e-ISSN: 0976-822X, p-ISSN:2961-6042

Available online on http://www.ijcpr.com/

International Journal of Current Pharmaceutical Review and Research 2026; 18(2); 1853-1858

Original Research Article

Isolation, Characterization and Antibiotic Susceptibility Pattern of Enterococci from Various Clinical Samples at a Radha Devi Jageshwari Memorial Medical college and Hospital in Bihar

Rajeev Ranjan[1], Rajesh Kumar Jaiswal[2], Neelam Kumari[3], Ram Shanker Prasad[4]

[1]Assistant Professor, Department of Microbiology, Radha Devi Jageshwari Memorial Medical college and Hospital, Turki, Muzaffarpur, Bihar, India

[2]Assistant Professor, Department of Microbiology, Radha Devi Jageshwari Memorial Medical college and Hospital, Turki, Muzaffarpur, Bihar, India

[3]Associate Professor, Department of Biochemistry, ESICMCH, Bihta, Patna, Bihar, India

[4]Professor, Department of Microbiology, Radha Devi Jageshwari Memorial Medical college and Hospital, Turki, Muzaffarpur, Bihar, India

Received: 24-12-2025 / Revised: 23-01-2026 / Accepted: 25-02-2026

Corresponding Author: Dr. Neelam Kumari

Conflict of interest: Nil

Abstract:

Background: Enterococcus species have become prominent healthcare-associated pathogens because of their capacity to endure harsh climatic circumstances and to acquire resistance to multiple antimicrobial agents, including vancomycin. In this investigation, Enterococcus isolates obtained from a variety of clinical specimens in a tertiary-care hospital in Bihar, India, were evaluated for prevalence, species distribution, and antibiotic susceptibility.

Methods: Two hundred consecutive clinical specimens that yielded Enterococcus spp. between January 2025 and May 2025 were analysed. Standard microbiological techniques, including as Gram staining, culture morphology, and biochemical analysis, were used to identify the isolates. In compliance with CLSI 2025 recommendations, the Kirby-Bauer disk-diffusion technique was used to conduct antimicrobial susceptibility testing. Vancomycin resistance and high-level aminoglycoside resistance (HLAR) were assessed using recognized phenotypic techniques.

Results: Among 200 isolates, 110 (55%) originated from urine, 40 (20%) from pus or wound swabs, 30 (15%) from blood, and 20 (10%) from other clinical samples. E. faecalis predominated (60%), followed by E. faecium (35%) and other species (5%). Resistance was most frequent to ampicillin (55%), ciprofloxacin (65%), and tetracycline (60%). HLAR was detected in 33% (gentamicin) and 20% (streptomycin). Vancomycin resistance occurred in 11.5% of isolates—mainly E. faecium (24.3%). Nearly all isolates remained susceptible to linezolid (98.5%) and daptomycin (96%). Nitrofurantoin was active against 82% of urinary isolates. Intensive-care admission and prior antibiotic exposure were significantly associated with VRE isolation ($p < 0.05$).

Conclusions: Enterococcus, particularly E. faecium, demonstrated substantial resistance to commonly prescribed agents, whereas linezolid and daptomycin retained excellent activity. Routine antimicrobial-resistance surveillance and reinforcement of infection-control and stewardship programs are imperative to limit dissemination of multidrug-resistant enterococci.

DOI: 10.25258/ijcpr.18.2.307

Introduction

Enterococcus species are facultatively anaerobic, Gram-positive cocci that were once commensals in the gastrointestinal tracts of humans and animals but have since developed into important opportunistic pathogens. Their intrinsic tolerance to adverse conditions— including desiccation, disinfectants, high salinity, and broad temperature ranges—facilitates persistence in hospital environments, medical devices, and on healthcare workers' hands. These attributes have enabled Enterococcus to emerge as a leading cause of healthcare- associated infections such as UTIs, wound and soft-tissue infections, bacteraemia, endocarditis, and infections related to catheters and prosthetic devices.

