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Prevalence and Antimicrobial Resistance Patterns of *Enterococcus* spp. with Special Reference to Vancomycin-Resistant Enterococci in Clinical Isolates from a Rural Tertiary Care Hospital in Indore, Central India.

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Abstract-

Introduction- Vancomycin-resistant enterococci (VRE) are opportunistic pathogens known to cause a wide range of clinical infections. Globally, VRE have emerged as a significant concern in healthcare settings, particularly among hospitalized patients, and have been implicated in numerous nosocomial outbreaks. The primary mechanism underlying vancomycin resistance in enterococci is the alteration of peptidoglycan precursors, which leads to reduced binding affinity for glycopeptide antibiotics, thereby diminishing their therapeutic efficacy.

Aim- The objective of this study was to assess the prevalence and antimicrobial resistance patterns of vancomycin-resistant enterococci (VRE) isolated from both outpatient attendees and inpatients admitted to various wards of the hospital.

Material and Methods- A prospective cross-sectional study was carried out over a period of two years in the Department of Microbiology at Index Medical College and Research Centre, Indore, Madhya Pradesh. All Enterococcus isolates recovered from clinical specimens—including blood, urine, pus, sputum, wound swabs, catheter tips, and other body fluids—were included in the study. A total of 112 Enterococcus isolates were obtained using standard conventional culture techniques and confirmed through biochemical identification. Antimicrobial susceptibility testing was performed using the Kirby-Bauer disc diffusion method in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines. Minimum inhibitory concentrations (MICs) for vancomycin were determined using the broth dilution method.

Results- Out of the 112 *Enterococcus* isolates, 69 (62%) were obtained from inpatients, while 43 (38%) were isolated from outpatients. The majority of isolates were from female patients (65%), compared to males (35%). The highest incidence of infection was observed in individuals aged 31–40 years. Among the species identified, *E. faecalis* was the most prevalent, accounting for 61% of the total isolates. Regarding sample distribution, the majority of isolates were recovered from urine samples (51%), followed by pus (15%), blood (14%), body fluids (7%), wound swabs (3%), and high vaginal swabs (HVS) (2%). Vancomycin resistance was detected in 23.2% of isolates using the disc diffusion method, while the broth dilution method confirmed resistance in 18.75% of cases.

Conclusion- Our findings underscore the prevalence of *Enterococcus* species and the proportion of isolates exhibiting vancomycin resistance, which remains a significant concern for healthcare professionals due to the therapeutic challenges posed by such infections. To effectively address the growing threat of vancomycin-resistant enterococci (VRE), it is imperative to encourage the rational use of antibiotics guided by antimicrobial susceptibility testing. Moreover, the implementation of stringent infection control measures—including early identification of carriers and adherence to contact precautions—is essential to limiting the nosocomial transmission of VRE and safeguarding patient outcomes.

***Key words*-** VRE, NSS, CLED, CLSI, AST, *Enterococcus faecalis*, vanA, vanB.

INTRODUCTION

Enterococci are Gram-positive, ovoid-shaped bacteria that typically appear in pairs or short chains under microscopic examination. Due to their morphological resemblance, they may be mistaken for *Streptococcus pneumoniae*. After 18–24 hours of incubation at 35°C to 37°C, *Enterococcus* species produce small colonies measuring 1–2 mm in diameter, which may exhibit α-, β-, or γ-haemolysis. These organisms grow optimally on 5–10% sheep blood agar, and most species can also thrive on nutrient agar at 45°C. Certain strains demonstrate notable environmental tolerance, including growth in the presence of 6.5% sodium chloride, survival at temperatures up to 50°C, and at a pH of 9.6. Remarkably, they can withstand exposure to 60°C for up to 30 minutes. Group D streptococci, which include enterococci, can be differentiated from other streptococcal groups by their rapid hydrolysis of aesculin in the presence of 40% bile salts, supporting growth on bile-containing media [1,2].

