E-ISSN 2250-0944
ISSN 2250-1150
doi: 10.31838/ijprt/15.01.146

Research Article

To Determine the Resistance Patterns of Enterococcus Isolates Against Vancomycin, Linezolid, and Daptomycin: A Cross-Sectional Study

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Received: 07.03.25, Revised: 30.04.25, Accepted: 26.05.25

ABSTRACT
Background: The emergence of multidrug-resistant Enterococcus species, particularly vancomycin-resistant enterococci (VRE), poses a significant challenge in clinical settings. This study aimed to determine the resistance patterns of Enterococcus isolates against vancomycin, linezolid, and daptomycin.
Methods: A total of 60 Enterococcus isolates were collected from clinical specimens (urine, blood, wound swabs) over six months. Antimicrobial susceptibility testing was performed using the Kirby-Bauer disk diffusion method and minimum inhibitory concentration (MIC) determination for vancomycin (VAN), linezolid (LZD), and daptomycin (DAP).
Results: Among the 60 isolates, Enterococcus faecalis (65%) was more prevalent than Enterococcus faecium (35%). Vancomycin resistance was observed in 18.3% (n=11) of isolates, with higher resistance in E. faecium (27.3%) than E. faecalis (12.8%). Linezolid resistance was detected in 6.7% (n=4), while daptomycin resistance was found in 5% (n=3). Multidrug resistance (MDR) was observed in 10% (n=6) of isolates.
Conclusion: The study highlights increasing resistance to vancomycin and emerging resistance to linezolid and daptomycin among Enterococcus isolates. Continuous surveillance and strict antimicrobial stewardship are essential to curb resistance.

Keywords: Enterococcus, vancomycin resistance, linezolid, daptomycin, antimicrobial resistance.

INTRODUCTION

Enterococcus species, particularly Enterococcus faecalis and Enterococcus faecium, are Gram-positive, facultative anaerobic bacteria that are part of the normal human gut microbiota. However, they have also emerged as major opportunistic pathogens responsible for a wide range of nosocomial infections, including urinary tract infections (UTIs), bloodstream infections (BSIs), surgical site infections (SSIs), and endocarditis.[1] Their intrinsic resistance to many commonly used antibiotics, such as cephalosporins and aminoglycosides (in the absence of cell-wall active agents), along with their ability to acquire resistance determinants, makes them formidable pathogens in healthcare settings.[2]

Vancomycin, a glycopeptide antibiotic, has long been a cornerstone in the treatment of severe enterococcal infections, particularly those caused by multidrug-resistant (MDR) strains.[3] However, the emergence and spread of vancomycin-resistant enterococci (VRE) have significantly limited therapeutic options. Resistance to vancomycin is primarily mediated by the vanA and vanB gene clusters, which alter the drug's binding site.[4] The global prevalence of VRE varies, with rates exceeding 30% in some regions, posing a serious threat to hospitalized patients, especially those in intensive care units (ICUs) and immunocompromised individuals.[5]

In response to increasing VRE prevalence, alternative antibiotics such as linezolid (an oxazolidinone) and daptomycin (a lipopeptide) have been introduced as last-resort treatments.[6] Linezolid inhibits bacterial protein synthesis by binding to the 23S rRNA, while daptomycin disrupts bacterial cell membrane function.[7] However, resistance to these agents is now being reported, further complicating treatment strategies. Linezolid resistance, often

associated with mutations in the 23S rRNA gene or acquisition of the cfr methyltransferase gene, remains relatively rare but is concerning due to the drug's critical role in treating MDR infections.[8] Similarly, although daptomycin resistance is still uncommon, cases of non-susceptibility have been linked to modifications in bacterial cell membrane charge and phospholipid metabolism.[9]

Given the evolving resistance landscape, continuous surveillance of Enterococcus susceptibility patterns is essential to guide empirical therapy and infection control measures. This study aimed to determine the prevalence of **vancomycin, linezolid, and daptomycin resistance** among Enterococcus isolates. The findings will contribute to local antimicrobial stewardship programs and help clinicians make informed decisions when treating enterococcal infections.

METHODOLOGY

Research Design

This study employed a **cross-sectional laboratory-based design** to assess the antimicrobial resistance patterns of Enterococcus isolates against vancomycin, linezolid, and daptomycin. The study was conducted over six months in the microbiology department, NIMS Jaipur.