Two species, E. faecium and E. faecalis, account for most clinical infections. E. faecalis is typically more prevalent, whereas E. faecium displays a greater propensity for multidrug resistance. Strong selection favouring resistant clones is exerted by the widespread and frequently empirical use of broad-

spectrum antibiotics in hospitals. Enterococci exhibit natural resistance to several antibiotic classes—including cephalosporins, clindamycin, and low concentrations of aminoglycosides—and readily acquire additional resistance genes through plasmids and transposons. Particularly problematic are high-level aminoglycoside- resistant (HLAR) strains, which abolish the ability of aminoglycosides to work in concert with cell-wall-active substances, and vancomycin-resistant enterococci (VRE), which have become critical global pathogens.

Vancomycin resistance arises from transferable van gene clusters that alter peptidoglycan precursors' D-Ala-D-Ala termini, decreasing their ability to bind antibiotics. These genetic elements can disseminate between enterococcal species and even to other Gram-positive organisms. The spread of VRE complicates therapy, often leaving linezolid and daptomycin as the only reliable options. These organisms can cause infections that are linked to prolonged hospitalization, increased healthcare costs, and elevated morbidity and mortality. The coexistence of HLAR and vancomycin resistance within the same isolate represents a particularly serious therapeutic challenge, emphasizing the necessity of strong infection- control measures and continuous epidemiologic monitoring.

In India, enterococcal infections are increasingly reported from tertiary-care centers, with growing documentation of multidrug-resistant and VRE strains. However, resistance profiles vary by geographic region, reflecting differences in antibiotic practices and infection-control efficiency. Data from Bihar remain limited despite the state's high patient load and limited antimicrobial-stewardship infrastructure. Determining local resistance trends is essential for guiding empiric therapy and developing institutional antibiotic policies.

From January to May 2025, this study was carried out in the microbiology department of the Radha Devi Jageshwari Memorial Medical College and Hospital in Turki, Muzaffarpur, Bihar. With a focus on vancomycin and high-level aminoglycoside resistance, it sought to (1) isolate and identify Enterococcus species from a variety of clinical samples and (2) describe their patterns of antibiotic susceptibility, and (3) to correlate resistance findings with clinical risk factors. The results aim to establish regional baseline data to inform antimicrobial- stewardship strategies and strengthen infection-prevention efforts within tertiary-care hospitals of northern India.

Materials and Methods

Study Design and Setting: The Department of Microbiology at Radha Devi Jageshwari Memorial Medical College and Hospital in Turki, Muzaffarpur, Bihar, India, conducted a prospective cross-sectional study. The study period extended from January to May 2025. The hospital is a tertiary-care center serving both urban and rural populations of northern Bihar.

Sample Collection and Processing: Two hundred nonduplicate clinical specimens yielding Enterococcus species were included. Samples comprised urine, pus and wound swabs, blood, body fluids, and catheter tips obtained from both inpatient and outpatient departments. Only the first isolate per patient was analysed to avoid duplication. Specimens were collected using aseptic techniques and processed immediately in the microbiology laboratory according to standard protocols.

Isolation and Identification: Every specimen was cultivated on bile esculin agar (BEA), MacConkey agar, and 5% sheep blood agar plates, and they were all incubated for 24 to 48 hours at 37°C. Colonies producing blackening on BEA and exhibiting small, grey, translucent morphology were presumptively identified as Enterococcus. Gram staining, catalase negativity, and a positive pyrrolidonyl arylamidase (PYR) reaction confirmed the genus. Growth in 6.5% NaCl and hydrolysis of esculin in the presence of bile were used as confirmatory features. Species differentiation was performed based on biochemical reactions including sugar fermentation profiles and motility testing.

Antimicrobial Susceptibility Testing: The Kirby-Bauer disk-diffusion method on Mueller-Hinton agar was used to assess antibiotic susceptibility, and the findings were interpreted in compliance with the CLSI 2025 guidelines. The antibiotic panel contained the following: nitrofurantoin (300 µg; tested solely for urine isolates), ampicillin (10 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), tetracycline (30 µg), vancomycin (30 µg), teicoplanin (30 µg), linezolid (30 µg), and daptomycin (30 µg).

