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Urinary Tract Infections by *Enterococcus* Species: A Retrospective Study at a Tertiary Care Hospital in Dhaka, Bangladesh

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Abstract

Background: *Enterococcus* species (*Enterococcus* species) have progressively transitioned from being commensals to causing severe, life-threatening hospital-acquired infections worldwide, largely due to their natural antimicrobial resistance and virulence potential. **Objective:** This retrospective study was carried out to determine the prevalence of *Enterococcal* infections in urine and assess their antibiotic susceptibility patterns at a tertiary care hospital in Dhaka, Bangladesh. **Methodology:** This six-month retrospective analysis was performed in the Microbiology Laboratory of Dhaka Medical College, Dhaka, Bangladesh. Urine specimens were processed to isolate *Enterococcus* species using culture methods, microscopic evaluation, and a series of biochemical assays. The antimicrobial resistance patterns of the isolates were examined using the Modified Kirby-Bauer disk diffusion technique. **Results:** Out of 5,957 urine samples, 872 (14.64%) yielded positive cultures. Among the culture-positive isolates, Gram-positive cocci accounted for 60(6.9%) of the isolates with 52(6.0%) identified as *Enterococcus* species. In this study, *Enterococcus* species showed high resistance to ciprofloxacin (82.7%), and doxycycline (65.4%). However, they were highly susceptible to linezolid (100.0%), vancomycin (94.2%), and teicoplanin (92.3%). **Conclusion:** Our study highlights a significant prevalence of resistance to ciprofloxacin, and doxycycline among *Enterococcal* isolates in urine. The prevalence of vancomycin-resistant *Enterococci* (VRE) continues to rise. Vancomycin and teicoplanin, both glycopeptide antibiotics, exhibit similar resistance rates, making teicoplanin a viable alternative to vancomycin.

Keywords: Antimicrobial resistance; Enterococcus species; urinary tract infection; VRE

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Introduction

Enterococcus species which are Gram-positive, facultative anaerobic, naturally found as harmless commensals in the gastrointestinal tract of humans and other warm-blooded animals; however, they harbor virulence factors and are considered opportunistic pathogens, responsible for a range of hospital-associated infections, including urinary tract infections, intra-abdominal infections, bacteremia, and endocarditis[1-2].

UTIs are a common cause of infections in people of all genders and ages worldwide[3]. They can be either

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asymptomatic or symptomatic, with symptoms ranging from mild painful urination to more severe conditions like bacteremia, sepsis, or even death[4]. The most common bacterial cause of urinary tract infections is Escherichia coli, responsible for nearly 80.0% of infections. The remaining 20.0% are primarily caused by *Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterococcus faecalis*, and *Staphylococcus saprophyticus*[5].

The rapid rise of antibiotic resistance among common pathogens is a major global health concern. Infections caused by *Enterococcus* species are particularly challenging due to their ability to thrive in extreme environments and their inherent and multidrug antibiotic resistance, making them a significant area of focus[6]. The increase in antibiotic resistance among *Enterococcus* species, particularly to vancomycin, is driven by the selective pressure from antibiotic exposure. This resistance develops through genetic mutations or horizontal gene transfer[7-8]. This study was conducted to assess the prevalence of *Enterococcal* infections in urine and evaluate their antibiotic susceptibility profile in a tertiary care hospital in Dhaka, Bangladesh.

Methodology

Study Settings and Population: This retrospective study was carried out in the Microbiology laboratory of Dhaka Medical College, Dhaka, Bangladesh between January and June 2024. A total of 5,957 urine samples were collected from patients of all age groups and both sexes, visiting the outpatient and inpatient departments of Dhaka Medical College Hospital.

Sample Collection and Processing[9-10]: Approximately 10 mL of midstream, clean-catch urine was collected in sterile, dry, wide-mouthed, leak-proof containers and promptly delivered to the laboratory. A 10 mL portion of urine was subjected to centrifugation at 1000X g for five minutes. Following centrifugation, the supernatant was carefully removed, and the remaining sediment was gently re-suspended by tapping the base of the tube. A single drop of the thoroughly mixed sediment was then transferred onto a clean glass slide, covered with a coverslip, and examined microscopically under both 10X and 40X magnifications.

