

PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF URINARY ENTEROCOCCUS ISOLATES IN A TERTIARY CARE HOSPITAL

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ABSTRACT

Background: Multidrug-resistant enterococci are emerging as prevalent nosocomial uropathogens. Before prescribing antibiotics, recent antibiogram of enterococci is essential for patients with urinary tract infection (UTI). **Materials and Method:** This cross-sectional study was conducted to determine the prevalence and antimicrobial susceptibility pattern of enterococci from urine samples between January 2023 and October 2023 in a tertiary care hospital. Isolation and identification of *enterococci* were done by standard microbiological procedures, and antimicrobial susceptibility test was performed by the Kirby-Bauer disk diffusion method. **Results:** Out of 1551 urine samples, 351 (22.63%) were culture positive and enterococci was the second most common (32.76%) isolated uropathogen among *Escherichia coli* (44.16%), *Klebsiella* spp. (11.40%), *Staphylococcus aureus* (3.13%), *Enterobacter* spp. (2.85%), *Pseudomonas* spp. (2.56%), *Proteus* spp. (1.99%), *Acinetobacter* spp. (0.85%), and *Staphylococcus saprophyticus* (0.28%). Female predominance (62.6%) was observed in UTI patients infected with Enterococci. Linezolid (92.17%) followed by Vancomycin (86.96%), Nitrofurantoin (82.61%), Ampicillin (75.65%) and Penicillin (63.48%) were found as most susceptible drugs. The enterococci were highly resistant to Tetracycline (60.87%), Ciprofloxacin (53.04%) and Levofloxacin (53.04%). **Conclusion:** Performing regular susceptibility tests could help in establishing preventative measures and treatment protocols for enterococcal infection.

Keywords: Enterococci, Urinary Tract Infection, Antibiotic Susceptibility.

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INTRODUCTION

Enterococci are Gram-positive cocci that can be arranged in pairs or in short chains. These are the normal flora of oral cavity, vagina, and gastrointestinal tract¹. Enterococci have been identified as important pathogenic bacteria in humans that can cause a variety of diseases, such as UTI, surgical wound infections, meningitis, bacteremia, genital tract infections, and bacterial endocarditis². Isolation rate of enterococcus is highest from urine (80%), followed by pus, blood and other specimen. In most of the studies,

Enterococci are reported as the most frequent uropathogen^{1,3,4}. After *Escherichia coli* (*E. coli*) and *Enterobacter*, *Enterococcus* species are found to be the third most common cause of UTI in studies conducted in Bangladesh^{5,6}. It has been found that Enterococci are the second most frequent Gram-positive bacteria that can cause UTI^{7,8}. Normal gut commensal enterococci have become nosocomial and community acquired pathogens due to the over-use of broad-spectrum antibiotics.

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The disease is primarily endogenous, but it can spread through cross-infection in hospitalized patients. Compromised host resistances, disruption of the integrity of the gastrointestinal or genito-urinary tract by instrumentation are the contributing factors^{2,9}. Among the enterococcal virulence factors, extracellular surface proteins, adhesins, aggregation substances, and biofilm formation play roles in the pathogenesis of UTI. The capacity of Enterococci to acquire extra chromosomal elements including antibiotic resistance genes explains their increasing importance as nosocomial pathogens^{10, 11}. Prolonged hospital stays, prior antimicrobial therapy, contact with contaminated medical equipment, proximity to a known patient, contact with a medical person assigned to another known patient, and use of parenteral or oral vancomycin are risk factors for enterococci infections¹².

In recent years, antibiotic resistance is a worldwide problem. In Bangladesh, the rate of indiscriminate use of antibiotics and spread of hospital acquired infection are higher¹³. Now a days, serious enterococcal infections are difficult to treat due to emergence of multidrug resistant and vancomycin resistant Enterococci (VRE). Enterococci exhibit intrinsic resistance to penicillinase-resistant penicillin, cephalosporins, low levels of aminoglycosides and clindamycin. Enterococci also have developed acquired resistance to tetracycline, high level of aminoglycosides, penicillin, fluoroquinolones; vancomycin, resulting from either DNA mutation or acquisition of new DNA. Enterococcus posing resistance to antimicrobial agents is a serious challenge for clinicians as it leads to treatment failure and spread of resistant strains^{2,10,13}.

Any stage of renal disease can be associated with enterococcal UTI, so it is vital to identify their sensitivity patterns promptly in order to ensure appropriate treatment, prevent long-term complications and reduce the risk of

morbidity¹⁴. Therefore, this study was designed to identify the prevalence of Enterococcus species in UTI patients and their antibiotic susceptibility pattern in a tertiary care hospital in Dhaka.

