

Examining Antimicrobial Resistance in Enterococcus Species: A Single-Center Cross-Sectional Study

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Abstract

Background: Antimicrobial resistance in Enterococcus species is a growing global concern. These bacteria, commonly found in the human gut, have resisted multiple antibiotics, making infections harder to treat. This poses a significant challenge for healthcare as it limits treatment options and highlights the urgent need for responsible antibiotic use and the development of new therapies. **Aim of the study:** The study aimed to determine the antimicrobial resistance pattern of Enterococcus Species at a tertiary care hospital in Bangladesh. **Methods:** This cross-sectional research occurred at Sample collected from deferent laboratories situated in rangpur and test/research perform, Department of Microbiology in prime institute of science and medical technology (PRISMET), Rangpur During March' 2022 to March' 2023. It spanned one year from [start date] to [end date]. The primary goal was to evaluate the antimicrobial susceptibility patterns of enterococci. Throughout the study, 1,450 samples were collected from various sources, including urine, blood, pus, swabs, and fluids. However, only 62 Enterococci isolates were successfully obtained. Standard microbiological protocols, such as microscopy and culture, were rigorously followed during sample processing. All clinical samples received at the Microbiology laboratory during the study were considered for inclusion. Data analysis was conducted using Microsoft Excel and presented with tables and graphs, while SPSS was employed for statistical analysis. **Result:** The study population primarily consisted of patients aged 36-75 (77.42%), averaging 52.01 years. Gender distribution was skewed towards males (59.68%). Enterococcus spp. They have dominated the microbial species (64.52%), with E. faecalis (14.52%) and E. faecium (11.29%) being prevalent. Urine samples were the most common (66.13%), followed by pus (11.29%) and swab (9.68%). Antibiotic susceptibility analysis showed varying results, with amoxicillin being sensitive in 87.10% of cases, while Cefixime, Cefotaxime, Ceftriaxone, and Cefotaxime exhibited over 96% resistance. **Conclusion:** This study highlights the rise of antimicrobial resistance (AMR) in Enterococcus species at a Bangladeshi tertiary care hospital. Once harmless, these bacteria now pose a severe risk to public health. Our research shows that E. faecalis and E. faecium have become resistant to many common antibiotics, particularly cephalosporins, urging immediate antibiotic stewardship and infection control. To combat this global health threat, we must explore novel treatments and employ a multidisciplinary approach to protect patients and healthcare systems from Enterococcus infections.

Keywords: Enterococcus species, antibiotic resistance, antimicrobial susceptibility pattern.

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INTRODUCTION

Antimicrobial resistance (AMR) poses an increasingly grave threat to global public health, leading to higher mortality rates, prolonged hospital stays, and increased healthcare costs. Enterococcus species have emerged as formidable foes among the myriad pathogens contributing to AMR. These Gram-positive bacteria are known for their resilience and adaptability, making them a concerning target for comprehensive study and

intervention. Enterococci are naturally found in the oral cavity, gastrointestinal tract (GIT), and the female genital tract in humans and animals [1]. While they are typically commensals in adult faeces, enterococci can become significant nosocomial pathogens [2]. The most prevalent nosocomial infections caused by these microorganisms include urinary tract infections, intraabdominal infections, and pelvic infections [3]. Enterococci have become increasingly concerning due to their antibiotic resistance, with E. faecalis being the most