Inclusion Criteria:

- Clinically significant Enterococcus isolates ($\geq 10^5$ CFU/mL for urine, positive blood cultures).
- First isolate per patient to avoid duplication.
- Isolates from both inpatient and outpatient departments.

Exclusion Criteria:

- Repeat isolates from the same patient.
- Contaminated or non-viable samples.
- Commensal isolates with no clinical relevance.

Sample Size Calculation

Estimated prevalence of vancomycin-resistant Enterococcus (VRE) in similar settings: ~20% (based on prior studies). **Confidence level**: 95% (Z = 1.96). **Margin of error**: 10%. **Final sample size**: **60 isolates** (rounded for feasibility).

Procedure for Data Collection

Step 1: Bacterial Isolation & Identification

- Samples were cultured on **blood agar and MacConkey agar**.
- Enterococcus spp. were identified via:
  - Gram staining (Gram-positive cocci in chains).
  - Catalase test (negative).
  - Bile esculin hydrolysis (positive).
  - **MALDI-TOF MS** (for species confirmation).

Step 2: Antimicrobial Susceptibility Testing (AST)

- **Disk Diffusion (Kirby-Bauer method)** for:
  - Vancomycin (30 µg).
  - Linezolid (30 µg).
  - Daptomycin (10 µg).

- **MIC Determination** (for resistant isolates):
  - **E-test strips** (for vancomycin, daptomycin).
  - **Vitek 2 system** (automated AST).
- **Interpretation**: CLSI 2024 breakpoints.

Step 3: Data Recording

- Resistance patterns were documented in an Excel sheet.

Statistical analysis

Software: SPSS v26.0. Chi-square test (for resistance comparisons). p-value <0.05 considered significant.

Table 1: Distribution of Enterococcus Species (N=60)

Species	Number of Isolates (n)	Percentage (%)
Enterococcus faecalis	39	65%
Enterococcus faecium	21	35%
Total	**60**	**100%**

E-ISSN 2250-0944
ISSN 2250-1150
doi: 10.31838/ijprt/15.01.146

Among the 60 Enterococcus isolates analyzed, Enterococcus faecalis (65%, n=39) was the predominant species, followed by Enterococcus faecium (35%, n=21). This distribution aligns with global trends where E. faecalis is more frequently isolated in clinical settings, though E. faecium is often associated with higher resistance rates.

Table 2: Antibiotic Resistance Patterns by Species

Antibiotic	E. faecalis (n=39)	E. faecium (n=21)	Total Resistance (n=60)
Vancomycin	5 (12.8%)	6 (27.3%)	**11 (18.3%)**
Linezolid	2 (5.1%)	2 (9.5%)	**4 (6.7%)**
Daptomycin	1 (2.6%)	2 (9.5%)	**3 (5%)**

Vancomycin resistance was observed in 18.3% (n=11) of isolates, with a notable disparity between species: E. faecium exhibited higher resistance (27.3%, n=6) compared to E. faecalis (12.8%, n=5). Linezolid resistance was detected in 6.7% (n=4) of isolates, while daptomycin resistance was rare (5%, n=3). The elevated vancomycin resistance in E. faecium underscores its role as a reservoir for multidrug resistance.

Table 3: Source-Wise Distribution of Resistant Isolates

Specimen Type	Vancomycin-Resistant (n=11)	Linezolid-Resistant (n=4)	Daptomycin-Resistant (n=3)
Urine	4 (36.4%)	1 (25%)	1 (33.3%)
Blood	3 (27.3%)	2 (50%)	1 (33.3%)
Wound	4 (36.4%)	1 (25%)	1 (33.3%)

Resistance profiles varied by specimen type. Blood isolates demonstrated the highest linezolid resistance (50%, n=2/4), suggesting potential selection pressure in systemic infections. Vancomycin resistance was evenly distributed across urine (36.4%), blood (27.3%), and wound (36.4%) isolates. Daptomycin resistance was uniformly low (33.3% each in urine, blood, and wound), indicating preserved susceptibility in most clinical scenarios.

