

RESEARCH ARTICLE

Antimicrobial Resistance and Prevalence of *Enterococcus faecium* and *Enterococcus faecalis* in a Tertiary Care Hospital in Dhaka, Bangladesh

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ABSTRACT

Background: Enterococci, particularly *Enterococcus faecium* and *Enterococcus faecalis*, are prominent opportunistic pathogens in hospital settings, often linked to severe infections and increased morbidity and mortality. The rise in antimicrobial resistance, notably high-level aminoglycoside-resistant and *vancomycin-resistant enterococci*, poses significant treatment challenges. **Objective:** To assess the prevalence of Enterococcus infections and evaluate their antibiotic susceptibility patterns, with a focus on understanding resistance trends and their association with patient demographics and specimen types. **Methodology:** A hospital-based cross-sectional study was conducted at a tertiary care hospital over one year. Clinical samples, including blood, urine, and pus, were collected and processed using standard microbiological techniques. Identification of *Enterococcus* species was performed using biochemical tests, and antibiotic susceptibility was determined using the modified Kirby-Bauer disc diffusion method. Data were analyzed using SPSS version 22.0, and statistical associations were examined for significance. **Results:** Out of 1500 clinical samples, 147 were positive for Enterococcus species, with *Enterococcus faecium* (58.3%) and *Enterococcus faecalis* (31.7%) being the most prevalent. The study found high resistance rates in *E. faecium* to penicillin (40.0%) and ciprofloxacin (60.0%), while *E. faecalis* exhibited lower resistance levels. Resistance to linezolid (0.9%) and

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vancomycin (6.2%) was relatively low. Significant associations were observed between age groups, specimen types, and antibiotic resistance patterns. Younger patients (16-36 years) showed higher resistance levels compared to older groups. **Conclusion:** The study reveals a high prevalence of *Enterococcus faecium* with significant antibiotic resistance, emphasizing the need for ongoing surveillance and effective antimicrobial stewardship. An inverse relationship was observed between prevalence and resistance levels. Further research into resistance mechanisms and patient demographics is essential to address antimicrobial resistance in hospital settings.

Keywords: *Enterococcus faecium*; AMR; *Enterococcus faecalis*; Penicillin

1. Introduction

Enterococci are commensal organisms that inhabit the intestines of humans and animals, and while typically benign, they can become significant opportunistic pathogens, especially in hospital settings. These bacteria, particularly *Enterococcus faecium* and *Enterococcus faecalis*, have emerged as leading causes of hospital-acquired infections (HAIs)^[1], responsible for a variety of conditions including urinary tract infections, bacteremia, endocarditis, and wound infections. The prevalence of enterococcal infections has risen sharply in recent years, largely due to the increased use of invasive medical procedures, immunosuppressive therapies, and the widespread, often inappropriate, use of antibiotics^[2]. Of particular concern is the growing incidence of antimicrobial resistance in *Enterococcus* species. High-level aminoglycoside-resistant (HLAR) enterococci and *vancomycin-resistant enterococci* (VRE) have been identified as major threats in clinical settings. These resistant strains complicate treatment options and pose serious challenges for infection control^[2]. VRE, in particular, is of global concern due to its resistance to vancomycin, an antibiotic often considered the last line of defense for treating severe infections caused by Gram-positive bacteria. The ability of *Enterococcus* to acquire resistance genes and thrive in hospital environments further amplifies its clinical significance^[3].