common species in clinical specimens and *E. faecium* exhibiting greater drug resistance than *E. faecalis* [4]. Antimicrobial resistance (AMR) is a major global health threat [5]. Moreover, the antimicrobial agents used to treat these infections are often costly, toxic, and progressively less effective, leading to higher mortality rates [6, 7]. Improper and excessive antibiotic use has been a leading cause of AMR development, and it is expected that antibiotic resistance will not only spread within hospitals but also in communities [8]. The misuse of antibiotics in factory farms contributes significantly to the rapid worldwide increase and dissemination of AMR [9]. In recent years, *Enterococcus* spp. has garnered significant attention among multidrug-resistant (MDR) bacteria responsible for nosocomial infections, primarily due to their ability to cause urinary tract infections, bacteremia, and infective endocarditis [10]. *Enterococci* are Gram-positive opportunistic microorganisms commonly residing in the human gastrointestinal tract [11]. Among *Enterococcus* spp., *E. faecium* and *E. faecalis* are the third leading cause of nosocomial infections after *S. aureus* and *P. aeruginosa* [12]. It has been estimated that *E. faecium* is responsible for 5-10% of these infections, while *E. faecalis* is responsible for 85-90% [13]. Infections caused by *Enterococci* have become a significant concern in recent years due to their ability to acquire resistance to many antimicrobial drugs used in clinical practice, posing severe life-threatening risks to patients with chronic illnesses or cancer [14]. The emergence of vancomycin-resistant *Enterococcus* (VRE) has led to increased use of alternative antibiotics such as daptomycin and linezolid. However, resistance to these alternatives is also rising [16]. The study aimed to determine the antimicrobial resistance pattern of *Enterococcus* Species at a tertiary care hospital in Bangladesh.

METHODOLOGY & MATERIALS

This cross-sectional study was conducted at Sample collected from deferent laboratories situated in rangpur and test/research perform, Department of Microbiology in prime institute of science and medical technology (PRISMET), Rangpur During March' 2022 to March' 2023. The study spanned one year from [start date] to [end date]. The primary objective of this investigation was to assess the antimicrobial susceptibility patterns of enterococci. Throughout the study, 1,450 samples were collected from various sources, including urine, blood, pus, swabs, and fluids. However, only 62 isolates of *Enterococci* were successfully obtained from these samples. The standard microbiological guidelines, including microscopy and culture, were strictly followed during sample processing. All clinical samples received at the Microbiology laboratory during the study period were considered for inclusion. Gram staining was performed for microscopy, while for culture, samples were inoculated on MacConkey agar and Blood agar and incubated at 37°C for 24 hours on the day of sample collection.

Identification of colonies was based on their macroscopic characteristics and Gram stain results. On MacConkey agar, magenta pink, small, round colonies were classified as *Enterococci*, while non-hemolytic translucent colonies on blood agar were also identified as *Enterococci*. Further confirmation was achieved through Gram staining, which revealed a gram-positive arrangement. Before collecting data, informed consent was taken from every participant.

Statistical Analysis

Data analysis was carried out using Microsoft Excel. The study results were presented in an organized manner using tables and graphs to enhance clarity and understanding. All statistical analyses were conducted using the Statistical Package for the Social Sciences (SPSS) program on a Windows platform. Continuous parameters were expressed as mean±SD, while categorical parameters were presented as frequency and percentage.

RESULT

Table 1 provides the age distribution within the study population. Most patients (41.94%) fell into the 56-75 age bracket, followed by the 36-55 age group at 35.48%. The 16-35 age group accounted for 19.35% of patients, while only two individuals (2.58%) were in the 75-95 age range. On average, the age of the study population was 52.01 years, with a standard deviation of 15.89. In terms of gender, the majority of participants were male (59.68%), while females comprised 40.32% of the population (Figure 1). Table 2 details the distribution of microbial species within the study. The predominant species identified was *Enterococcus* spp., representing 64.52% of the total. Among *Enterococcus* species, *E. faecalis* was the most prevalent, accounting for 14.52% of cases, followed closely by *E. faecium* at 11.29%. Smaller proportions included *E. galinarum* (3.23%, 2 cases), *E. casseliflavus* (1.61%, 1 case), *E. avium* (1.61%, 1 case), *E. durans* (1.61%, 1 case), and *E. hirae* (1.61%, 1 case). Table 3 highlights the types of clinical samples collected, with urine being the most commonly obtained (66.13%). Pus and swab samples constituted 11.29% and 9.68% of the samples, respectively. Blood samples were less frequent, representing 4.84% of the total samples, while stool and fluid samples had a lower representation, each accounting for approximately 1.61% of the samples. Table 4 presents a comprehensive analysis of antibiotic susceptibility for various antibiotics, providing insights into the number and percentage of cases that were sensitive, resistant, or showed intermediate responses. For example, amoxicillin exhibited sensitivity in 87.10% of cases and resistance in 12.90% of cases, with no instances of an intermediate response. Conversely, antibiotics like Cefixime, Cefazidime, Ceftriaxone, and Cefotaxime displayed high resistance percentages exceeding 96%, with minimal sensitivity or intermediate responses. This table serves as a valuable reference for

assessing the effectiveness of these antibiotics against different strains or pathogens.