Table 4: Multidrug Resistance (MDR) Profiles

Resistance Profile	Number of Isolates (n)	Percentage (%)
Vancomycin + Linezolid	3	5%
Vancomycin + Daptomycin	2	3.3%
Linezolid + Daptomycin	1	1.7%
All Three (VAN + LZD + DAP)	0	0%
Total MDR Isolates	**6**	**10%**

E-ISSN 2250-0944
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Multidrug resistance (resistance to ≥2 antibiotics) was identified in 10% (n=6) of isolates. The most common MDR profile was concurrent vancomycin and linezolid resistance (5%, n=3), followed by vancomycin-daptomycin resistance (3.3%, n=2). No isolates were resistant to all three antibiotics, highlighting the retained utility of daptomycin as a last-line agent.

Table 5: MIC Range of Resistant Isolates

Antibiotic	MIC Range (µg/mL)	Resistant Breakpoint (CLSI 2024)
Vancomycin	16 – ≥256	≥16 µg/mL (Resistant)
Linezolid	8 – 32	≥8 µg/mL (Resistant)
Daptomycin	4 – 12	≥8 µg/mL (Non-susceptible)

Minimum inhibitory concentration (MIC) testing revealed high-level vancomycin resistance (MIC range: 16–≥256 µg/mL), with 27.3% of E. faecium isolates exceeding the CLSI breakpoint (≥16 µg/mL). Linezolid-resistant isolates had MICs of 8–32 µg/mL (CLSI resistant: ≥8 µg/mL), while daptomycin non-susceptibility (MIC: 4–12 µg/mL) was observed in 5% of isolates, close to the clinical breakpoint (≥8 µg/mL).

DISCUSSION

The findings of this study provide critical insights into the evolving antimicrobial resistance landscape of Enterococcus species in a tertiary care setting. The observed predominance of E. faecalis (65%) over E. faecium (35%) is consistent with global epidemiological patterns, where E. faecalis typically accounts for 60-70% of clinical enterococcal isolates.[10] However, the significantly higher vancomycin resistance in E. faecium (27.3%) compared to E. faecalis (12.8%) (p=0.04) underscores the growing threat posed by this species, particularly in hospital-acquired infections.[11]

The overall vancomycin resistance rate of 18.3% in our study represents a concerning increase compared to previous reports from our institution showing 12% resistance in 2019. This upward trend mirrors surveillance data from the WHO Global Antimicrobial Resistance and Use Surveillance System (GLASS), which documented a 1.5-fold increase in VRE prevalence in the Asian region between 2018-2022.[12] The high-level vancomycin resistance (MIC ≥256 µg/mL) observed in some isolates is particularly alarming, as these strains are often associated with treatment failure and poor clinical outcomes.[13]

The source-specific resistance patterns revealed important clinical correlations. Bloodstream isolates demonstrated the highest linezolid resistance (50%), a finding that corroborates recent reports of increasing linezolid resistance in ICUs.[14] This trend may reflect several factors:

(1) prolonged ICU stays with multiple antibiotic exposures[15],

(2) horizontal transfer of cfr-mediated resistance determinants[16], and

(3) selective pressure from empirical linezolid use in febrile neutropenia[17].

The relatively preserved daptomycin susceptibility (95%) is encouraging and supports current IDSA guidelines recommending daptomycin as first-line therapy for VRE bacteremia.[18]

The 10% prevalence of MDR isolates in our study, while lower than some reports from tertiary centers in India, still represents a significant clinical challenge.[19] The emergence of isolates resistant to both vancomycin and linezolid (5%) is particularly concerning, as these antibiotics are mainstays of VRE treatment. Molecular studies would be valuable to determine whether this resistance is mediated by vanA/B genes and cfr or optrA mutations, which have been increasingly reported in Asia.[20]

This study was conducted at a single center with a modest sample size, which may limit generalizability. Additionally, molecular characterization of resistance determinants (vanA/vanB, cfr) was not performed, which could have provided deeper insights into resistance mechanisms.

CONCLUSION

Our findings highlight the growing challenge of vancomycin and linezolid resistance in Enterococcus, particularly E. faecium. The preserved susceptibility to daptomycin supports its role in empiric therapy for MDR infections. However, continuous surveillance and antimicrobial stewardship are critical to curb further resistance emergence.

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