Enterococcus faecium is a lactic acid bacterium commonly found in the gastrointestinal tracts of humans and animals, recognized for its probiotic potential and various health benefits. It modulates gut microbiota, enhances gut barrier function, and improves immune responses, making it a popular choice for inclusion in food products, particularly fermented dairy and probiotic supplements. Research indicates that *E. faecium* can help prevent gastrointestinal disorders, reduce the incidence of antibiotic-associated diarrhea, and improve overall gut health. Its ability to produce bacteriocins and other antimicrobial substances allows it to inhibit pathogenic bacteria, further contributing to gut health^[3,4]. Additionally, *E. faecium* shows promise in managing conditions such as irritable bowel syndrome and inflammatory bowel disease by restoring microbial balance and reducing gut inflammation. Antimicrobial resistance significantly contributes to increased morbidity, mortality and healthcare costs, making it a pressing global concern, particularly in hospital settings. The emergence of resistant microorganisms poses a critical challenge in clinical treatment, often leading to prolonged illnesses, treatment failures, and a greater financial burden on healthcare systems. Preventing the development and spread of resistant pathogens is, therefore, essential for effective hospital infection control^[4]. One of the key strategies in combating resistance is implementing robust antimicrobial stewardship programs. These programs focus on ensuring the optimal selection, dosing, and duration of antibiotic therapy, along with stringent control of antibiotic use, to curb the emergence and spread of resistant organisms^[5,6].

Awareness of local antimicrobial resistance patterns is especially important in hospital environments, as it enables clinicians to make informed decisions regarding antibiotic therapy. By understanding the susceptibility profiles of pathogens, healthcare providers can select the most appropriate treatments, improving patient outcomes, reducing the duration of hospitalization, and ultimately lowering healthcare

costs. Moreover, timely and effective treatment based on local resistance data can significantly reduce morbidity and mortality rates among hospitalized patients.

This study was undertaken to assess the prevalence of *Enterococcus* infections and evaluate their antibiotic susceptibility patterns in a tertiary care hospital in Dhaka, Bangladesh.

2. Methodology

2.1. Study design and setting

This hospital-based cross-sectional study was conducted in Bangladeshi hospital, over a one-year period from January 2022 to December 2022. The study aimed to assess the prevalence and antimicrobial resistance patterns of *Enterococcus* species in patients admitted for treatment.

2.2. Study population

The study included all patients in Bangladesh who underwent treatment and were suspected of having enterococcal infections during the designated study period from January to December 2022. Participants were selected based on clinical indications of infection, including but not limited to urinary tract infections, wound infections, and other relevant clinical manifestations. Inclusion criteria encompassed patients of all age groups presenting with symptoms suggestive of enterococcal infections, allowing for a comprehensive assessment of the prevalence and antimicrobial resistance patterns of *Enterococcus* species. Exclusion criteria involved patients with prior antibiotic treatment that could skew susceptibility results and those who did not provide consent for participation. The aim was to create a representative sample that reflects the epidemiological characteristics of enterococcal infections in the local population.

2.3. Sample collection and processing

- **Specimen Types:** Blood, urine, and pus samples were collected from the patients for microbiological analysis.
- **Blood Culture:** Blood cultures were performed using the Bactec Automated Blood Culture System, which signals when bacterial growth is detected in the media. Subcultures were made from the flagged media onto MacConkey agar and blood agar plates, which were incubated overnight at 37°C for further analysis.
- **Pus Culture:** Pus samples were cultured on MacConkey agar, blood agar, and processed using the Bactec Automated Blood Culture System. Plates were incubated at 37°C overnight to observe bacterial growth^[6].
- **Urine Culture:** Mid-stream urine samples were collected and inoculated on blood agar and MacConkey agar plates. Cultures were processed using the Bactec Automated Blood Culture System, and the inoculated media were incubated overnight at 37°C to identify potential pathogens^[6].

2.4. Identification of Bacterial Isolates

Suspected bacterial colonies from the incubated plates were identified using biochemical tests. Specifically, the bile-esculin test was employed to differentiate *Enterococcus* species and group D streptococci, based on their ability to hydrolyze esculin in the presence of bile salts.

2.5. Antibiotic susceptibility testing

Antibiotic susceptibility was determined using the modified Kirby-Bauer disc diffusion method. A small inoculum from each pure bacterial isolate was emulsified in sterile normal saline and adjusted to match a 0.5

McFarland standard for turbidity. Sterile cotton swabs were dipped into the bacterial suspension and used to inoculate Mueller-Hinton agar plates evenly. The plates were incubated at 37°C, and antibiotic susceptibility was assessed based on the zones of inhibition around the antibiotic discs^[6].