MATERIALS AND METHOD

This cross-sectional study was conducted in the Department of Microbiology of Medical College for Women and Hospital between January 2023 and October 2023. A total of 1551 urine samples were collected from in-patient and out-patient department of the hospital. The demographic information of the urine sample (age, gender) was recorded. Patients with improper information on age, gender, urine culture report were excluded. Proper permission was taken from the department for the study. The confidentiality of each patient was assured and anonymity was maintained. Identification of enterococcus spp. and antimicrobial susceptibility test were done according to standard microbiological procedure and Clinical and Laboratory Standard Institution (CLSI) guideline- 2023¹⁵.

Semi-quantitative urine culture was done and enterococci colonies were identified by colony morphology on chromogenic agar media, which was incubated at 37° C for overnight. Further, suspected enterococci colonies were confirmed by catalase negative and positive bile esculin test. The antimicrobial susceptibility test of enterococci isolates was done by modified Kirby- Bauer disk diffusion method on Muller Hinton agar plate. The plates were incubated at 37° C for 16 -18 hours. Next day susceptibility profile of Enterococci was measured and recorded. The antibiotic discs used were penicillin (10 U), ampicillin (10 µg), vancomycin (30 µg), linezolid (30 µ), ciprofloxacin (5 µg), nitrofurantoin (300 µg) levofloxacin (5 µg) and tetracycline (30 µ). Statistical analysis of the data was performed by SPSS software version 27 and the results were expressed as number (percentage).

RESULTS

A total of 1551 urine samples were collected from patients and 351 (22.63%) were culture positive (Figure 1). Table 1 shows *E. coli* was the commonest (44.16%) bacteria followed by *Enterococcus* spp. (32.76%), *Klebsiella* spp. (11.40%), *S. aureus* (3.13%), *Enterobacter* spp. (2.85%), *Pseudomonas* spp. (2.56%), *Proteus* spp. (1.99%), *Acinetobacter* spp. (0.85%), and *S. saprophyticus* (0.28%). Among the UTI patients infected with *Enterococcus*, 72

(62.6%) were female and 43 (37.4%) were male. Most of the female (30, 41.66%) were from age group of 21-40 years and male (32, 74.41%) were from age group of below 20 years (Figure 2). Antimicrobial susceptibility pattern of *Enterococcus* spp. is summarized in Table 2. High level of sensitivity showed against linezolid (92.17%), vancomycin (86.96%), nitrofurantoin (82.61%), ampicillin (75.65%) and penicillin (63.48%). Most of the enterococcus was resistant to tetracycline (60.87%). ciprofloxacin and levofloxacin were found 53.04% resistant.

Table 1 : Distribution of culture positive isolates (n= 351)

Isolated bacteria	Number (%)
<i>E. coli</i>	155 (44.16%)
<i>Enterococcus</i> spp.	115 (32.76%)
<i>Klebsiella</i> spp.	40 (11.40%)
<i>S. aureus</i>	11 (3.13%)
<i>Enterobacter</i> spp.	10 (2.85%)
<i>Pseudomonas</i> spp.	9 (2.56%)
<i>Proteus</i> spp.	7 (1.99%)
<i>Acinetobacter</i> spp.	3 (0.85%)
<i>S. saprophyticus</i>	1 (0.28%)

n: Total number of culture positive samples; *E. Coli*: *Escherichia Coli*.

Table 2 : Antimicrobial susceptibility pattern of *Enterococcus* spp. (n=115)

Antimicrobial drug	Susceptible number (%)	Intermediate number (%)	Resistant number (%)
Penicillin	73 (63.48%)	0 (0%)	42 (36.52%)
Ampicillin	87 (75.65%)	0 (0%)	28 (24.35%)
Vancomycin	100 (86.96%)	2 (1.74%)	13 (11.30%)
Linezolid	106 (92.17%)	1 (0.87%)	8 (6.96%)
Nitrofurantoin	95 (82.61%)	1 (0.87%)	19 (16.52%)
Ciprofloxacin	50 (43.48%)	4 (3.48%)	61 (53.04%)
Levofloxacin	53 (46.09%)	1 (0.87%)	61 (53.04%)
Tetracycline	45 (39.13%)	0 (0%)	70 (60.87%)

