

Antimicrobial Resistance Profile of Enterococcus Species Isolated from Clinical Specimens in a Tertiary Care Hospital in Eastern India

Monalisa Panigrahi¹, Bimoch Projna Paty², Banojini Parida³

¹Assistant Professor, Department of Microbiology, SJMCH, Puri, Odisha, India

²Professor and HOD, Department of Microbiology, SJMCH, Puri, Odisha, India

³Ex. HOD, Microbiology Department, MKCG, MCH, Berhampur, Odisha, India

Received: 27-07-2025 / Revised: 25-08-2025 / Accepted: 27-09-2025

Corresponding Author: Monalisa Panigrahi

Conflict of interest: Nil

Abstract:

Background: Enterococcus are important opportunistic pathogens that cause diverse healthcare-associated infections. The rise of antimicrobial resistance, especially the spread of vancomycin-resistant enterococci (VRE), has created a major global treatment challenge. Monitoring regional resistance patterns is crucial for optimizing therapy and strengthening infection control strategies.

Aim: "To identify the species distribution and assess the antimicrobial resistance patterns of Enterococcus isolates recovered from clinical specimens in a tertiary care hospital in Eastern India."

Methods: "A laboratory-based cross-sectional descriptive study was carried out in the Department of Microbiology, MKCG Medical College and Hospital, Berhampur, between 2017 and 2019. A total of 100 Enterococcus isolates were recovered from urine, pus, blood, and other body fluids. Species identification was done using standard biochemical methods, while antimicrobial susceptibility testing was performed by the Kirby–Bauer disk diffusion technique on Mueller–Hinton agar in accordance with CLSI 2019 guidelines. Resistance patterns by species and multidrug resistance (MDR) were evaluated using SPSS version 23.0."

Results: Among the 100 isolates, *E. faecalis* (64%) was the most prevalent species, followed by *E. faecium* (31%) and *E. durans* (5%). The highest resistance rates were noted for ciprofloxacin (77%), ampicillin (66%), and tetracycline (61%). Glycopeptide resistance was detected in 23% of isolates for vancomycin and 18% for teicoplanin. High-level aminoglycoside resistance was observed in 46% of isolates for gentamicin and 40% for streptomycin. All isolates remained susceptible to linezolid. *E. faecium* demonstrated significantly higher resistance compared to *E. faecalis*. MDR was present in 47% of isolates, predominantly among *E. faecium*. Notably, 69.6% of the vancomycin-resistant isolates were *E. faecium*."

Conclusion: Enterococcus species, particularly *E. faecium*, are emerging as multidrug-resistant nosocomial pathogens in Eastern India. While linezolid remains universally effective, the high prevalence of resistance to commonly used antibiotics, along with increasing VRE, underscores the urgency of robust antibiotic stewardship and infection control strategies.

Recommendations: Routine species-level identification and antimicrobial susceptibility testing should be carried out for all Enterococcus isolates. Continuous monitoring of resistance patterns, judicious use of antibiotics, and stringent infection control practices are crucial to prevent the dissemination of VRE and multidrug-resistant strains. Additionally, prioritizing research into new therapeutic alternatives is imperative.

Keywords: Enterococcus, antimicrobial resistance, vancomycin-resistant enterococci (VRE), multidrug resistance, Eastern India.

This is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>) and the Budapest Open Access Initiative (<http://www.budapestopenaccessinitiative.org/read>), which permit unrestricted use, distribution, and reproduction in any medium, provided original work is properly credited.

Introduction

Enterococcus species are notable opportunistic pathogens and have become a leading cause of hospital-acquired infections globally. Although they normally reside in the gastrointestinal tract, they can lead to serious illness in high-risk patients, especially those with extended hospitalizations, implanted medical devices, or prior antibiotic treatment. Among them, *Enterococcus faecalis* and *Enterococcus faecium* are the most clinically significant, frequently implicated in urinary tract

infections, bacteremia, endocarditis, intra-abdominal infections, and wound infections [1,2].