Enterococcus species are commensal organisms commonly found in the gastrointestinal tracts of humans and animals [3]. Their environmental presence is often associated with fecal contamination, and their capacity to endure harsh conditions makes them resilient and widespread in various ecological niches [4]. Clinically, they are recognized as opportunistic pathogens capable of causing

a wide range of infections. According to data from the Centers for Disease Control and Prevention (CDC)'s National Nosocomial Surveillance Survey (NSS), enterococci are responsible for approximately 16% of hospital-acquired urinary tract infections (UTIs) [5]. Beyond UTIs, they have been implicated in a variety of infections including biliary tract infections, intra-abdominal and pelvic abscesses, bacteremia, sepsis, infections associated with intravascular catheters, and infections of wounds, burns, bones, and the pleural cavity. Additionally, they can cause endocarditis, hydroceles, and infections in peripartum women and neonates. Although less common, respiratory and central nervous system infections have also been reported [6,7,8].

The principal mechanism of vancomycin resistance in enterococci involves alterations in peptidoglycan precursors, which diminish the binding affinity of glycopeptide antibiotics [9]. To date, eight phenotypic variants of acquired vancomycin resistance have been identified in *Enterococcus* species: VanA, VanB, VanD, VanE, VanG, VanL, VanM, and VanN [10,11].

The present study aims to evaluate the antimicrobial susceptibility profiles of clinical *Enterococcus* isolates, perform species-level identification, determine the prevalence of vancomycin-resistant enterococci (VRE), and detect the presence of vancomycin resistance genes. The outcomes of this research are expected to support the development of robust infection control strategies and antimicrobial stewardship programs, particularly within healthcare facilities in and around Indore, Madhya Pradesh.

AIMS AND OBJECTIVES

The objective of this study was to assess the prevalence and antimicrobial resistance patterns of vancomycin-resistant enterococci (VRE) isolated from both outpatient attendees and inpatients admitted to various wards of the hospital.

MATERIAL AND METHODS

A prospective cross-sectional study was conducted in the Department of Microbiology, Index Medical College, Indore M.P. for a period of 2 years.

Inclusion Criteria: All *Enterococcus* isolates obtained from clinical specimens—including blood, urine, pus, sputum, wound swabs, catheter tips, and other body fluids—were included in the study.

Exclusion criteria: All commensal *Enterococcus* isolates obtained from anatomical sites such as the gastrointestinal tract, female genital tract, stool, and oropharyngeal (throat) swabs were excluded from the study.

SAMPLE COLLECTION AND PROCESSING

Clinical samples including pus, wound swabs, blood, urine, endotracheal aspirates, sputum, and other body fluids were collected from patients. Pus and wound discharge were obtained using sterile cotton swabs (HiMedia), while blood samples were collected aseptically and transported to the laboratory in brain heart infusion (BHI) broth. Clean-catch midstream urine samples were collected in sterile, wide-mouthed containers and transported to the laboratory immediately after collection.

All samples were inoculated on appropriate culture media: blood agar and MacConkey agar for most specimens, while urine samples were specifically inoculated on cystine lactose electrolyte-deficient (CLED) agar. *Enterococcus* species were identified based on colony morphology, Gram staining, motility testing, and standard biochemical methods as described in established microbiological protocols [12,13,14].

Antimicrobial susceptibility testing: AST were performed by Kirby -Bauer disc diffusion method on MHA for all enterococci isolates result were interpreted according to the guideline laid down by the CLSI Guidelines 2024. Antimicrobial susceptibility testing in the presence of any potential growth was determined using the disc diffusion method according to the CLSI guidelines. The antimicrobial which was tested included: Discs of Ampicillin (10 µg), erythromycin (30µg), High Level Gentamicin (120µg), High Level streptomycin (300µg), Linezolid (10 µg), Teicoplanin (30 µg), Tetracycline (10µg), Tigecycline (15µg), Ciprofloxacin (5µg), Norfloxacin (10 µg), and Nitrofurantoin (300 µg) Vancomycin (30 µg), Levofloxacin (5µg).