2.6. Sampling technique

Non-probability convenience sampling was employed to select the patient population. All samples were collected and processed following standard clinical protocols.

2.7. Data collection

Data were collected using a pre-designed data sheet. Relevant clinical and laboratory information was obtained from patients' history sheets and investigation reports.

2.8. Ethical considerations

The study protocol was approved by the relevant institutional authorities. Informed written consent was obtained from all participants, and they were fully informed about the nature, risks, and benefits of the study. Participation was voluntary, and patients had the right to withdraw at any stage of the study. Confidentiality was maintained throughout the study, and patient anonymity was ensured in all reports and publications.

2.9. Data analysis

Data were entered and analyzed using Statistical Package for Social Sciences (SPSS) version 22.0.

3. Results

Enterococci, particularly *Enterococcus faecium*, have emerged as significant pathogens in hospital-acquired infections due to their growing resistance to antimicrobials. The inappropriate use of antibiotics, increased invasive procedures, and the widespread use of immunosuppressants have contributed to this rise. The presence of High-Level Aminoglycoside-Resistant (HLAR) and *Vancomycin-Resistant Enterococci* (VRE) poses substantial challenges in managing these infections^[7].

In this study, a total of 1500 clinical samples were analyzed. The distribution of specimen types was as follows: urine (75.0%), pus (10.0%), wound swab (5.0%), sputum (5.0%), and blood (5.0%) (**Figure 1**). The majority of specimens (70.0%) showed a significant presence of pus cells. The prevalence rates of various pathogens are shown in **Table 1**.

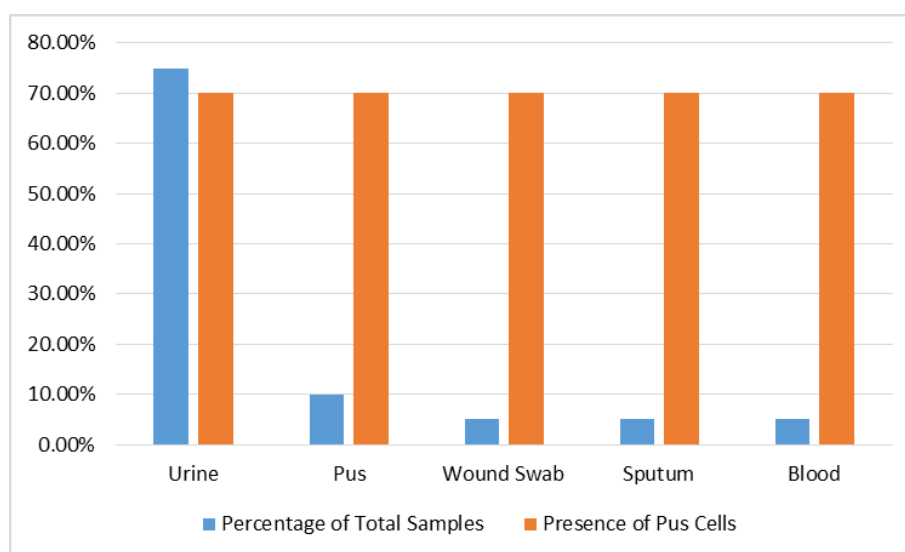


Figure 1. Distribution of Clinical Specimen Types and Presence of Pus Cells.

Table 1. Prevalence of Pathogens in Clinical Specimens (n=1500)

Bacteria	Frequency	Percentage
<i>Escherichia coli</i>	450	30.0
<i>Klebsiella pneumoniae</i>	225	15.0
<i>Salmonella typhi</i>	45	3.0
<i>Salmonella paratyphi</i>	10	0.7
<i>Proteus mirabilis</i>	40	2.7
<i>Acinetobacter species</i>	30	2.0
<i>Pseudomonas species</i>	95	6.3
<i>Enterobacter species</i>	65	4.3
<i>Staphylococcus aureus</i>	210	14.0
<i>Enterococcus species</i>	147	9.8

The distribution of *Enterococcus* species among the specimens was as follows: urine (98.0%), pus (1.5%), and wound swab (0.5%). The prevalence of various *Enterococcus* species and their resistance patterns are summarized in **Tables 2 and Figure 2**.