"Over the last twenty years, managing Enterococcal infections has become increasingly difficult due to rising antimicrobial resistance. While *E. faecalis* was historically more common and generally susceptible to standard treatments, *E. faecium* has emerged as a high-risk, multidrug-resistant pathogen, showing greater resistance to β -lactams,

fluoroquinolones, and aminoglycosides. Of particular concern is the global emergence and spread of (VRE), especially *E. faecium*, which further restricts therapeutic options and represents a serious public health challenge."

The occurrence of VRE differs worldwide, with the United States and certain European regions showing higher rates than Asia. In India, recent studies have shown a rising trend in VRE, especially in tertiary care hospitals, where irrational antibiotic use and inadequate infection control practices contribute to the problem [6,7]. Linezolid and daptomycin remain the most effective agents against resistant enterococci; however, sporadic reports of resistance to these last-line drugs are also emerging, raising serious therapeutic concerns [8].

The impact of antimicrobial resistance in enterococci goes beyond treatment failure in individual patients. These bacteria serve as reservoirs for resistance genes, such as van A and van B, which can be transmitted to other pathogens, including *Staphylococcus aureus*, increasing the risk of infections that are difficult to treat. Therefore, ongoing surveillance of resistance patterns is vital to inform empirical therapy and support effective antimicrobial stewardship programs.

Although many studies have been conducted worldwide, information on the antimicrobial resistance patterns of enterococci in Eastern India is still scarce. Knowledge of local epidemiology and resistance trends is essential, as these can differ regionally due to variations in antibiotic use, infection control practices, and healthcare infrastructure. Therefore, this study was conducted to assess the species distribution and antimicrobial resistance profiles of *Enterococcus* isolates obtained from clinical specimens in a tertiary care hospital in Eastern India.

Methodology

Study Design: To ascertain the antibiotic resistance profile of *Enterococcus* species isolated from clinical specimens, a cross-sectional descriptive investigation was carried out in a laboratory.

Study Setting: The study was conducted at the MKCG Medical College and Hospital's Department of Microbiology in Berhampur, Odisha, Eastern India. This tertiary care facility serves a sizable population from both urban and rural areas.

Study Duration: The study was conducted over a period of two years, from 2017 to 2019.

Study Participants: A hundred clinical isolates of *Enterococcus* species were extracted from a variety of specimens, such as bodily fluids, urine, pus, and blood. To prevent redundancy, duplicate isolates from the same patient were eliminated, and each isolate represented a single patient sample.

Inclusion Criteria

- Clinical specimens yielding *Enterococcus* species.
- Samples collected from patients admitted to or attending MKCG Medical College and Hospital.
- Only one isolate per patient was included.

Exclusion Criteria

- Duplicate isolates from the same patient.
- Mixed growth with no significant yield of *Enterococcus*.

Bias: To minimize selection bias, all consecutive *Enterococcus* isolates meeting the inclusion criteria during the study period were included. Observer bias in identification and susceptibility testing was reduced by following standardized CLSI 2019 guidelines and using quality control strains.

Data Collection: Relevant clinical information was collected from laboratory requisition forms. A routine procedure was used to identify the isolates, and testing for antibiotic susceptibility was carried out. All results were documented systematically in laboratory records before statistical analysis.

Processing and Identification: Typical microbiological procedures were used to process each specimen. After being cultivated on blood agar and MacConkey agar, the samples were incubated aerobically for 24 to 48 hours at 37°C. Gram staining (Gram-positive cocci in pairs or chains) was used to identify colonies that appeared to be *Enterococcus*.

- *Enterococcus* isolates were identified based on a negative catalase test, hydrolysis of bile-esculin, growth in 6.5% NaCl, and their ability to develop at 45°C and 10°C.
- Additionally, a positive Pyrrolidonyl Arylamidase (PYR) test, along with arginine deamination and fermentation of sugars such as raffinose, mannitol, sorbitol, arabinose, and pyruvate, was used for species-level identification.

