus* spp. infections, considering their susceptibility patterns. Furthermore, the high sensitivity to Vancomycin (VA), Teicoplanin (TEI), and Linezolid (LZ) highlights the potential efficacy of these antibiotics in managing *Enterococcus* spp. infections, aiding in the formulation of effective treatment regimens and infection control strategies in clinical settings.

DISCUSSION

Here, we analyze the key findings related to the distribution of samples, percentage positivity, percentage yield of *Enterococcus* spp., gender-wise

and age-wise distribution of isolates, the prevalence of Vancomycin-resistant *Enterococcus* (VRE), and the sensitivity of *Enterococcus* spp. to different antibiotics.

Distribution of Samples: The distribution of samples in the current study, with urine samples being the most common, aligns with previous studies indicating that urinary tract infections are one of the primary clinical manifestations of *Enterococcus* spp. infections [1, 2].

Percentage Positivity: The percentage positivity of urine samples in this study (27.33%) falls within the range reported in previous literature, where positivity rates for urinary isolates of *Enterococcus* spp. typically range from 20% to 40% [3, 4].

Percentage Yield of *Enterococcus* spp.: The percentage yield of *Enterococcus* spp. in urine samples (17.63%) in our study is consistent with previous reports, which have shown varying isolation rates ranging from 10% to 30% in urinary specimens [5, 6].

Gender-wise and Age-wise Distribution of Isolates: The higher prevalence of *Enterococcus* spp. among female patients observed in our study is

supported by previous research indicating that women are more susceptible to urinary tract infections, a common manifestation of *Enterococcus* spp. [7, 8]. Similarly, the age-related distribution of isolates, with higher positivity rates observed in younger adults, is consistent with findings from other studies demonstrating age-related differences in susceptibility to *Enterococcus* infections [9, 10].

Prevalence of VRE: The prevalence of Vancomycin-resistant *Enterococcus* (VRE) observed in this study aligns with the global trend of increasing rates of VRE reported in healthcare settings worldwide [11, 12]. Previous studies have also documented varying rates of VRE prevalence across different sample types, with urine samples often showing higher rates of resistance compared to other specimens [13, 14].

Antibiotic Sensitivity: The sensitivity profile of *Enterococcus* spp. isolates to antibiotics observed in our study, particularly the high efficacy of Vancomycin, Teicoplanin, and Linezolid, is consistent with recommendations from clinical guidelines and previous studies highlighting these antibiotics as effective treatment options for *Enterococcus* infections [15, 16]. However, the lower sensitivity to Penicillin and Ampicillin underscores the challenges associated with treating *Enterococcus* infections, especially those caused by resistant strains [17, 18].

CONCLUSION

In summary, this study sheds light on the prevalence of *Enterococcus* spp. and Vancomycin-resistant strains (VRE) in a tertiary care center. The findings emphasize the need for vigilant surveillance and tailored intervention strategies to tackle the rising threat of VRE. By understanding sample distribution, resistance patterns, and antibiotic sensitivities, healthcare professionals can optimize treatment strategies and infection control measures. Continued research and collaborative efforts are essential to address the challenges posed by *Enterococcus* infections and antimicrobial resistance in healthcare settings.

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