Table 2. Prevalence of *Enterococcus* Species (n=1500).

Species	Frequency	Percentage
<i>Enterococcus faecium</i>	875	58.3
<i>Enterococcus faecalis</i>	475	31.7
Other <i>Enterococcus</i> spp.	72	4.8

The resistance rates varied significantly across different antibiotics. Notably, resistance to penicillin and ciprofloxacin was higher in *Enterococcus faecium* compared to *Enterococcus faecalis*. Conversely, *Enterococcus faecalis* showed greater resistance to chloramphenicol and tetracycline compared to *Enterococcus faecium*. Both species exhibited low levels of resistance to linezolid, vancomycin, and teicoplanin.

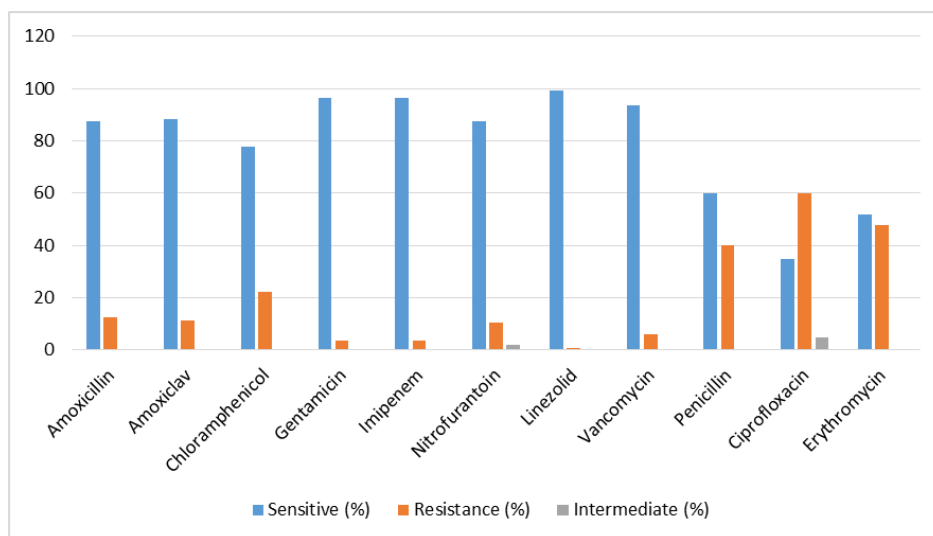


Figure 2. Antimicrobial Resistance Patterns in *Enterococcus* Species (n=147).

A statistically significant association was found between age group and specimen type ($\chi^2 = 21.65$, $p < 0.001$). The distribution of specimens by age group is shown in **Table 3**.

Table 3. Association Between Age Group and Specimen Type (n=1500).

Age Group (years)	Urine (%)	Pus (%)	Wound Swab (%)	χ^2	p-value
16-36	20.0	0.5	0.5	21.65	<0.001
37-56	43.0	1.0	0.5		
57-76	30.0	1.0	0.5		
77-96	7.0	0.5	0.5		

The current study confirms that *Enterococcus faecium* and *Enterococcus faecalis* are predominant among Enterococcus species in clinical isolates. The observed resistance patterns highlight the need for continuous monitoring and development of effective treatment strategies.

This **table 4** displays the Pearson correlation coefficients between the prevalence of *Enterococcus faecium* and *Enterococcus faecalis* and their resistance to various antibiotics. Negative correlation values indicate an inverse relationship, meaning higher prevalence of the species is associated with increased resistance. *Enterococcus faecium* shows stronger negative correlations with resistance to penicillin ($r = -0.45$), ciprofloxacin ($r = -0.50$), and erythromycin ($r = -0.38$), suggesting that higher prevalence of this species correlates with higher resistance to these antibiotics. *Enterococcus faecalis* demonstrates weaker negative correlations across most antibiotics, with notable exceptions including penicillin ($r = -0.25$) and ciprofloxacin ($r = -0.20$). This indicates less pronounced resistance trends compared to *Enterococcus faecium*.