Antimicrobial Susceptibility Testing

Using Mueller-Hinton agar and the Kirby-Bauer disk diffusion method, antimicrobial susceptibility testing was conducted and evaluated in accordance with CLSI 2019 standards. Among the antibiotics tested were:

- Ampicillin (10 µg), Ciprofloxacin (5 µg), Tetracycline (30 µg), Vancomycin (30 µg), Teicoplanin (30 µg), Linezolid (30 µg), High-level gentamicin (120 µg), High-level streptomycin (300 µg).
- For urinary isolates, Nitrofurantoin (300 µg) was additionally tested.

E. faecalis ATCC 51299 was used as a quality control strain.

Statistical Analysis: All data were compiled in Microsoft Excel and analyzed using SPSS version 23.0. Results were expressed as percentages of resistant isolates. Resistance patterns were analyzed species-wise (*E. faecalis*, *E. faecium*, *E. durans*).

Descriptive statistics were applied to present distribution and resistance trends.

Results

Species Distribution: Out of 5672 clinical specimens, 2268 (39.9%) were culture-positive. Among these, 100 isolates (4.41%) were identified as *Enterococcus* species.

Table 1: Distribution of Enterococcus species (n = 100)

Species	Number of isolates (n)	Percentage (%)
<i>E. faecalis</i>	64	64%
<i>E. faecium</i>	31	31%
<i>E. durans</i>	5	5%
Total	100	100%

E. faecalis was the predominant species (64%), followed by *E. faecium* (31%), while *E. durans* (5%) was least frequent.

Specimen-wise Distribution: The distribution of isolates according to type of clinical specimen is shown in Table 2.

Table 2: Specimen-wise distribution of Enterococcus isolates (n = 100)

Specimen type	Number of isolates (n)	Percentage (%)
Urine	61	61%
Pus/wound swab	18	18%
Blood	12	12%
Body fluids	9	9%
Total	100	100%

Urine was the most common source (61%), followed by pus/wound swabs (18%) and blood (12%).

Antimicrobial Resistance Profile: The overall resistance pattern among the isolates is shown in Table 3.

Table 3: Antimicrobial resistance profile of Enterococcus isolates (n = 100)

Antibiotic	Resistant isolates (n)	Percentage (%)
Ciprofloxacin (5 µg)	77	77%
Ampicillin (10 µg)	66	66%
Tetracycline (30 µg)	61	61%
Vancomycin (30 µg)	23	23%
Teicoplanin (30 µg)	18	18%
Linezolid (30 µg)	0	0% (all sensitive)
High-level Gentamicin	46	46%
High-level Streptomycin	40	40%
Nitrofurantoin* (300 µg)	12	19.67%

* Nitrofurantoin tested only in urinary isolates (n = 61).

The greatest resistance to ampicillin (66%) and ciprofloxacin (77%), respectively, was noted. Linezolid sensitivity was present in every isolate.

Species-wise Resistance: A comparison of resistance rates between *E. faecalis*, *E. faecium*, and *E. durans* is presented in Table 4.

Table 4: Species-wise resistance pattern of Enterococcus isolates

Antibiotic	<i>E. faecalis</i> (n=64)	<i>E. faecium</i> (n=31)	<i>E. durans</i> (n=5)
Ciprofloxacin	70%	90%	60%
Ampicillin	58%	85%	40%
Aminoglycosides (HLG/HLS)	40%	65%	20%
Vancomycin	20%	30%	0%
Teicoplanin	15%	22%	0%
Linezolid	0%	0%	0%

E. faecium exhibited highest resistance across most antibiotics, particularly ampicillin, ciprofloxacin, and glycopeptides. *E. faecalis* showed moderate

resistance, while *E. durans* isolates were less frequent but showed moderate levels of resistance.

Distribution of Vancomycin-Resistant Enterococcus (VRE): Species-specific vancomycin resistance was as follows

Table 5: Distribution of Vancomycin-Resistant Enterococcus (VRE) isolates (n = 23)

Species	Number of VRE isolates (n)	Percentage (%)
<i>E. faecalis</i>	6	26.1%
<i>E. faecium</i>	16	69.6%
<i>E. durans</i>	1	4.3%
Total	23	100%

Vancomycin resistance was highest among *E. faecium* (69.6%), followed by *E. faecalis* (26.1%). Only one isolate of *E. durans* was resistant.

