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Original Research Article

A Five-Year Retrospective Analysis of Antimicrobial Resistance Trends in Enterococcus Species

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Abstract:

Background: Antimicrobial resistance (AMR) poses a major global health challenge, with Enterococcus species increasingly implicated in nosocomial infections.

Aim: To evaluate five-year trends in antimicrobial resistance among Enterococcus faecalis and Enterococcus faecium isolates in a tertiary care hospital.

Methodology: A retrospective observational study was conducted at Nalanda Medical College and Hospital, Patna, India, analyzing 70 Enterococcus isolates. Clinical samples included urine, blood, pus, catheter tips, and respiratory specimens. Species identification and antimicrobial susceptibility testing were performed using standard microbiological methods and automated systems, following CLSI guidelines. Data was analyzed with SPSS v27; trends were assessed using chi-square tests.

Results: E. faecalis accounted for 58.6% and E. faecium 41.4% of isolates, with urine being the predominant source (45.7%). Resistance was highest to ciprofloxacin (68.6%), levofloxacin (60%), and ampicillin (54.3%). E. faecium exhibited significantly higher resistance than E. faecalis across most antibiotics, though resistance to vancomycin (7.1%), linezolid (4.3%), teicoplanin (5.7%), and tigecycline (1.4%) remained low.

Conclusion: E. faecium is emerging as a multidrug-resistant pathogen, highlighting the importance of continuous surveillance, species-level identification, and strengthened antimicrobial stewardship to guide empirical therapy and limit nosocomial spread.

Keywords: Enterococcus, antimicrobial resistance, E. faecalis, E. faecium, vancomycin-resistant Enterococci (VRE), nosocomial infections.

Introduction

Antimicrobial resistance has emerged as one of the greatest global health challenges of the 21st century. Infections once readily treatable with standard antibiotics are now becoming increasingly difficult to manage in health systems around the world [1]. For more than a decade, international organizations have warned that continued rises in drug-resistant pathogens threaten to undermine the advances of modern medicine in surgery, cancer therapy, and intensive care medicine. The increasing burden of AMR not only imperils patient outcomes but also places unprecedented stressors on healthcare infrastructure.

International projections have painted a sobering picture of the future trajectory of AMR. If current trends continue, drug-resistant infections could be responsible for millions of deaths annually by mid-century, surpassing mortality from today's major non-communicable diseases [2]. Beyond the clinical implications, the economic impact of AMR is enormous. Rising healthcare costs, extended lengths of stay in hospitals, and the need for more expensive second-line or third-line treatments combined to increase the financial burden on health systems, patients, and communities [3]. These considerations underscore the urgent need for strong surveillance and stewardship programs.

The spread of AMR is closely related to the heavy misuse and overuse of antibiotics in both clinical and non-clinical uses. Inappropriate prescribing, inadequate diagnostic support, and self-medication have hastened the selection of resistant strains among human populations. Added to this, extensive antimicrobial use in agriculture has further heightened selective pressure, facilitating not only the spread of resistant organisms within the environment and the community but also their survival [4]. These factors have created a setting in which resistant bacteria can develop, spread, and endure.

Among the pathogens of most concern, the place of Enterococcus species is outstanding for their increasing role in nosocomial infections and remarkable ability to acquire resistance determinants [5]. Enterococci are Gram-positive commensals commonly residing in the gastrointestinal tract, but they easily turn into opportunistic pathogens under favorable conditions. They have turned into major etiological agents in urinary tract infections, bacteremia, intra-abdominal infections, wound infections, and infective endocarditis, particularly among hospitalized and immuno-compromised patients [6].

Most clinically significant infections are caused by two species: Enterococcus faecalis and Enterococcus faecium. Although E. faecalis still predominates, E. faecium has become more problematic due to its inherent resistant characteristics and increased propensity to acquire further resistance determinants. Its ability to adapt has made it one of the leading causes of potentially life-threatening nosocomial infections [7]. In recent years, an increase in resistance rates to major therapeutic classes, such as aminoglycosides and β-lactams, has been noted, making treatment more difficult.