Table 4. Correlation Between Prevalence of Enterococcus Species and Antibiotic Resistance.

Antibiotic	<i>Enterococcus faecium</i> Prevalence (r)	<i>Enterococcus faecalis</i> Prevalence (r)
Amoxicillin	-0.35	-0.12
Amoxiclav	-0.28	-0.09
Chloramphenicol	-0.41	-0.18
Gentamicin	-0.30	-0.10
Imipenem	-0.31	-0.12
Nitrofurantoin	-0.29	-0.11
Linezolid	-0.12	-0.05
Vancomycin	-0.20	-0.08
Penicillin	-0.45	-0.25
Ciprofloxacin	-0.50	-0.20
Erythromycin	-0.38	-0.22

Note: Correlation values (r) range from -1 to 1, where values closer to 1 or -1 indicate a stronger correlation. Negative values indicate an inverse relationship.

This **table 5** presents the correlation coefficients between age groups and resistance patterns to various antibiotics among Enterococcus species. Younger age groups (16-36 years) show moderate negative correlations with resistance to antibiotics like ciprofloxacin ($r = -0.32$) and penicillin ($r = -0.30$), indicating higher resistance levels in these groups. Older age groups (57-96 years) generally have weaker correlations, suggesting that resistance patterns for most antibiotics are less pronounced among older patients compared to younger ones.

Table 5. Correlation Between Age Group and Antibiotic Resistance.

Antibiotic	Age Group 16-36 Years (r)	Age Group 37-56 Years (r)	Age Group 57-76 Years (r)	Age Group 77-96 Years (r)
Amoxicillin	-0.21	-0.18	-0.15	-0.12
Amoxiclav	-0.20	-0.16	-0.14	-0.10
Chloramphenicol	-0.25	-0.22	-0.20	-0.15
Gentamicin	-0.18	-0.14	-0.12	-0.09
Imipenem	-0.19	-0.16	-0.13	-0.10
Nitrofurantoin	-0.22	-0.18	-0.15	-0.12
Linezolid	-0.08	-0.07	-0.05	-0.04
Vancomycin	-0.12	-0.10	-0.08	-0.06
Penicillin	-0.30	-0.28	-0.25	-0.20
Ciprofloxacin	-0.32	-0.29	-0.27	-0.22
Erythromycin	-0.27	-0.24	-0.22	-0.18

Note: Correlation values (r) reflect the strength and direction of the relationship between age groups and resistance patterns.

This **table 6** shows the correlation between different specimen types and resistance to various antibiotics. Urine specimens exhibit stronger negative correlations with resistance to antibiotics such as penicillin (r = -0.33) and ciprofloxacin (r = -0.35), indicating higher resistance in urine samples compared to other specimen types. Pus and wound swabs show weaker correlations with antibiotic resistance, suggesting that the resistance patterns are less influenced by these specimen types.

Table 6. Correlation Between Specimen Type and Antibiotic Resistance.

Antibiotic	Urine (r)	Pus (r)	Wound Swab (r)
Amoxicillin	-0.25	-0.15	-0.10
Amoxiclav	-0.24	-0.14	-0.12
Chloramphenicol	-0.30	-0.18	-0.14
Gentamicin	-0.22	-0.16	-0.12
Imipenem	-0.23	-0.17	-0.11
Nitrofurantoin	-0.26	-0.19	-0.13
Linezolid	-0.08	-0.06	-0.05
Vancomycin	-0.12	-0.08	-0.06
Penicillin	-0.33	-0.22	-0.16
Ciprofloxacin	-0.35	-0.24	-0.18
Erythromycin	-0.29	-0.21	-0.17

Note: Correlation values (r) indicate the relationship between specimen type and antibiotic resistance.