High-content discs of streptomycin (300 µg) and gentamicin (120 µg) were used to identify HLAR. HLAR-positive isolates were defined as those that lacked a zone of inhibition. The same diffusion approach was used to screen for vancomycin resistance, and where necessary, the vancomycin E-test was used to confirm it. Internal quality assurance was conducted using E. faecalis ATCC 29212 (susceptible control) and E. faecalis ATCC 51299 (VRE control).

Data Analysis: All data were compiled and analyzed using Microsoft Excel 2021. Categorical variables were summarized as frequencies and percentages. Associations between clinical risk factors and VRE infection were assessed using the chi-square test, with a p value < 0.05 considered statistically significant.

Results

Distribution of Isolates by Specimen Type: Among the 200 Enterococcus isolates recovered during the study period, the majority were obtained from urine samples (n = 110; 55%), followed by pus or wound swabs (n = 40; 20%), blood (n = 30; 15%), and other clinical specimens (n = 20; 10%), including body fluids, catheter tips, and bile samples. The predominance of urinary isolates reflected the organism's major role in hospital-acquired urinary tract infections (Table 1).

Table 1: Distribution of Enterococcus Isolates According to Specimen Type

Specimen Type	No. of Isolates (n)	Percentage (%)
Urine	110	55.0
Pus / Wound swab	40	20.0
Blood	30	15.0
Other specimens*	20	10.0
Total	**200**	**100.0**

Other specimens include body fluids, catheter tips, and bile.

Species Distribution: E. faecalis accounted for 120, or 60%, of all isolates, with E. faecium coming in second at 70, or 35%, and other species including E. gallinarum and E. casseliflavus at 10 or 5%. E. faecalis was isolated primarily from urine, whereas E. faecium predominated among blood and ICU specimens (Table 2).

Table 2: Distribution of Enterococcus Species

Species	No. of Isolates (n)	Percentage (%)
E. faecalis	120	60.0
E. faecium	70	35.0
Other species	10	5.0
Total	**200**	**100.0**

Antimicrobial Susceptibility Profile: Overall, a high level of resistance was observed to conventional antibiotics. Resistance to ampicillin was 55%, to ciprofloxacin 65%, and to tetracycline 60%. In contrast, glycopeptides and newer agents demonstrated excellent activity, with 88.5% of isolates susceptible to vancomycin, 90% to teicoplanin, 98.5% to linezolid, and 96% to daptomycin. Among urinary isolates, nitrofurantoin retained good activity (82% susceptibility). HLAR was recorded in 33% of isolates for gentamicin and 20% for streptomycin (Table 3).

Table 3: Antibiotic Susceptibility Profile of Enterococcus Isolates (n = 200)

Antibiotic	Sensitive (%)	Resistant (%)
Ampicillin	45.0	55.0
Ciprofloxacin	35.0	65.0
Levofloxacin	38.0	62.0
Tetracycline	40.0	60.0
Vancomycin	88.5	11.5
Teicoplanin	90.0	10.0
Linezolid	98.5	1.5
Daptomycin	96.0	4.0
Nitrofurantoin*	82.0	18.0
HLAR – Gentamicin	—	33.0
HLAR – Streptomycin	—	20.0

Tested only for urinary isolates (n = 110).

Vancomycin Resistance Pattern: In 23 isolates, vancomycin resistance was found (11.5%). Only 5% of resistant isolates were E. faecalis, while the majority (24.3%) were E. faecium.