Multidrug Resistance (MDR): Multidrug resistance (resistance to ≥ 3 antibiotic classes) was observed more frequently among *E. faecium* compared to other species.

Table 6: Multidrug resistance among Enterococcus isolates

Species	MDR isolates (n)	Percentage (%)
<i>E. faecalis</i>	24	37.5%
<i>E. faecium</i>	22	71.0%
<i>E. durans</i>	1	20.0%
Total	47	47%

E. faecium had the highest proportion of MDR isolates (71%), while *E. faecalis* showed moderate

MDR (37.5%). Importantly, all isolates remained sensitive to linezolid.

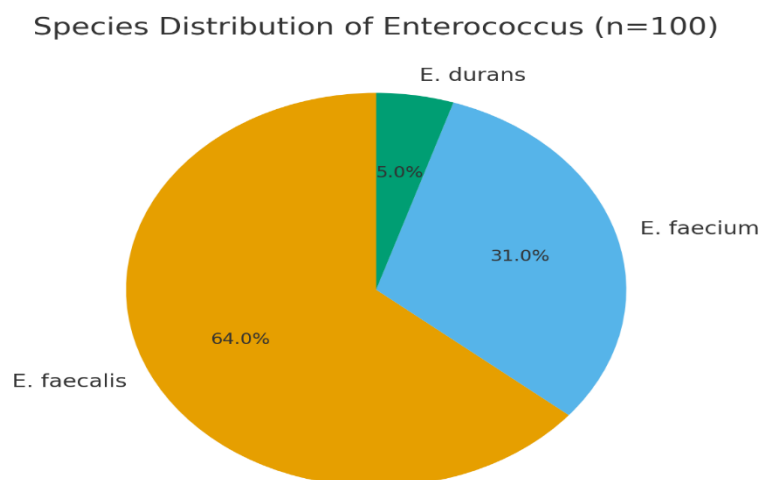


Figure 1: Species distribution of enterococcus

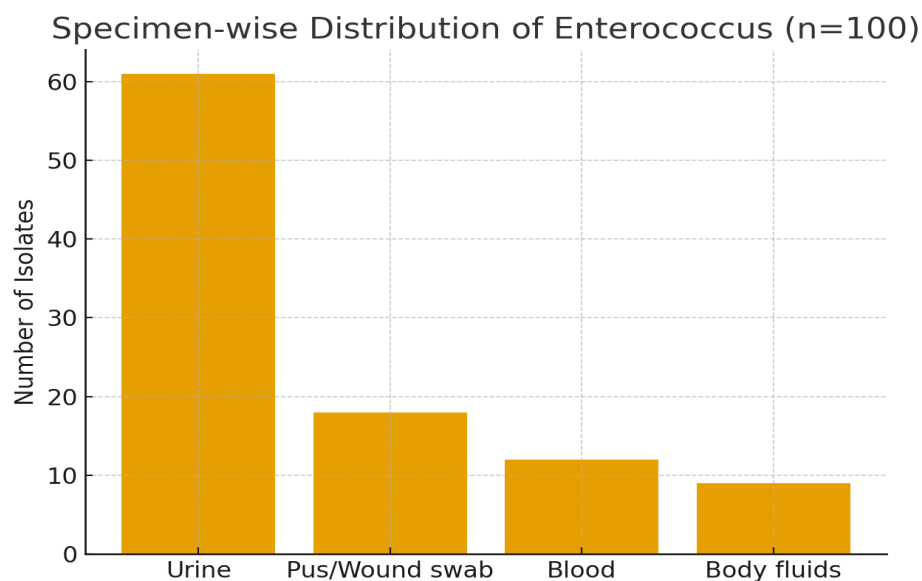


Figure 2: Specimen-wise distribution of enterococcus

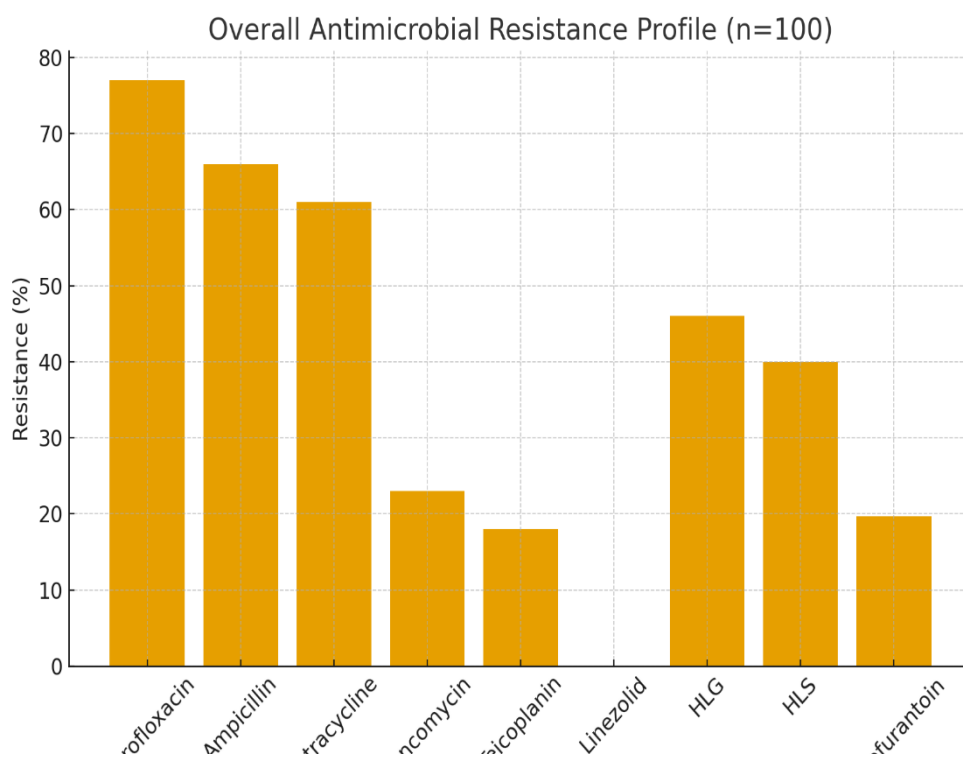


Figure 3: Overall antimicrobial resistance profile (n=100)

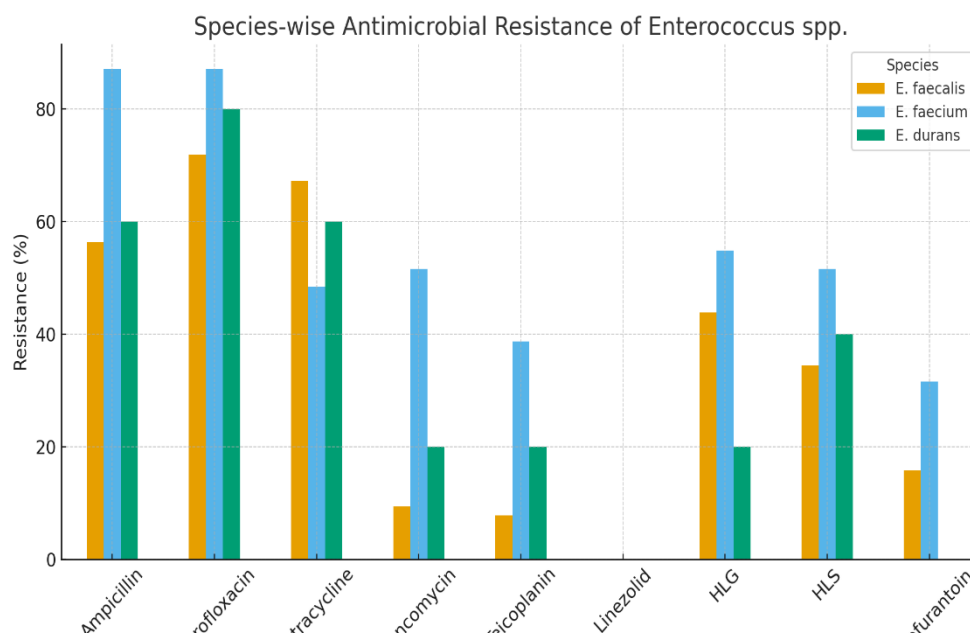


Figure 4: Species-wise antimicrobial resistance of enterococcus spp.

