Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 32s (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

Isolation, Phenotypic Characterization and speciation of Vancomycin Resistant Enterococci from Clinical Samples

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Cite this paper as: Muskan Bhardwaj, Siva Prasad Reddy, (2025) Isolation, Phenotypic Characterization and speciation of Vancomycin Resistant Enterococci from Clinical Samples. *Journal of Neonatal Surgery*, 14 (32s), 6923-6928.

ABSTRACT

Background: Vancomycin-resistant enterococci (VRE) represent a growing concern in nosocomial infections due to limited treatment options and their intrinsic and acquired antimicrobial resistance. This study focused on the isolation, phenotypic characterization, and speciation of VRE from diverse clinical samples collected at a tertiary care center in Northern India.

Objectives: To identify and differentiate Enterococcus species from clinical specimens, determine their antimicrobial susceptibility patterns, and estimate the prevalence of vancomycin resistance among these isolates.

Methods:A cross-sectional observational study was conducted from June 2023 to September 2024. Enterococci were isolated using conventional culture methods from blood, urine, pus, cerebrospinal fluid, and other body fluids. Biochemical tests and selective media such as bile esculin azide agar and potassium tellurite agar were used for speciation. Antimicrobial susceptibility testing was performed by the Kirby–Bauer disk diffusion method according to CLSI M100 standards. Vancomycin resistance was confirmed using vancomycin screen agar.

Results:A total of 259 Enterococcus isolates were recovered; Enterococcus faecalis constituted 65.25% (n=169) and Enterococcus faecium 34.75% (n=90). The isolates predominantly originated from urine (47.8%) and blood (21.6%). Vancomycin resistance was observed in 4.1% of E. faecalis and 3.3% of E. faecium isolates. High resistance rates were noted for erythromycin (64.6%), penicillin (59.8%), and ampicillin (36.7%). Linezolid, teicoplanin, and vancomycin retained high sensitivity (>95%) among isolates.

Conclusion:E. faecalis was the predominant species isolated with a low but notable prevalence of vancomycin resistance. Judicious antibiotic use, early detection, and strict infection control measures are essential to controlling the spread of multidrug-resistant enterococci in healthcare settings..

Keywords: *Vancomycin-resistant enterococci, Enterococcus faecalis, Enterococcus faecium, antimicrobial susceptibility, nosocomial infections.*

1. INTRODUCTION

Enterococci are currently thought to be a significant cause of nosocomial infections because they are primarily commensals found in human feces. Among the infections, urinary tract infections are the most frequently seen, followed by abdominal infections,

endocarditis, meningitis, bacteremia, and surgical site infections seldom.[1,2] Originally identified as gram-positive cocci, enterococci were subsequently categorized under the Streptococcus genus. Enterococci were categorized as group D based on a battery of biochemical tests and Lancefield serological typing. Group D streptococci, which distinguishes them from non-enterococcal ones.[2,3] E. faecalis (80–90%) and E. faecium (5–10%) are the two most common Enterococci that infect humans [4,5]. Their growing significance is caused by their inherent resistance to a number of common antimicrobials as well as their capacity to develop resistance to a number of others through mutation or the transfer of plasmids and transposons.[6,7] Vancomycin-resistant enterococci (VRE) have become one of the main causes of nosocomial infections worldwide since their first report in 1988. This is especially concerning because it has reduced the number of treatment options available to clinicians.[8,9] According to genotypic analyses, nine different genes—vanA, vanB, vanC, vanD, vanE, vanG, vanL, vanM, and vanN—mediate vancomycin resistance. E. faecalis and E. faecium are primarily linked to Van A and Van B.[11,12] A study was carried out at a tertiary care facility in Northern Rajasthan with the goal of characterizing

the phenotypic resistance to vancomycin isolates and estimating the drug resistance to various antimicrobials through an analysis of the antibiogram, given the growing prevalence of VRE in the Indian healthcare system.[13,14]

2. MATERIALS AND METHODS

The present study was an observational, cross-sectional study conducted at a tertiary care center from North India from June 2023 to September 2024 in which Enterococci isolates from different clinical specimen such as blood, pus, urine and other body fluids were included in the study

Standard procedures were followed in processing the specimens using conventional microbiological culture techniques. In short, items other than blood and urine were inoculated on MacConkey agar (HiMedia Laboratories, India) and 5% sheep blood agar (HiMedia Laboratories, India). In addition, Enterococcus species were grown using bile esculin azide agar as a selective medium. Aerobic incubation of the culture media lasted 48–72 hours, depending on the material.