Of particular concern is the emergence and global spread of vancomycin-resistant Enterococci. Vancomycin, once considered a last-resort therapy, has become increasingly ineffective against resistant strains, prompting greater reliance on alternative agents such as daptomycin, linezolid, and tigecycline [8]. However, early signs of reduced susceptibility to these agents have begun to appear in clinical surveillance reports, raising alarms about the dwindling therapeutic arsenal available to combat severe Enterococcal infections. The rapid evolution of VRE underlines the need for constant monitoring and timely information on resistance patterns [9].

Active epidemiological surveillance plays a critical role in guiding clinicians toward appropriate empirical therapy and informing hospital infection control policies. Knowledge of local and regional resistance trends allows healthcare practitioners to make more apt choices of initial treatments and to revise antimicrobial guidelines to mirror contemporary microbiological realities. Temporal analyses provide perspectives on changes over time, the emergence of resistance, and alterations in species distribution across hospital populations [10].

In this perspective, the current study focused on characterizing the trend of antimicrobial resistance exhibited by E. faecium and E. faecalis isolates from clinical specimens for five years. It further explores emerging resistance patterns between 2020 and 2024 that may influence empirical therapy options, thus informing strategic future stewardship interventions in the university hospital setting. Its results will add to the broader understanding of Enterococcal dynamics of resistance and assist in optimizing patient care through evidence-based management of antimicrobials.

Methodology

Study Design: This study was a retrospective observational study conducted to analyze the trends in antimicrobial resistance patterns of Enterococcus species isolated

Study Area: The study was conducted in the Department of Microbiology at Nalanda Medical College and Hospital (NMCH), Patna, Bihar, India.

Study Duration: The study was carried out over a period of five months from March 2025 to July 2025.

Study Participants

Inclusion Criteria

- Clinical samples submitted to the Microbiology laboratory that yielded growth of Enterococcus spp.
- Samples from patients of all age groups and both sexes.
- Only Enterococcus faecalis and Enterococcus faecium isolates with complete antimicrobial susceptibility data.

Exclusion Criteria

- Duplicate isolates from the same patient during the same infection episode.
- Samples show mixed growth of multiple organisms.
- Incomplete records lacking susceptibility profiles or demographic data.

Sample Size: A total of 70 Enterococcus isolates were included in the study.

Procedure: This retrospective study involved reviewing the laboratory records of the Department of Microbiology, NMCH, Clinical samples such as urine, blood, wound swabs, pus, catheter tips, respiratory specimens, and other body fluids submitted to the bacteriology section were included. Samples were processed as per standard microbiological guidelines. Urine samples were inoculated onto

CLED agar, while blood cultures were incubated in automated systems and subsequently subcultured on Blood agar and MacConkey agar. Other clinical specimens were plated on appropriate culture media and incubated at 37°C for 18–24 hours.

Isolated colonies suggestive of Enterococcus were identified by Gram staining, catalase testing, bile esculin hydrolysis, growth in 6.5% NaCl, and other conventional biochemical tests. Species-level identification and antimicrobial susceptibility testing (AST) were performed using automated systems such as VITEK-2 or Kirby–Bauer disc diffusion according to CLSI guidelines. Antibiotics tested included ampicillin, high-level gentamicin, high-level streptomycin, ciprofloxacin, levofloxacin, linezolid, vancomycin, teicoplanin, tigecycline, and nitrofurantoin. The antimicrobial resistance patterns of E. faecalis and E. faecium were documented and compared across the 5-year period. Quality control strains such as Enterococcus faecalis ATCC 29212 were used to ensure accuracy of testing.

Statistical Analysis: Data regarding patient demographics, types of clinical samples, isolated Enterococcus species, and antimicrobial susceptibility profiles were entered into Microsoft Excel and analyzed using IBM SPSS version 27. Descriptive statistics such as frequencies and percentages were used to summarize categorical variables. Trends in antimicrobial resistance over five years were evaluated using the chi-square test. A p-value of <0.05 was considered statistically significant.

Result

Table 1 shows the five-year distribution of 70 Enterococcus isolates having relatively steady number of cases each year, ranging from 12 to 16 isolates. E. faecalis remained the predominant species throughout the period, accounting for 58.6% (41/70) of all isolates, while E. faecium made up 41.4% (29/70). Although E. faecalis consistently outnumbered E. faecium each year, the gap narrowed over time, with 2024 showing an equal distribution (8 each). Overall, the data indicates a gradual increase in total isolates and a rising proportion of E. faecium toward the later years.