Culture of Urine[9,10]: Urine samples having more than 5 polymorphonuclear cells (PMNs/HPF) were inoculated in chromogenic agar media (HiMedia, India) and incubated aerobically for 24 hours at 37°C. After incubation, the plates were inspected for bacterial colony growth. The bacterial load was interpreted as 10,000 colony-forming units (CFU) per mL. More than 1,00,000 CFU /mL (105/mL) was considered as significant bacteriuria.

Samples identification: All suspected colonies were identified by standard microbiological methods such as Gram staining, examination of colonial morphology, growth on selective media, lactose and mannitol fermentation, hemolytic property, pigment production, biochemical tests including catalase, coagulase, oxidase, bile esculin hydrolysis, H2S production, indole and citrate utilization, motility, and urease activity[11-13].

Antimicrobial Susceptibility Test: Antimicrobial susceptibility testing of clinical isolates was conducted on Muller-Hinton agar (MHA) (HiMedia, India) using standard disc diffusion technique like modified Kirby-Bauer method[14]. The antibiotic discs, sourced commercially (Oxoid Ltd, UK), were used according to the standard antibiotic panel for the identified organisms. Antibiotic discs used for sensitivity tests were ciprofloxacin, nitrofurantoin, fosfomycin, doxycycline, ampicillin, vancomycin and linezolid and teicoplanin. Zones of inhibition diameter were interpreted according to the CLSI (2024) guidelines[15].

Results

During the study period, 5,957 urine samples were analyzed for culture and sensitivity, with 872 (14.64%) yielding positive cultures (Figure I).

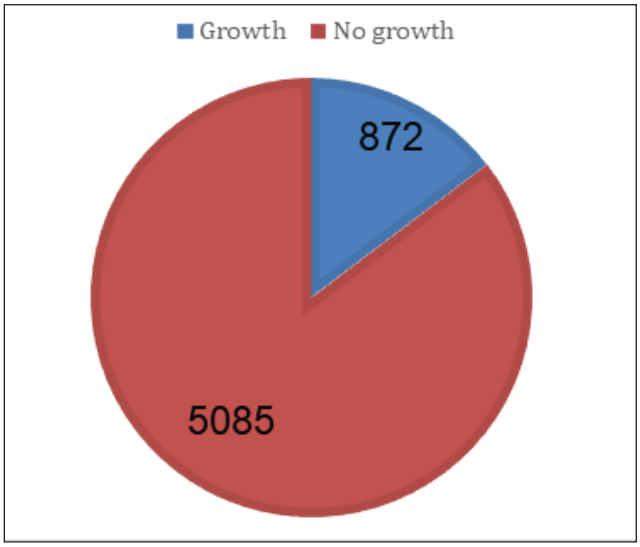

Figure I: Culture Positivity of the Study Population (N= 5,957)

Among the 872 culture-positive isolates, Gram-negative bacteria were the predominant group, comprising 812

(93.12%) isolates. In contrast, Gram-positive bacteria accounted for 60 (6.88%) isolates of the total (Figure II).

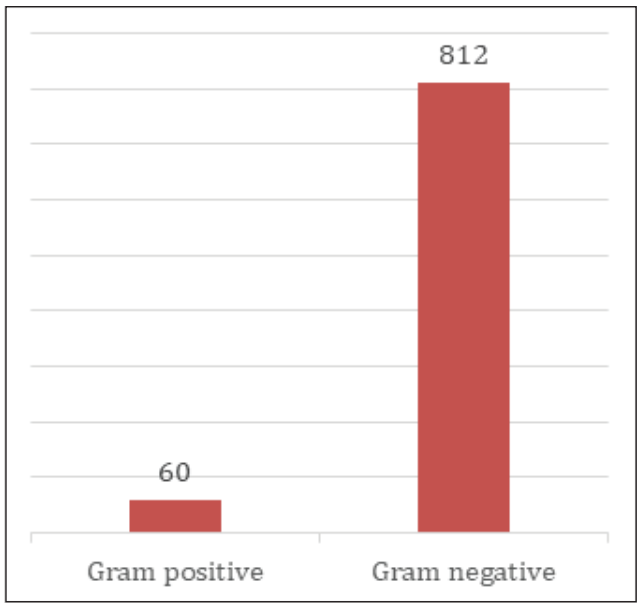

Figure II: Organism distribution according to Gram positivity among culture positive isolates (N= 872)