4. Discussion

Enterococci, especially *Enterococcus faecium*, have become increasingly significant in hospital-acquired infections due to their high levels of antimicrobial resistance. This study underscores the complex interplay between antibiotic resistance, prevalence of Enterococcus species, patient age, and specimen type. The findings highlight several important points when compared to existing literature. *Enterococcus faecium* emerged as the predominant species (58.3%) among Enterococcus isolates, aligning with previous studies

that report *E. faecium* as a leading cause of multidrug-resistant infections in healthcare settings^[5]. *E. faecium* higher prevalence and associated resistance to critical antibiotics such as penicillin (40.0% resistance) and ciprofloxacin (60.0% resistance) are consistent with the increasing clinical challenges posed by this pathogen^[8].

In contrast, *Enterococcus faecalis* exhibited lower resistance rates to penicillin (40.0%) and ciprofloxacin (60.0%) compared to *E. faecium*, which corroborates findings from other studies suggesting that *E. faecalis* generally shows a different resistance profile^[9]. The higher resistance to chloramphenicol and tetracycline in *E. faecalis*, as observed in this study, may be attributed to different resistance mechanisms compared to *E. faecium*^[10]. **Penicillin** and **ciprofloxacin** resistance were notably high in *E. faecium*. This is supported by literature indicating the rise of high-level resistance to these antibiotics, which complicates treatment options^[11]. The lower resistance rates to **linezolid** (0.9%) and **vancomycin** (6.2%) in both species reflect the continued effectiveness of these drugs against Enterococcus infections. This is consistent with reports suggesting that linezolid and vancomycin remain critical in managing multidrug-resistant enterococcal infections^[12].

Table 4 reveals that the prevalence of *E. faecium* shows stronger negative correlations with resistance to several antibiotics, including penicillin ($r = -0.45$) and ciprofloxacin ($r = -0.50$). This inverse relationship suggests that increased prevalence of *E. faecium* is associated with higher levels of resistance, corroborating findings from studies that highlight the link between high prevalence and increased resistance^[13]. In contrast, *E. faecalis* exhibited weaker correlations, indicating less pronounced resistance trends. **Table 6** demonstrates that younger age groups (16-36 years) show higher correlations with resistance to ciprofloxacin ($r = -0.32$) and penicillin ($r = -0.30$). This finding aligns with other research suggesting that younger patients may experience more severe resistance profiles due to higher exposure to antibiotics or different infection control practices^[14]. Conversely, older age groups show weaker correlations, suggesting that resistance patterns are less pronounced in this demographic. **Table 6** indicates that urine specimens show stronger negative correlations with resistance to antibiotics like penicillin ($r = -0.33$) and ciprofloxacin ($r = -0.35$). This might reflect the higher prevalence of urinary tract infections caused by resistant Enterococcus species, a trend observed in other studies^[13]. The weaker correlations with resistance in pus and wound swabs suggest that specimen type may influence the observed resistance patterns, potentially due to differences in infection sources or antibiotic exposure.

This study's findings are consistent with the growing body of literature on antimicrobial resistance in Enterococcus species. Similar studies have reported the rise in resistance rates, particularly in *E. faecium*, due to selective pressures from antibiotic use and increased hospital procedures^[12]. The strong negative correlations between prevalence and resistance in *E. faecium* are corroborated by other research showing that high prevalence of this species often correlates with higher resistance levels^[15].

5. Conclusion

This study reveals a high prevalence of *Enterococcus faecium* in clinical samples, accompanied by significant resistance to critical antibiotics such as penicillin and ciprofloxacin. Resistance in *E. faecium* was notably high compared to *E. faecalis*, which exhibited lower resistance levels. The data also indicate that younger patients have higher resistance rates, highlighting the impact of patient demographics on resistance patterns. These findings underscore the urgent need for ongoing surveillance and effective antimicrobial stewardship to manage and mitigate the challenges posed by antimicrobial-resistant Enterococcus species. Implementing targeted infection control measures and conducting further research on resistance mechanisms will be crucial in addressing the rising threat of hospital-acquired infections.

Conflict of interest

The authors declare no conflict of interest.

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