Cysteine Lactose Electrolyte Deficient (CLED) medium (HiMedia Laboratories, India) was used for urine culture, and it was incubated aerobically for up to 24 hours. Brain heart infusion (BHI) broth (HiMedia Laboratories, India) was used to inoculate 5–10 ml of blood for blood culture, and the mixture was then cultured aerobically for up to 5 days.

All specimens except urine, which was subjected to wet mount examination to determine the type and quantity of cells, such as pus cells (≥104 CFU/ml correlated with pyuria), were examined under a microscope for correlation with the culture isolates using Gram's staining. Enterococci were observed as Gram-positive cocci grouped in pairs on Gram's staining.

Conventional biochemical tests were used to identify the isolates in accordance with published standard techniques [16]. In order to show a lack of effervescence consistent with a negative result suggesting the presence of Streptococci/Enterococci, colonies identified as Gram-positive cocci were exposed to 3% hydrogen peroxide. Enterococci were biochemically identified using the bile esculin hydrolysis test and growth in 6.5% NaCl. Additionally, the isolates were subcultured onto potassium tellurite agar (HiMedia Laboratories, India) in order to differentiate between Enterococcus faecalis, which generates black colonies, and Enterococcus faecium/other spp., which produces negative or no black colonies.

Using the Kirby Bauer disk diffusion method and Muller Hinton agar (HiMedia Laboratories, India), the Antimicrobial Susceptibility test (AST) was conducted in accordance with CLSI standards M100 [17]. Ampicillin (10µg), High level gentamicin (120µg), Erythromycin (15µg), Vancomycin (30µg), Teicoplanin (30µg), and Linezolid (15µg) were the antimicrobial discs utilized for disc diffusion tests. Ampicillin (30µg), High level gentamicin (120µg), Levofloxacin (5µg), Norfloxacin (10µg), Nitrofurantoin (300µg), Vancomycin (30µg), Teicoplanin (30µg), and Linezolid (30µg) antibacterial discs were employed for urine isolates. The plates were read under transmitted light after being kept at 37°C for the entire day.

When the zone size surrounding vancomycin was less than 14 mm, the isolate was deemed resistant to the antibiotic. Vancomycin screen agar, which was made by mixing 6 µg/ml vancomycin with brain heart BHI agar, was also used to test for vancomycin resistance. One or more Enterococcus spp. colonies growing was thought to be a sign of vancomycin resistance. Using strains of E. faecalis ATCC 29212 and E. faecium ATCC 51559 that were accessible in the lab, appropriate controls were employed during culture and AST.

3. RESULTS

A Total of 259 Enterococci isolates were extracted from clinical specimens. With 59.46% of the patients being male and 40.5% being female, the patients' mean age was 44.3 years (range: 2–83 years). 4.6% of the patients were OPD patients, while 95.4% were the hospitalized patients.

Out of these 259 isolates, 169(65.25%) were identified as *Enterococcus faecalis* while the rest 90(34.75%) were *Enterococcus faecium* [Table/Fig:1]. These enterococci isolates were obtained from various specimens, the commonest being urine (*n*=124, 47.8%), blood (*n*=56, 21.6%),ET (n=1,0.4%), body fluids from normally sterile sites (*n*=12, 4.25%), pus (*n*=56, 21.6%) and CSF (*n*=10, 3.86%). Among these isolates, a total of 10 were resistant to vancomycin.

Sample	**E. Faecalis**		**E. Faecium**		
	Number (n)	Percentage (%)	Number (n)	Percentage (%)	P Value
urine	86	33.2	38	14.67	

Blood	36	13.9	20	7.72	0.36
CSF	04	1.54	06	2.32	
Pus	34	13.13	22	8.5	
Body Fluid	08	3.1	04	1.54	
ET	01	0.4	00	00	
Total	169	65.25	90	34.75	