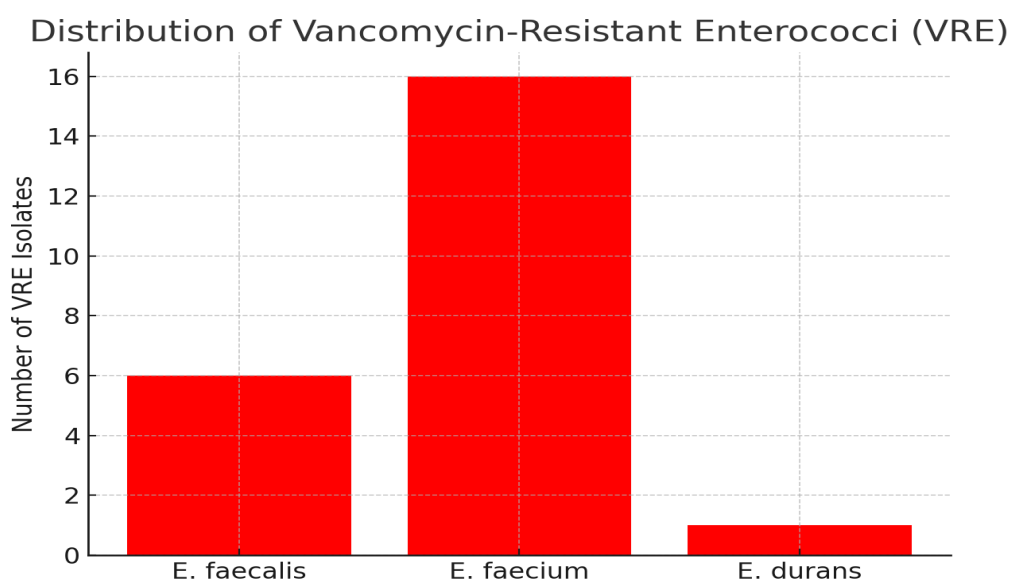


Figure 5: Distribution of Vancomycin-Resistant Enterococci (VRE)