Among the Gram-positive bacteria, *Enterococcus* spp. were the most common, representing 52 (86.7%) isolates followed by *Staphylococcus aureus* 7(11.7%), and *Staphylococcus saprophyticus* 1 (1.7%) (Table 1).

Table 1: Distribution of Gram- positive bacteria (N= 60)

Isolated Gram- positive bacteria	Frequency	Percent
Enterococcus species	52	86.7
Staphylococcus aureus	7	11.7
Staphylococcus saprophyticus	1	1.7

Enterococcus species exhibited high resistance to ciprofloxacin (82.7%), doxycycline (65.4%), while showing strong susceptibility to linezolid (100.0%), vancomycin (94.2%), and teicoplanin (92.3%) (Table 2).

Table 2: Antimicrobial Resistance Pattern of Isolated *Enterococcus species* (N = 52))

Antibiotics	Frequency	Percent
Ciprofloxacin	43	82.7
Doxycycline	34	65.4
Ampicillin	22	42.3
Nitrofurantoin	18	34.6
Fosfomycin	16	30.8
Teicoplanin	4	7.7
Vancomycin	3	5.8
Linezolid	0	0.0

Discussion

Enterococcal infections caused a range of diseases, including UTIs, wound infections, soft tissue infections, and bacteremia. Factors such as urinary tract instrumentation, catheterization, genitourinary issues, previous antibiotic use, and prolonged hospital stays contributed to the risk of these infections[16-17]. *Enterococcus faecalis* accounts for 80.0% to 90.0% of enterococcal infections, which can include severe, life-threatening conditions. It is a significant cause of nosocomial (hospital-acquired) infections worldwide. The growing prevalence of drug-resistant *Enterococcus faecalis* strains, particularly those resistant to most antimicrobial agents such as vancomycin, poses a global health threat. These resistant strains contribute to the increasing challenge of managing hospital infections[18].

In this study, the majority of culture-positive isolates were Gram-negative bacteria, accounting for 93.1%, while Gram-positive bacteria represented 6.9%. Among the Gram-positive isolates, *Enterococcus* species were the most frequently identified.

In this study, 52(6.0%) *Enterococcus* spp. were isolated from urine samples, a result similar to a related study in Bangladesh, which reported 8.4% *Enterococcus* species[19]. Additionally, another study in Brazil found that 6.2% of *Enterococcus* species were isolated[20]. The growing antibiotic resistance of *Enterococcus* species is complicating the treatment of serious infections caused by this pathogen, despite the extensive use of antimicrobial agents.

In this study, the highest resistance percentage in *Enterococcus* species causing UTIs were observed for ciprofloxacin (82.7%). This result was similar to the related previous study[19]. Another study showed that 53.5% ciprofloxacin resistance for *Enterococcus* species[21]. The high resistance to ciprofloxacin observed in this study may be linked to its widespread use as a first-line UTI treatment in Bangladesh. Reports indicate a growing resistance of Enterococci to Ciprofloxacin in both Bangladesh and India[19,21]. The higher resistance of *Enterococcus* species to fluoroquinolones (Ciprofloxacin) may be attributed to intrinsic mechanisms, such as reduced drug uptake or the acquisition of resistance through foreign genetic material[22].

In the current study, 42.3% of *Enterococcus* were resistant to ampicillin. Wu et al[23] reported that all Enterococcus faecium (100.0%) isolates were resistant to ampicillin, whereas only 6.2% of *Enterococcus faecalis* exhibited resistance. Another study found that

94.0% of *Enterococcus faecium* isolates were resistant to ampicillin, compared to only 4.3% of *Enterococcus faecalis* isolates[24]. However, as we did not perform genetic profiling, species-level identification, as described in the reference studies, could not be carried out.