Detection of Vancomycin resistant enterococci: The broth dilution method was employed to determine the minimum inhibitory concentration (MIC) of vancomycin. This method is considered more accurate and reliable than the disc diffusion technique for assessing vancomycin susceptibility [15-19]. Mueller-Hinton agar plates were prepared with varying concentrations of vancomycin. A 10 µL aliquot of standardized bacterial suspension was inoculated onto each plate, followed by incubation at 37°C for 18–24 hours. The MIC was defined as the lowest concentration of vancomycin that completely inhibited visible bacterial growth, in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines, 2025. According to CLSI breakpoints, *Enterococcus* isolates were categorized as vancomycin-resistant if the MIC was ≥32 µg/mL, intermediately resistant if the MIC ranged from 8 to 16 µg/mL, and susceptible if the MIC was ≤4 µg/mL.

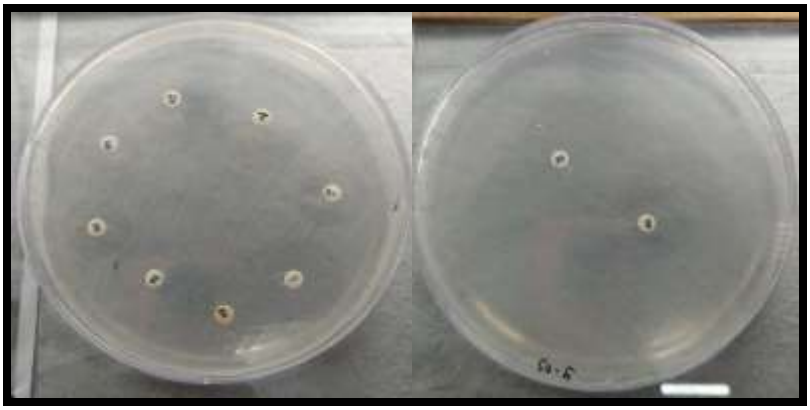

Fig 1: Vancomycin resistant and sensitive result by disc diffusion method

RESULTS

During the course of this prospective study, a variety of clinical specimens were collected from patients and processed in the Department of Microbiology, Index Medical College, Indore (Madhya Pradesh). A total of 112 *Enterococcus* isolates were recovered from these samples. Of these, 69 isolates (62%) were obtained from inpatients, while the remaining 43 (38%) were from outpatients.

The isolation rate was higher among female patients (65%) compared to males (35%). The highest prevalence of infection was observed in the 31–40-year age group. Among the isolates, *E. faecalis* was the most frequently identified species, accounting for 61% of the total, followed by *E. faecium* (38.2%) and *E. gallinarum* (0.8%). In terms of sample distribution, the majority of isolates were recovered from urine samples (51%), followed by pus (15%), blood (14%), body fluids (7%), wound swabs (3%), and high vaginal swabs (2%). Vancomycin resistance among the clinical *Enterococcus* isolates was assessed using both the disc diffusion and broth dilution methods. Resistance was detected in 26 isolates (23.2%) by the disc diffusion method, while 21 isolates (18.75%) were confirmed as vancomycin-resistant by the broth dilution method. Among the 21 vancomycin-resistant isolates, *Enterococcus faecium* was the predominant species, representing 71.4% of the total. This was followed by *E. faecalis* (23.8%) and *E. gallinarum* (4.8%).