Table 1: Age distribution of the study population (N=62).

Age range (Year)	Frequency (n)	Percentage (%)
16-35	12	19.35
36-55	22	35.48
56-75	26	41.94
75-95	2	3.23
Mean±SD	52.01±15.89	
Total	62	100.00

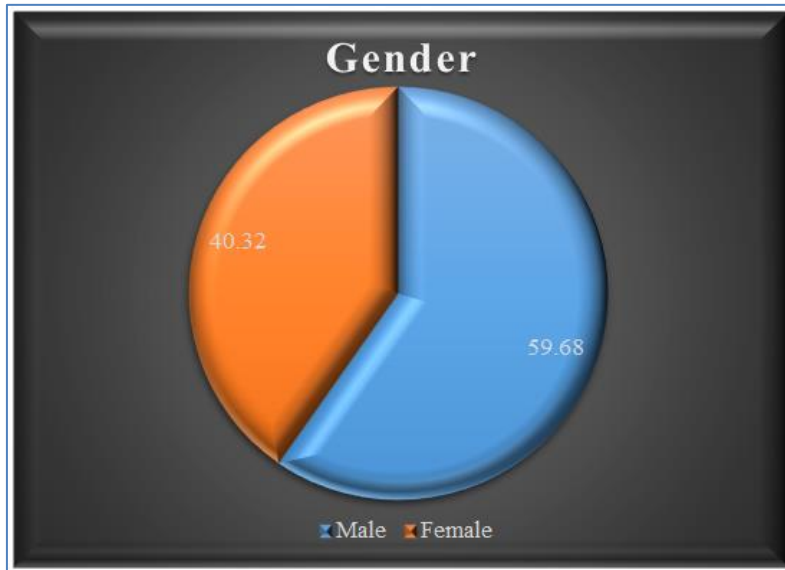


Figure 1: Gender distribution of the study population (N=62).

Table 2: Species detection (N=62)

Species	Frequency (n)	Percentage (%)
Enterococcus Spp.	40	64.52
E. faecalis	9	14.52
E. faecium	7	11.29
E. galinarum	2	3.23
E. casseliflavus	1	1.61
E. avium	1	1.61
E. durans	1	1.61
E. hirae	1	1.61

Table 3: Clinical samples for isolates of Enterococci

Clinical sample	Frequency (n)	Percentage (%)
Urine	41	66.13
Pus	7	11.29
Swab	6	9.68
Blood	3	4.84
Sputum	1	1.61
Stool	1	1.61
Fluid	1	1.61
Others	2	3.23

Table 4: Antibiotic resistance pattern of Enterococci of the study population

Antibiotics	Sensitive		Resistant		Intermediate	
	n	%	n	%	n	%
Amoxicillin	54	87.10	8	12.90	0	0.00
Amoxiclave	55	88.71	7	11.29	0	0.00
Cefixime	2	3.23	60	96.77	0	0.00
Ceftazidime	2	3.23	60	96.77	0	0.00
Ceftriaxone	2	3.23	60	96.77	0	0.00
Cefotaxime	2	3.23	60	96.77	0	0.00
Ciprofloxacin	24	38.71	35	56.45	3	4.84
Cefotaxime	1	1.61	61	98.39	0	0.00
Chloramphenicol	48	77.42	14	22.58	0	0.00
Co-trimoxazole	1	1.61	61	98.39	0	0.00
Gentamycin	60	96.77	2	3.23	0	0.00
Imipenem	60	96.77	2	3.23	0	0.00
Nalidixci acid	4	6.45	58	93.55	0	0.00
Nitrofurantoin	55	88.71	6	9.68	1	1.61
Doxycycline	31	50.00	30	48.39	1	1.61
Liniazolid	61	98.39	1	1.61	0	0.00
Levofloxacin	27	43.55	35	56.45	0	0.00
Vancomycine	58	93.55	4	6.45	0	0.00