Table 1: Year-wise Distribution of Enterococcus Isolates (n = 70)

Year	Total Isolates (n)	E. faecalis	E. faecium
2020	12	8	4
2021	14	9	5
2022	15	9	6
2023	13	7	6
2024	16	8	8
Total	70	41 (58.6%)	29 (41.4%)

Table 2 shows that Enterococcus species were most frequently isolated from urine samples, which accounted for nearly half of all cases (45.7%). Blood was the second most common source (20%), followed by pus or wound swabs (17.1%). Less frequent sources included catheter tips (8.6%) and respiratory samples (5.7%), while other body fluids contributed only a small proportion (2.9%). Overall, the distribution highlights urine as the predominant sample type yielding Enterococcus spp. among the 70 clinical isolates analyzed.

Table 2: Distribution of Clinical Samples Yielding Enterococcus spp.

Sample Type	Number of Isolates (n)	Percentage (%)
Urine	32	45.7
Blood	14	20
Pus / Wound Swab	12	17.1
Catheter Tip	6	8.6
Respiratory Samples	4	5.7
Body Fluids (other)	2	2.9
Total	70	100

Table 3 shows the Enterococcus isolates between 2021 and 2025, displaying varying degrees of antimicrobial resistance. The highest resistance was observed against ciprofloxacin (68.6%) and levofloxacin (60%), followed by ampicillin (54.3%). Moderate resistance was seen with high-level streptomycin (38.6%), nitrofurantoin among urine isolates (37.5%), and high-level gentamicin (34.3%). In contrast, resistance to last-resort or critical antibiotics was relatively low, with vancomycin at 7.1%, linezolid at 4.3%, teicoplanin at 5.7%, and tigecycline at 1.4%, indicating that these agents remain largely effective against Enterococcus in this period. Overall, resistance patterns highlight significant fluoroquinolone and aminopenicillin resistance, while glycopeptides and newer agents retain high activity.

Table 3: Antimicrobial Resistance Patterns of Enterococcus Isolates (Overall 2021–2025)

Antibiotic	Resistant (n)	Resistant (%)
Ampicillin	38	54.3
High-level Gentamicin (HLG)	24	34.3
High-level Streptomycin (HLS)	27	38.6
Ciprofloxacin	48	68.6
Levofloxacin	42	60
Linezolid	3	4.3
Vancomycin	5	7.1
Teicoplanin	4	5.7
Tigecycline	1	1.4
Nitrofurantoin (for urine isolates, n=32)	12	37.5

Table 4 shows the comparative antibiotic resistance of E. faecalis and E. faecium from 2021 to 2025. Overall, E. faecium exhibits significantly higher resistance rates across most antibiotics compared to E. faecalis. Notably, E. faecium shows high resistance to ampicillin (79%), high-level gentamicin (HLG, 52%), high-level streptomycin (HLS, 52%), and fluoroquinolones such as ciprofloxacin (89%) and levofloxacin (83%), whereas E. faecalis shows comparatively lower resistance for the same drugs (39%, 22%, 29%, 54%, and 46%, respectively). Resistance to linezolid, vancomycin, teicoplanin, and tigecycline remains relatively low for both species, though E. faecium again shows slightly higher rates (10–14%) compared to E. faecalis (0–2%). This pattern highlights that E. faecium presents a greater therapeutic challenge due to its broader resistance profile.

Table 4: Comparative Resistance Between E. faecalis and E. faecium (2021–2025)

Antibiotic	E. faecalis Resistant (%) (n=41)	E. faecium Resistant (%) (n=29)
Ampicillin	39%	79%
HLG	22%	52%
HLS	29%	52%
Ciprofloxacin	54%	89%
Levofloxacin	46%	83%
Linezolid	0%	10%
Vancomycin	2%	14%
Teicoplanin	2%	10%
Tigecycline	0%	3%

Discussion

The results of the five-year surveillance of Enterococcus isolates investigated here indicated a relatively constant number of cases per year, ranging between 12 and 16 isolates, totaling 70 throughout 2020–2024. E. faecalis consistently dominated the total number of isolates, with 58.6%, whereas E. faecium comprised 41.4%. Importantly, despite E. faecalis outnumbering E. faecium over most years, the difference narrowed over time, reaching an equal representation of both species in 2024. This follows the trend that there may be a gradual shift in the prevalence of the species, with the clinical importance of E. faecium increasing, which can have implications with respect to the management of infections due to a more extensive resistance profile.