Table/Fig:1 Distribution of Enterococcus species in various clinical specimens

Among the 259 isolates, rate of resistance for erythromycin (64.6%) then for penicillin (59.8%), and E. Faecium also shows maximum resistance for erythromycin (76.5%) then for penicillin (57.8%). E. faecalis showed resistance mainly for ampicillin (36.7%), Fosfomycin (32.6%) norfloxacin (45.3%) doxycycline (41.1%) and E. faecium showed resistance mainly for ampicillin (31.1%), Fosfomycin (34.2%), norfloxacin (44.7%), chloramphenicol (38.5%). Maximum sensitivity of E. Faecium is for linezolid (96.7%), vancomycin (96.7%) and teicoplanin (96.7%), but in E. Faecalis it is showing maximum sensitivity linezolid (97.6%), vancomycin (95.9%) and teicoplanin (97.6%). Resistance to vancomycin was 4.1% in E. Faecalis and 3.3 % are resistant in E. Faecium [Table/Fig:2]

AST	**E. Faecalis (n)**		**Percentage (%)**		**E. Faecium (n)**		**Percentage (%)**		**p- Value**
	R	S	R	S	R	S	R	S	
P	101	68	59.8	40.2	52	38	57.8	42.2	0.76
AMP	62	107	36.7	63.3	28	62	31.1	68.9	0.37
VA	07	162	4.1	95.9	03	87	3.3	96.7	0.75
LZ	04	165	2.4	97.6	03	87	3.3	96.7	0.65
TEI	04	165	2.4	97.6	03	87	3.3	96.7	0.65
TE	22	147	13.0	87.0	06	84	6.7	93.3	0.12
HLG	42	127	24.9	75.1	15	75	16.7	83.3	0.13
CIP	17	152	10.1	89.9	08	82	8.9	91.1	0.75
LE	13	156	7.7	92.3	06	84	6.7	93.3	0.76
FO	28	58	32.6	67.4	13	25	34.2	65.8	0.85
NX	39	47	45.3	54.7	17	21	44.7	55.3	0.94
NIT	12	46	20.7	79.3	07	31	18.4	81.6	0.78

E	53	29	64.6	35.4	39	12	76.5	23.5	0.15
DO	69	99	41.1	58.9	29	61	32.2	67.8	0.16
MI	24	59	28.9	71.1	16	36	30.8	69.2	0.82
C	25	58	30.1	69.9	20	32	38.5	61.5	0.32
TGC	14	33	29.8	70.2	06	25	19.4	80.6	0.30

Table/Fig-2: Antimicrobial susceptibility patterns of enterococci by Kirby- Bauer disc diffusion method

4. DISCUSSION

The present study reports a higher prevalence of Enterococcus faecalis (65.25%) as compared to Enterococcus faecium (34.75%). In a systemic review and meta- analysis by Smout et al, a similar hospital based research in which Enterococcus faecalis was found to be the most prevalent isolate[15]. Similar findings were reported by Sivaradjy et al [16] and Phukan et al [17]. While In another investigation conducted in New Delhi Enterococcus faecalis was isolated more frequently than Enterococcus faecium [18].

The selection pressure imposed by the usage of cell wall acting drugs, such as vancomycin, is responsible for the development of drug resistance in enterococci [19], Vancomycin-resistant enterococci pose a concern to people for additional reasons. In addition to being one of the main contributors to drug-resistant organisms that cause potentially fatal nosocomial infections, they can also use horizontal gene transfer mechanisms to spread vancomycin resistance gene clusters to strains of Staphylococcus aureus, including methicillin-resistant Staphylococcus aureus (MRSA) [20,21].

According to earlier research, the isolates exhibited extremely high resistance (over 90%) to tetracycline, fluoroquinolones like ciprofloxacin and levofloxacin, and beta lactam medicines like ampicillin and penicillin [12]. 80% of VRE isolates also showed high levels of gentamicin resistance, which is consistent with earlier research [22].

In the current investigation, over 95% of the isolates of enterococci were susceptible to linezolid, teicoplanin, tigecycline, doxycycline, and minocycline, demonstrating reasonable susceptibility patterns. These medications remain the cornerstone of treatment despite prior research showing inconsistent outcomes from these antimicrobials when applied to VRE isolates [12].

5. CONCLUSION

The prevalent isolates in the present study were *E. faecalis* and *E. faecium* that demonstrated approximately 4.1% and 3.3% resistance to vancomycin by phenotypic method. The prevention and control of the spread of multidrug-resistant Enterococcal infections can be achieved through the efforts of the hospital's various departments, including staff education, the rational use of antibiotics, laboratory early detection and reporting, and the implementation of suitable infection control measures.

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