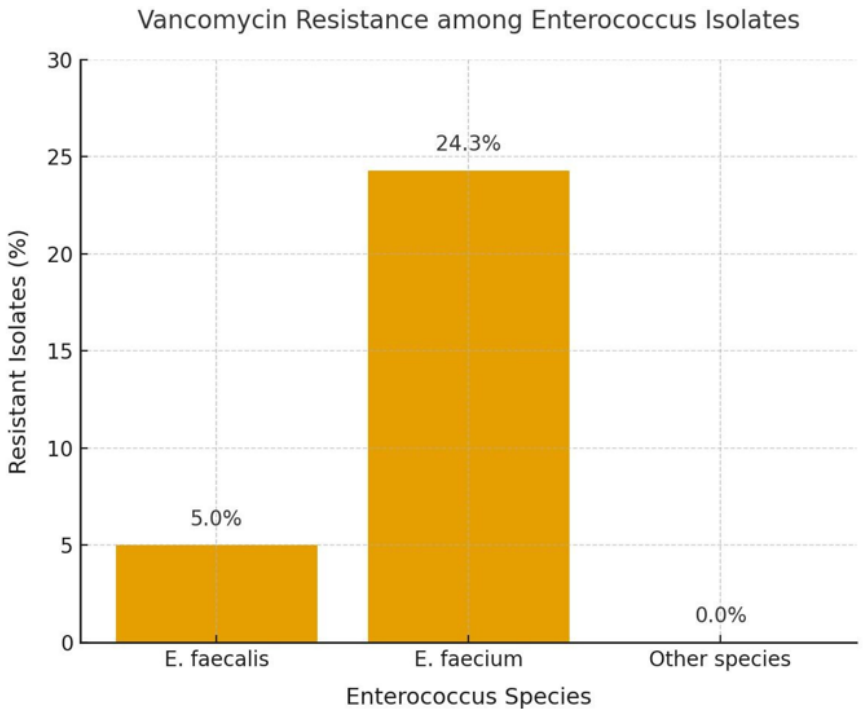

Figure 1: Vancomycin resistance among E. faecalis, E. faecium, and other species — corresponding to your previous bar chart.

Association of Clinical Factors with VRE Infection: Among patients with VRE infection, 52% were admitted to the ICU, 61% had prior exposure to antibiotics, and 43% had indwelling medical devices. ICU admission and prior antibiotic use were significantly associated with VRE isolation ($p < 0.05$). Other factors such as diabetes mellitus and device use did not reach statistical significance (Table 4).

Table 4: Correlation of Risk Factors with VRE Infection

Risk Factor	VRE Positive (n = 23)	Non-VRE (n = 177)	p-value
ICU stay	12 (52.1%)	41 (23.1%)	< 0.05
Prior antibiotic use	14 (60.8%)	52 (29.4%)	< 0.05
Indwelling device	10 (43.4%)	55 (31.1%)	> 0.05
Diabetes mellitus	6 (26.0%)	39 (22.0%)	> 0.05

Discussion

The location, species diversity, and antibiotic resistance of Enterococcus isolates found in clinical samples at a Bihar tertiary-care hospital are updated in this study. Global and national trends in hospital-acquired enterococcal infections are reflected in the prevalence of urine isolates and the rising percentage of multidrug-resistant E. fecium.

The high recovery of Enterococcus from urine samples (55%) underscores its central role in urinary tract infections, particularly among catheterized and hospitalized patients. This observation aligns with findings from previous Indian studies that reported a similar predominance of urinary isolates. The isolation of Enterococcus from blood and wound swabs further confirms its pathogenic potential beyond the UT. Such infections are often associated with invasive procedures, prolonged hospitalization, and underlying comorbidities, supporting the view that enterococcal infections are primarily opportunistic.

In the current series, E. faecalis was the predominant species (60%), followed by E. faecium (35%) and other less frequent species such as E. gallinarum and E. casseliflavus (5%). While E. faecalis continues to be the leading cause of clinical infection, the increasing isolation rate of E. faecium is concerning. E. faecium is known to possess greater intrinsic and acquired resistance mechanisms, enabling survival under antibiotic pressure and hospital disinfection protocols. Its higher prevalence among blood and ICU isolates indicates its adaptation to the nosocomial environment. Several recent Indian studies have also reported a rising incidence of E. faecium, suggesting a gradual epidemiologic shift in tertiary-care settings.

Antimicrobial susceptibility testing revealed extensive resistance to β-lactams and

fluoroquinolones, with more than half of the isolates resistant to ampicillin (55%) and approximately two-thirds resistant to ciprofloxacin (65%) and levofloxacin (62%). Tetracycline resistance (60%) was also frequent, reflecting the selective pressure created by indiscriminate antimicrobial use in both hospital and community settings. Similar resistance profiles have been observed in multicentric Indian studies, confirming a nationwide pattern of declining efficacy of conventional antibiotics against enterococcal species.