The analysis of the clinical sources of Enterococcus isolates revealed that the predominant sources of the organisms were urine samples, accounting for nearly half of the cases (45.7%), followed by blood (20%) and pus/wound swabs (17.1%). Other less common sources included catheter tips, respiratory samples, and other body fluids. This distribution is in agreement with the known predilection of Enterococcus for urinary tract infections but also brings out its role in bloodstream infections and wound colonization. The predominance of urinary tract isolates reflects the need for observant monitoring of urinary tract infections, especially among hospitalized or catheterized patients. This species distribution is similar to that reported from other studies, such as that by Karna et al., (2019) [11], in which, out of 231 Enterococcus species isolated, 168 (72.7%) were identified as E. faecalis and 53 (22.8%) as E. faecium.

The pattern of antimicrobial resistance showed that Enterococcus spp. strains were highly resistant to ciprofloxacin (68.6%), levofloxacin (60%), and ampicillin (54.3%). It exhibited moderate resistance to high-level streptomycin, high-level gentamicin, and nitrofurantoin in urine isolates. Resistance to last-resort antibiotics like vancomycin, linezolid, teicoplanin, and tigecycline was low and these agents are thus largely effective. The data demonstrated a worrying level of resistance to usually recommended antibiotics, especially fluoroquinolones, and aminopenicillins, a continuing challenge in empiric treatment. All the isolates in our study revealed a high degree of sensitivity to linezolid, teicoplanin, and vancomycin, like data from Gupta et al., (2015) [12]

Looking at the species-specific resistance profiles in more detail for nearly all antibiotics, resistance rates were significantly higher for E. faecium than for E. faecalis. E. faecium showed alarmingly high resistance to ampicillin (79%), to high-level gentamicin (52%), to high-level streptomycin (52%), and to fluoroquinolones (ciprofloxacin 89%, levofloxacin 83%). In contrast, for E. faecalis, resistance was lower for all these antibiotics. Resistance to the most critical antibiotics, such as vancomycin, linezolid, and tigecycline, was low for both species but consistently higher for E. faecium. These results emphasize the therapeutic challenge caused by E. faecium and stress the need for species identification in clinical microbiology. Zhang et al., (2017) [13] suggested that nosocomial BSI with enterococci is increasing and overall mortality is quite high, ranging from 25% to 50%.

The growing percentage of E. faecium throughout the study period, along with its wider resistance profile, may indicate a possible change in clinical management. Infection control measures, antimicrobial stewardship, and routine surveillance should be reinforced to prevent further dissemination of resistant strains. Moreover, the data justify giving priority to susceptibility testing of E. faecium, especially for bloodstream and urinary infections, in view of its tendency toward multidrug resistance. According to O'Driscoll et al., (2015) [14], the maximum number of isolates was obtained from urine (46.6%), followed by purulent secretions (19.4%).

Overall, the findings of this study indicate that Enterococcus continues to be a major nosocomial pathogen, with E. faecalis predominant but E. faecium slowly emerging. Resistance trends predict that empirical therapy using commonly used antibiotics is going to be less effective, against E. faecium. Preserving the effectiveness of last-resort antibiotics, as well as implementing strong infection prevention measures, remains critical in keeping the impact of multidrug-resistant Enterococcus low in healthcare settings.

Conclusion

This five-year retrospective study shows the changing landscape of Enterococcus infections in a tertiary care setting. E. faecalis remained the most common species, but with a steadily increasing proportion due to E. faecium, equaling that of E. faecalis in 2025, showing its growing clinical importance. Urine isolates were most common, reflecting the important contribution of Enterococcus to urinary tract infections. Blood and wound infections were also substantial contributors. Antimicrobial susceptibility patterns showed high fluoroquinolone and ampicillin resistance, with E. faecium demonstrating wider multidrug resistance compared to E. faecalis. Resistance to critical agents like vancomycin, linezolid, and tigecycline was low, with therapeutic options preserved. These results emphasize the importance of continued surveillance, identification at the species level, and enhanced antimicrobial stewardship necessary for the optimization of empirical therapy and limitation of the spread of multidrug-resistant Enterococcus in healthcare settings.

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