In this study, 65.4% and 34.6% of *Enterococcus* species were resistant to doxycycline and nitrofurantoin respectively. Barros et al[20] reported that 50.0% of *Enterococcus* species were resistant to nitrofurantoin. Another study showed that 16.5% nitrofurantoin resistance for *Enterococcus* species[25]. The differences in antibiotic resistance patterns are influenced by various factors, including geographic regions, local infection control measures, antibiotic prescribing practices, and the specific characteristics of the bacterial strains being studied[26]. As our institute is a tertiary care hospital, resistant organisms are more prevalent here.

In our study, 5.8% *Enterococcus* species were resistant to vancomycin. This result was consistent with the study of Mazhar et al[25] showed that 11.30% of *Enterococcus* species. were resistant to vancomycin. As our institute is a tertiary care hospital, resistant organisms are more prevalent here. Since molecular identification of drug resistance genes was not performed, the underlying causes of vancomycin-resistant Enterococci could not be determined. Enterococci have developed resistance to glycopeptides like vancomycin through a two-step process. They alter the end terminals of their pentapeptide precursors to D-Ala-D-Lac or D-Ala-D-Ser, which reduces glycopeptide binding by about 1000-fold[27]. This change is due to gene clusters associated with vancomycin resistance. Nine variants of these clusters exist like *vanA, vanB, vanC, vanD, vanE, vanG, vanL, vanM,* and *vanN*. All but *vanC* (which is intrinsically resistant) result from acquired resistance. The vanA gene cluster is the most common and is often found in *Enterococcus* faecium[28].

In this study, *Enterococcus* spp. showed significantly very high susceptibility against teicoplanin (92.3%). All *Enterococcus species* were found to be sensitive to linezolid, which is consistent with the finding of previous study[21]. Resistance to several antimicrobial agents was prevalent among the Enterococci isolates recovered in the hospital. Knowledge of the antimicrobial resistance profile is essential to formulate treatment guidelines for UTIs caused by *Enterococcus* species. The unavailability of species and drug resistance gene identification were limitations of the study.

Conclusion

Our study reveals a high prevalence of resistance to ciprofloxacin and doxycycline among Enterococcal isolates in urine, which limits the therapeutic use of these antibiotics. Although vancomycin, teicoplanin, and linezolid are considered reserve drugs, the prevalence of vancomycin-resistant Enterococci (VRE) is steadily increasing. Both vancomycin and teicoplanin are glycopeptide antibiotics, and their resistance rates are nearly identical, teicoplanin can serve as an effective alternative to vancomycin. However, given that no resistance to linezolid has been identified, its use should be approached with caution to preserve its effectiveness.

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None

Conflict of Interest
The authors have no conflicts of interest to disclose.

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Authors' contributions
Noor-E-Jannat Tania conceived and designed the study, analyzed the data, interpreted the results, and wrote up the draft manuscript. Rubaiya Binte Kabir, Md. Asaduzzaman, Avizit Sarker contributed to the formal analysis, methodology, investigation, resources. Md. Faizur Rahman, Nusrat Noor Tanni, Maherun Nesa, Farjana Binte Habib, Azmeri Haque, Umme Saoda contributed to the investigation and resources. Nadira Akter, Rozina Aktar Zahan, Kakali Halder involved in the manuscript review and editing. Mahbuba Chowdhury involved in the manuscript review and editing, administration. Sazzad Bin Shahid contributed to the overall supervision, validation, funding acquisition, the manuscript review and editing. All authors read and approved the final manuscript.

Data Availability
Any inquiries regarding supporting data availability of this study should be directed to the corresponding author and are available from the corresponding author on request.

Ethics Approval and Consent to Participate
Ethical approval for the study was obtained from the Institutional Review Board. As this was a prospective study the written informed consent was obtained from all study participants. All methods were performed in accordance with the relevant guidelines and regulations.

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