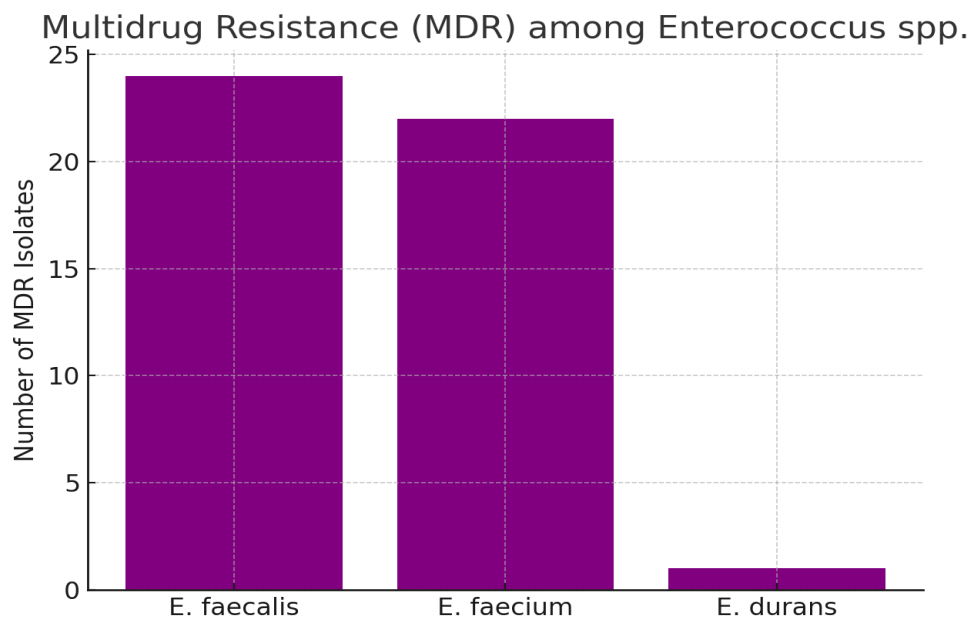


Figure 6: Multidrug resistance (MDR) among enterococcus spp.

Discussion

In the present study, out of 5672 clinical specimens processed, 2268 (39.9%) were culture-positive, and 100 isolates (4.41%) were identified as Enterococcus species. Among these, *E. faecalis* was the predominant species (64%), followed by *E. faecium* (31%), while *E. durans* accounted for only 5%. The majority of isolates were recovered from urine (61%), followed by pus/wound swabs (18%) and blood (12%), highlighting the role of Enterococcus as a frequent urinary pathogen in hospitalized patients.

Analysis of antimicrobial resistance patterns revealed high levels of resistance to commonly used antibiotics. Ciprofloxacin resistance was most frequent (77%), followed by ampicillin (66%) and tetracycline (61%). Glycopeptide resistance was also notable, with vancomycin resistance detected in 23% and teicoplanin resistance in 18% of isolates. Alarming, 46% and 40% of isolates demonstrated high-level resistance to gentamicin and streptomycin, respectively, limiting the usefulness of aminoglycosides in synergy-based therapy. On a positive note, all isolates remained uniformly sensitive to linezolid, making it the most effective therapeutic option.

Species-wise comparison demonstrated that *E. faecium* was significantly more resistant than *E. faecalis*, particularly against ampicillin, ciprofloxacin, aminoglycosides, and glycopeptides. *E. durans* isolates showed moderate resistance levels but were infrequently isolated. Among vancomycin-resistant Enterococcus (VRE) isolates, *E. faecium*

accounted for the majority (69.6%), followed by *E. faecalis* (26.1%) and *E. durans* (4.3%). This finding highlights the clinical significance of *E. faecium* as a challenging nosocomial pathogen with limited therapeutic options.

(MDR), defined as resistance to three or more classes of antibiotics, was observed in nearly half of the isolates (47%). *E. faecium* had the highest proportion of MDR isolates (71%), followed by *E. faecalis* (37.5%). The persistence of linezolid sensitivity across all isolates provides an important therapeutic safeguard; however, the rising prevalence of VRE and MDR strains underscores the urgent need for strict antibiotic stewardship and continuous surveillance.

Recent studies from India and other regions highlight the growing challenge of multidrug-resistant Enterococcus species in hospital settings. A study from Southern India demonstrated that *E. faecalis* and *E. faecium* were the predominant isolates, showing high resistance to erythromycin, ciprofloxacin, and tetracycline, although susceptibility to linezolid and vancomycin was preserved [10]. Similarly, research in Nepal revealed that clinical Enterococcus isolates exhibited very high resistance to erythromycin and ciprofloxacin, but all isolates remained susceptible to vancomycin and linezolid, underscoring their role as last-resort options [11].

In a large tertiary hospital in India, *E. faecium* isolates were significantly more resistant than *E. faecalis*, particularly to ampicillin, aminoglycosides, and fluoroquinolones, while vancomycin and

linezolid retained good efficacy [12]. Comparable resistance patterns were observed in Ethiopia, where multidrug resistance was widespread, especially against ampicillin and tetracycline, with preserved susceptibility to linezolid and vancomycin [13].