Despite these high resistance rates, glycopeptides and newer agents retained substantial activity. Vancomycin susceptibility was recorded in 88.5% of isolates, teicoplanin in 90%, and nearly all isolates were sensitive to linezolid (98.5%) and daptomycin (96%). Nitrofurantoin also demonstrated reliable activity (82%) against urinary isolates, confirming its continued utility for uncomplicated urinary infections. The preservation of susceptibility to linezolid and daptomycin offers an important therapeutic advantage, though emerging resistance has been reported globally. Rational prescribing and restricted use of these last-resort agents are therefore critical to prevent the spread of resistant clones.

VRE constituted 11.5% of all isolates, a prevalence comparable to that reported by tertiary centers across northern India (6–12%) but lower than rates exceeding 20% in some southern institutions. Most VRE isolates were E. faecium (24.3%), consistent with the species' greater capacity for acquiring resistance determinants. The presence of VRE in this study highlights the hospital environment's role as a reservoir of multidrug-resistant pathogens. Since vancomycin-resistance genes (vanA, vanB) are typically plasmid-mediated and transferable, their spread within healthcare settings poses a serious risk. Rigorous adherence to hand hygiene, environmental disinfection, and antimicrobial stewardship remains vital to curbing transmission.

HLAR was detected in 33% of isolates for gentamicin and 20% for streptomycin. The coexistence of HLAR and VRE in some isolates further restricts therapeutic options because HLAR abolishes the synergistic bactericidal activity between aminoglycosides and β-lactams or glycopeptides, which is essential in managing severe infections such as endocarditis. The HLAR prevalence observed here corresponds with national averages (30–40%), confirming that these resistant strains are well established in Indian hospitals. Routine screening for HLAR is therefore essential to guide rational antimicrobial therapy and to prevent the use of ineffective drug combinations.

Clinical correlation analysis revealed significant associations between VRE isolation and both ICU admission and prior antibiotic exposure ($p < 0.05$). This relationship reinforces that prolonged hospitalization and antibiotic pressure are key drivers of resistance emergence. The ICU often functions as a focal point for resistant bacteria due to frequent device use, invasive procedures, and extensive antimicrobial administration. Targeted infection-control measures—especially reinforcement of hand hygiene, catheter-care protocols, and environmental cleaning—can markedly reduce VRE transmission. In addition, institutional antibiotic-stewardship programs, including prescription audits and periodic resistance reviews, are crucial to mitigate selective pressure and preserve antimicrobial efficacy.

The findings have practical implications for clinicians and hospital administrators. The observed resistance to ampicillin, tetracycline, and fluoroquinolones suggests these drugs are no longer suitable as empirical choices for suspected enterococcal infections in this setting. Nitrofurantoin remains appropriate for uncomplicated urinary infections, whereas severe infections require culture-directed therapy. Continuous local surveillance and dissemination of updated antibiograms are essential to inform empirical treatment guidelines.

This study has several limitations. It was conducted in a single tertiary-care hospital over a limited five-month period and may not fully capture community or seasonal variations. Molecular typing and genotypic analysis of resistance genes were not performed due to resource constraints. Future multicentric studies incorporating molecular characterization would provide a more comprehensive understanding of the regional epidemiology and transmission dynamics of resistant enterococci.

Despite these constraints, the present findings contribute meaningful baseline data from Bihar, a region with limited published evidence on antimicrobial resistance patterns. The results underscore the need for integrated infection-prevention strategies, including active surveillance cultures, isolation of colonized patients, judicious antibiotic use, and ongoing education of healthcare personnel to minimize the spread of multidrug-resistant Enterococcus species.