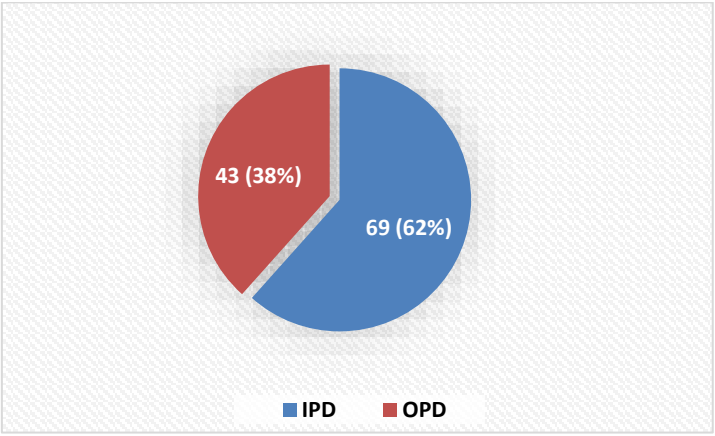

Fig 2: Distribution of clinical isolates of *Enterococci* among IPD and OPD Patients (n=112)

Sample	Number	Percent
Urine	62	51%
Blood	17	14%
Pus	19	15%
Wound swab	03	3%
High vaginal swab	02	2%
Body Fluid	09	7%

Table 1: Sample-wise prevalence of *Enterococci* (n=112)

Antibiotic agent	Sensitive No (%)	Intermediate sensitive No (%)	Resistant No (%)
Ampicillin	37(33%)	-	75(66.9%)
Erythromycin	29(26%)	-	83(77.6%)
Ciprofloxacin	31(29.4%)	-	81(72.3%)
Daptomycin	47(42%)	-	65(58%)
High level Gentamicin	73(65.1%)	-	39(34.8%)
High level streptomycin	82(73.2%)	-	30(26.7)
Linezolid	109(97.3%)	-	3(2.6%)
Teicoplanin	97(86.6%)	-	15(13.3%)
Tetracycline	92(82.1%)	-	20(77.6%)
Tigecycline	78(69.6%)	-	34(30.3%)
Vancomycin	98(87.5%)	3(2.6%)	21(18.7%)
Nitrofurantoin	76(67.8%)	4(3.5%)	32(28.5%)
Norfloxacin	37(33%)	-	75(66.9%)
Levofloxacin	9(8%)	1(0.8%)	7(6.2%)

Table 2- AST pattern of Enterococci

Name Of antibiotics	Sensitive- No.	Intermediate sensitive- No	Resistant No.
Ampicillin	1	-	20
Erythromycin	1	-	20
Ciprofloxacin	5	-	16
Daptomycin	5	-	16
High level Gentamicin	4	-	17
High level Streptomycin	19		2
Linezolid	7	-	14
Teicoplanin	7	-	14
Tetracycline	6	-	15
Tigecycline	6	-	15
Vancomycin	0	-	21

Table 3- AST pattern of VRE

Method	Resistant	Percentage
Disc diffusion	26	23.2%
Broth Dilution Microtiter Plate	21	18.8%

Table 4: Comparison of Broth Dilution Method and Disc Diffusion method for Vancomycin resistance detection in *Enterococcus species*