DISCUSSION

A total of 63 patients were enrolled and analyzed in this study. Enterococci are commensal bacteria that inhabit the intestines of both humans and animals. They are the major conditionally pathogenic bacteria responsible for hospital-acquired infections. The increasing inappropriate use of antimicrobial agents, the rise in invasive therapy, and the widespread use of immunosuppressants have led to many clinical infections caused by *Enterococcus* spp., particularly *Enterococcus faecium* [17]. Our study yielded similar results to Mesbah *et al.*'s study in 2020, with a mean±SD of 52.01±15.89 [18]. In our study, 59.68% of patients were male, and 40.32% were female. Radadiya *et al.*, reported 59% male and 41% female participants in their study [19]. Out of the 62 samples in our study, 40 (64.52%) were *Enterococcus* spp., 9 (14.52%) were *E. faecalis*, and 7 (11.29%) were *E. faecium*. Elham Jannati *et al.*, reported 118 *Enterococcus* spp., 235 *E. faecium*, and 56 *E. faecalis* in their study [20]. Barreto *et al.*, and Poeta *et al.*, found that *E. faecium* accounted for over 50% of *Enterococcal* isolates recovered from patients [21]. This species distribution aligns with other studies' findings [22, 23]. The majority of isolates in our study came from urine (41, 66.13%), followed by pus (7, 11.29%), and swab (6, 9.68%). Radadiya *et al.*, reported a similar trend in their study, with most isolates coming from urine, followed by pus, swab, and blood [19]. Globally, there is a significant level of ampicillin resistance in clinical isolates. Approximately 87.10% of species were sensitive to amoxicillin, 88.71% were sensitive to amoxiclave, 77.42% to chloramphenicol, and 96.77% to gentamycin and imipenem. However, a high percentage, 98.39%, of species showed resistance to Cefotaxime and Co-trimoxazole, as well as 96.77% resistance to Cefixime, Ceftazidime, Ceftriaxone, and Cefotaxime.

Mesbah *et al.*, obtained similar results in their 2020 study [18].

Limitations of the Study

This study's main limitation stems from its relatively small sample size. Out of 1,450 collected samples, only 62 *Enterococci* isolates were successfully obtained, potentially limiting the representation of *Enterococcus* species' diversity and prevalence in the population. The research occurred exclusively at a single tertiary care hospital in Bangladesh, which might not capture broader regional or national antimicrobial resistance trends for *Enterococcus*. Additionally, the study's cross-sectional design offers only a momentary view of antimicrobial resistance patterns, needing more ability to establish causality or track changes over time. Longitudinal studies or extended surveillance would provide more comprehensive insights into antimicrobial resistance dynamics.

CONCLUSION AND RECOMMENDATIONS

In conclusion, this study sheds light on the alarming prevalence of antimicrobial resistance (AMR) among *Enterococcus* species in a tertiary care hospital in Bangladesh. Once considered commensal bacteria, *Enterococci* have evolved into significant nosocomial pathogens, posing a substantial threat to public health. Our findings reveal that *Enterococcus* species, particularly *E. faecalis* and *E. faecium*, have developed resistance to a wide range of antibiotics commonly used in clinical practice. Antibiotic susceptibility analysis highlights the concerning resistance levels, with several antibiotics showing limited effectiveness against *Enterococcus* species. Notably, high resistance rates were observed for cephalosporins, emphasizing the urgency of appropriate antibiotic stewardship in clinical

settings. These findings underscore the pressing need for enhanced infection control measures, judicious antibiotic prescribing practices, and the development of alternative treatment strategies to combat AMR in Enterococcus infections. Future research should focus on understanding the molecular mechanisms underlying resistance and exploring novel therapeutic options to address this growing global health threat. In the face of increasing AMR, a multidisciplinary approach is imperative to mitigate the impact of Enterococcus-related infections on patient outcomes and healthcare systems.

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