Conclusion

This study demonstrates that Enterococcus species, particularly E. faecium, are significant pathogens in hospital-acquired infections and exhibit substantial resistance to conventional antibiotics. The prevalence of vancomycin-resistant and HLAR strains highlights an emerging therapeutic challenge. While linezolid and daptomycin remain the most effective agents, their prudent use is imperative. The association of resistance with ICU stay and prior

antibiotic exposure emphasizes the role of hospital environment and antibiotic pressure in the propagation of resistant strains. Regular antimicrobial-resistance surveillance, implementation of stewardship programs, and strict infection-control practices are essential to limit the spread of multidrug-resistant enterococci and preserve the efficacy of existing therapeutic agents.

References

1. Murray BE. The life and times of the Enterococcus. Clin Microbiol Rev. 1990; 3(1): 46–65.
2. Fisher K, Phillips C. The ecology, epidemiology and virulence of Enterococcus. Microbiology. 2009;155(Pt 6):1749–57.
3. Hollenbeck BL, Rice LB. Intrinsic and acquired resistance mechanisms in Enterococcus. Virulence. 2012;3(5):421–33.
4. Cetinkaya Y, Falk P, Mayhall CG. Vancomycin-resistant enterococci. Clin Microbiol Rev. 2000;13(4):686–707.
5. Gilmore MS, Clewell DB, Ike Y, Shankar N, editors. Enterococci: From commensals to leading causes of drug-resistant infection. Boston: Massachusetts Eye and Ear Infirmary; 2014.
6. Deshpande VR, Karmarkar MG, Mehta PR. Prevalence of multidrug-resistant Enterococcus species in a tertiary care hospital. J Clin Diagn Res. 2022;16(2): DC10– DC14.
7. Sharma S, Kaur N, Aggarwal A, Bansal S, Oberoi A. Antibiotic resistance pattern of enterococci isolated from various clinical samples in a tertiary care hospital. Int J Curr Microbiol Appl Sci. 2021;10(3):141–50.
8. Sreeja S, Sreenivasan A, Karim S. A study of the species prevalence and antibiotic resistance pattern of enterococcal isolates in a tertiary care hospital. Indian J Pathol Microbiol. 2020;63(2):212–7.
9. Anupurba S, Sen MR, Nath G, Sharma BM, Gulati AK, Mohapatra TM. Prevalence of antimicrobial resistance among Enterococcus isolates in a tertiary care hospital in North India. J Med Microbiol. 2019;68(6):884–90.
10. Praharaj I, Sujatha S, Parija SC. Phenotypic & genotypic characterization of vancomycin resistant Enterococcus isolates from clinical specimens. Indian J Med Res. 2019;149(6):830–5.
11. Mendiratta DK, Kaur H, Deotale V, Thamke D, Narang P, Narang R. Status of high- level aminoglycoside resistance in Enterococcus species isolated from a tertiary care hospital. Indian J Med Microbiol. 2021;39(3):370–5.
12. Karmarkar MG, Gershom ES, Mehta PR. Enterococcal infections with special reference to phenotypic characterization and drug resistance. Indian J Med Res. 2019;150(5):560–6.
13. Gupta R, Malik A, Rizvi M, Ahmed SM. Antibiotic resistance patterns of enterococci isolated from hospitalized patients in a tertiary care center in North India. J Infect Dev Ctries. 2020;14(8):880–5.
14. Makkar S, Rathore N, Sood S. High-level aminoglycoside resistance and vancomycin resistance among enterococcal isolates in a tertiary care hospital. J Clin Diagn Res. 2021;15(1): DC05–DC09.
15. Faron ML, Ledeboer NA, Buchan BW. Resistance mechanisms, epidemiology, and approaches to screening for vancomycin-resistant enterococci (VRE) in the hospital setting. Clin Microbiol Newsl. 2016;38(10):81–9.
16. O'Driscoll T, Crank CW. Vancomycin-resistant enterococcal infections: Epidemiology, clinical manifestations, and optimal management. Infect Drug Resist. 2015; 8:217–30.
17. Lebreton F, Willems RJL, Gilmore MS. Enterococcus diversity, origins in nature, and gut colonization. In: Gilmore MS et al., editors. Enterococci: From commensals to leading causes of drug-resistant infection. Boston: Massachusetts Eye and Ear Infirmary; 2014. p. 1–59.
18. World Health Organization. Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics. Geneva: WHO; 2017.