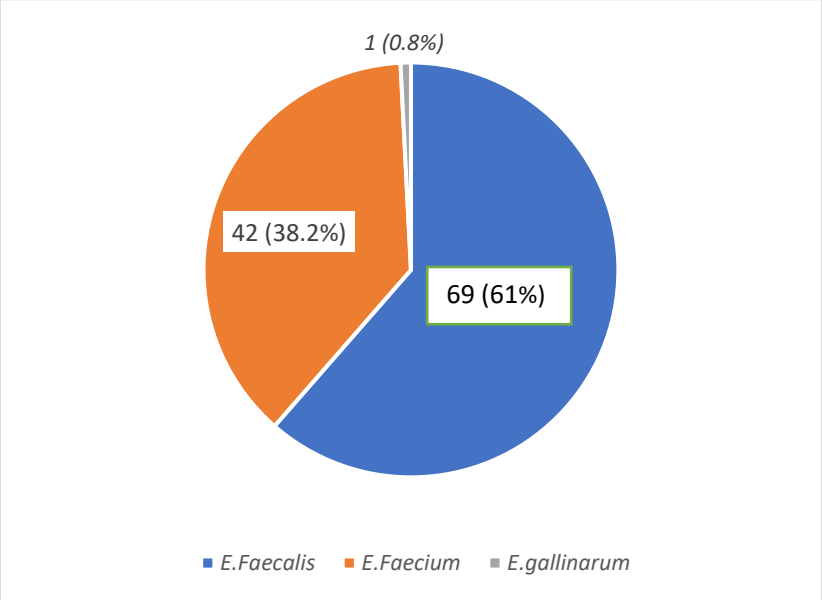

Fig 3: Different Enterococci species isolated (n=112)

Species	Number	Percentage
E. feacium	15	71.4%
E. faecalis	5	23.8%
E. gallinarum	1	4.8%

Table 5: Distribution of VRE Species (n=21)

Gender	Number	Percentage
Male	9	(42.8%)
Female	12	(57.2%)

Table 6: Gender wise distribution of VRE (n=21)

DISCUSSION

In this study, a total of 112 *Enterococcus* isolates were recovered, with 69 (62%) obtained from inpatient (IPD) samples and 43 (38%) from outpatient (OPD) specimens. Species-level identification revealed *E. faecalis* as the predominant organism (61%), followed by *E. faecium* (38.2%) and *E. gallinarum* (0.8%). These findings are consistent with those reported by Verma B.S. et al. (2024), who documented *E. faecalis* as the most common species (64.2%), followed by *E. faecium* (23.58%), *E. durans* (13.2%), and *E. avium* (8.49%). In our study, 55% of the *E. faecalis* isolates were derived from urine samples.

Antimicrobial susceptibility testing showed that 66.9% of the isolates were resistant to ampicillin, 34.8% to high-level gentamicin, and 26.7% to high-level streptomycin. A high level of resistance was also observed against erythromycin (77.6%) and ciprofloxacin. In contrast, linezolid and teicoplanin demonstrated the highest levels of susceptibility. Among urinary isolates specifically, 66.9% were resistant to norfloxacin and 38.5% to nitrofurantoin. These resistance patterns are comparable to findings by Ohri S. et al. (2023), who studied *Enterococcus* spp. in Amritsar, Punjab.

With respect to vancomycin resistance, *E. faecium* demonstrated the highest rate (13.3%), followed by *E. faecalis* (4.46%) and *E. gallinarum* (0.8%), resulting in an overall vancomycin-resistant *Enterococcus* (VRE) prevalence of 18.75%. These findings align

with those reported by Shrestha et al. (2021). Additionally, our study confirmed that *E. faecium* exhibited higher resistance overall compared to *E. faecalis*.

Vancomycin resistance was assessed using both disc diffusion and broth dilution methods. The disc diffusion technique identified 26 resistant isolates (23.2%), while the broth dilution method confirmed vancomycin resistance in 21 isolates (18.75%).

CONCLUSION

In our study, the high prevalence of *Enterococcus* species and their multidrug resistance patterns underscore a significant clinical concern, particularly due to the therapeutic difficulties they pose in treating *Enterococcal* infections. Vancomycin resistance among these isolates has remained relatively steady in recent years, with reported rates ranging between 8% and 18%. While vancomycin was historically the antibiotic of last resort for *Enterococcal* infections, newer agents such as linezolid, teicoplanin, and quinupristin-dalfopristin are now being widely used to manage Vancomycin-resistant Enterococcus (VRE) infections.

Our findings reaffirm the need for continuous surveillance of antimicrobial resistance and reinforce the importance of antimicrobial stewardship. Rational antibiotic use guided by susceptibility testing is essential to prevent further resistance development. Moreover, timely identification of VRE carriers is vital, as delayed diagnosis can facilitate nosocomial transmission. Implementing stringent infection control measures—including contact precautions, active screening, and isolation protocols—is crucial to reducing the spread of VRE within healthcare facilities.

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