Studies beyond South Asia reinforce this global concern. In Iran, researchers found that over half of the *Enterococcus* isolates were multidrug-resistant, with notable resistance to erythromycin and tetracycline, though vancomycin resistance remained relatively rare [14]. In Nigeria, hospital isolates showed extensive resistance to conventional antibiotics including ampicillin, erythromycin, and tetracycline, but retained activity against linezolid and vancomycin [15]. Overall, the evidence indicates that since 2018, multidrug-resistant *Enterococcus* species have been consistently reported across diverse regions, with resistance particularly high against macrolides, aminoglycosides, fluoroquinolones, and beta-lactams, while linezolid and vancomycin remain the most effective treatment options.

Conclusion

Enterococcus species are significant clinical pathogens that exhibit high levels of resistance to widely used antibiotics, especially *E. faecalis* and *E. faecium*. All isolates were still susceptible to linezolid, but the highest rates of resistance were observed to ampicillin, ciprofloxacin, and aminoglycosides. The increasing hazard of these organisms in hospital settings is highlighted by multidrug resistance and the introduction of vancomycin-resistant *E. faecium*, underscoring the necessity of ongoing surveillance and responsible antibiotic usage.

References

1. Fiore E, Van Tyne D, Gilmore MS. Pathogenicity of *Enterococci*. *Microbiol Spectr*. 2019;7(4).
2. Weiner-Lastinger LM, Abner S, Edwards JR, et al. Antimicrobial-resistant pathogens associated with healthcare-associated infections. *Infect Control Hosp Epidemiol*. 2020;41(1):1-18.
3. Hollenbeck BL, Rice LB. Intrinsic and acquired resistance mechanisms in *Enterococcus*. *Virulence*. 2019;10(1):421-433.
4. Lee T, Pang S, Abraham S, Coombs GW. Antimicrobial-resistant *Enterococcus faecium*: the past, the present, and the future. *J Glob Antimicrob Resist*. 2019;16:36-47.
5. Zahedi Bialvaei A, Rahbar M, Yousefi M, Asgharzadeh M, Samadi Kafil H. Linezolid: a promising option in the treatment of Gram-positive resistant bacterial infections. *Biomed Pharmacother*. 2018;106:1027-1038.
6. Praharaj I, Sujatha S, Parija SC. Phenotypic and genotypic characterization of vancomycin-resistant *Enterococcus* isolates from clinical specimens. *Indian J Med Res*. 2019; 149(2): 185-191.
7. Bose S, Ghosh AK, Baral R, Banerjee S. Species distribution and antimicrobial resistance of enterococci in a tertiary care hospital in Eastern India. *J Clin Diagn Res*. 2020; 14(6):DC07-DC11.
8. Miller WR, Munita JM, Arias CA. Mechanisms of antibiotic resistance in enterococci. *Expert Rev Anti Infect Ther*. 2020;18(2):137-147.
9. Werner G, Coque TM, Hammerum AM, et al. Emergence and spread of vancomycin resistance among enterococci in Europe. *Euro Surveill*. 2018;23(48):1800348.
10. Kavitha R, Udaya Shankar C, Prasad SR. Antimicrobial susceptibility pattern of enterococci isolates in a tertiary care hospital, Southern India. *J Lab Physicians*. 2020;12(2):120-4.
11. Acharya KP, Wilson RT. Antibiotic resistance pattern of *Enterococcus* species isolated from clinical samples in Nepal. *Trop Med Health*. 2019; 47:27-32.
12. Mehrotra S, Singh R, Saxena A. Antimicrobial resistance trends in *Enterococcus* species from a tertiary care centre in North India. *Indian J Med Microbiol*. 2020;38(3-4):451-6.
13. Mengesha RE, Tekele SG, Baraki AG. *Enterococci* clinical isolates in Ethiopia: antimicrobial resistance patterns. *BMC Res Notes*. 2021;14:99-104.
14. Abadi ATB, Mobarez AM, Goudarzi H. Antimicrobial resistance of *Enterococcus* species isolated from clinical samples in Tehran, Iran. *Iran J Microbiol*. 2019;11(5):410-6.
15. Olayinka AT, Oni AA, Adesiji YO. High-level resistance in *Enterococcus* isolates from hospitals in Nigeria. *Afr J Clin Exp Microbiol*. 2021; 22(3):327-33.