n: Total number of samples with enterococcus SPP

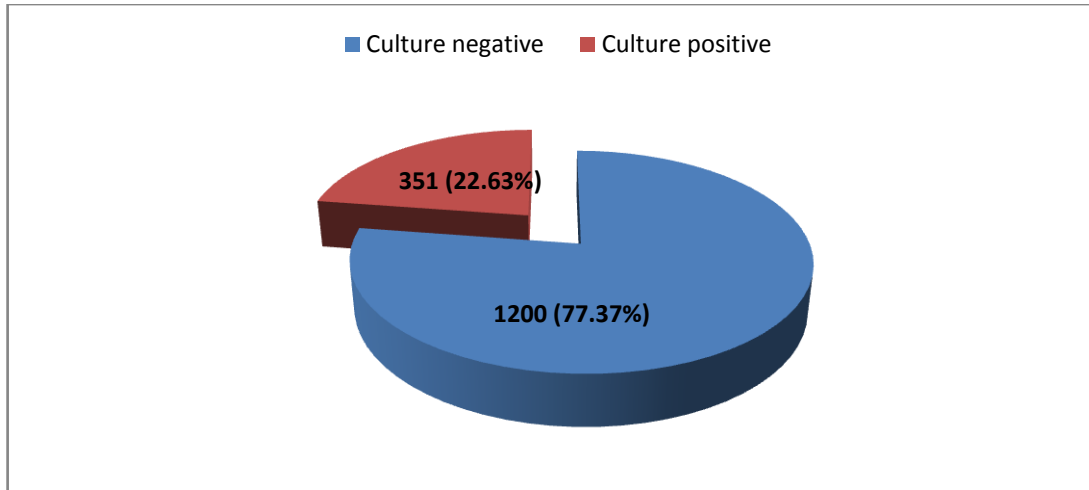


Figure 1: Bacterial growth among total urine sample.

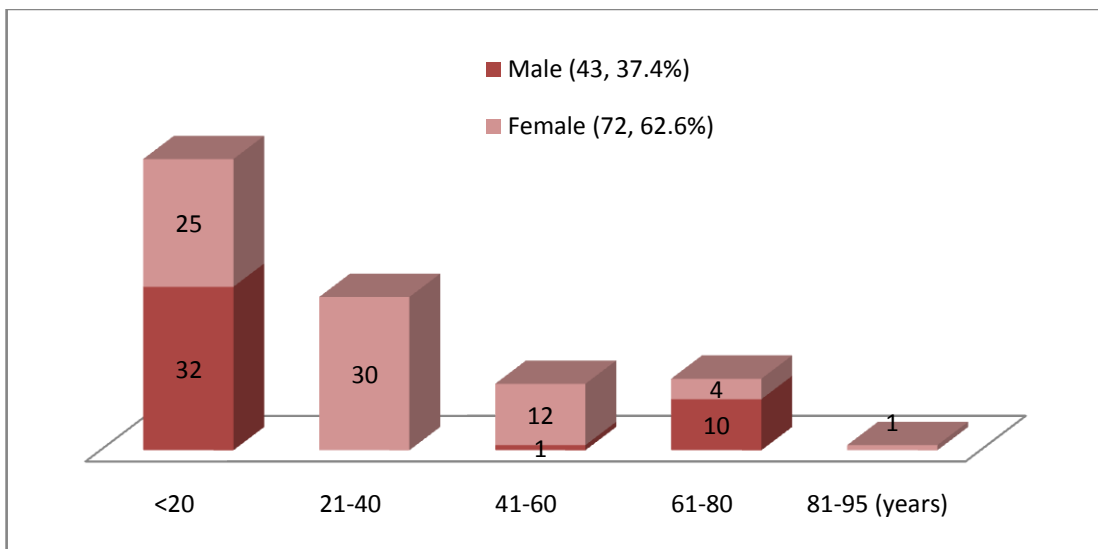


Figure 2 : Age group and gender distribution of UTI patients infected with enterococci (n=115)

DISCUSSION

The prevalence of nosocomial infections with enterococci and increasing rate of their resistance to antimicrobial agents are a major concern at many hospitals throughout the world including in Bangladesh. Identification of enterococci in urine sample with their sensitivity pattern is very important for early initiation of proper treatment. In the present study, 351 (22.63%) samples were culture positive among 1551 urine samples and enterococci was found as second commonest (32.76%) isolated uropathogen. In Bangladesh, the frequency of enterococci as uropathogen has increased significantly from 11.38% to 13.29% over 5 years (2003-2008)⁶. In 2017,

the prevalence of enterococci in urine was found to be 36.06%, which is consistent with current study¹⁶. In Germany, enterococci was the second most frequent bacteria isolated from UTI patients. Furthermore, recurrent UTI was significantly caused by enterococci compared to *E. coli*¹⁷. Higher rate of enterococcal UTI might be explained by its transmission from a healthcare worker's hands to a patient, urinary tract instrumentation, indwelling catheterization, genitourinary tract disease, prolonged stay in hospital or Intensive Care Unit and previous multiple antimicrobial therapy^{12, 18}.

In present study, female (62.6%) had higher enterococci infection rate and most of the female (41.66%) were from age group of 21-40 years, which is similar with the report of other studies^{5,19}. The reason behind this high prevalence of UTI in young females is due to female genito-urinary structures, reproductive function, poor hygienic conditions and negligence to treat urine infection^{5,19}. The sensitivity pattern of enterococci isolates showed that linezolid was the most sensitive drug (92.17%). Similar sensitivity (99.1%- 99.4%) of linezolid to enterococci was observed in other studies^{12, 20}. Moreover, 100% sensitivity to linezolid was reported by authors from Bangladesh and India^{1, 10}. Vancomycin resistance has been increasingly reported worldwide, however in the current study, vancomycin was the second most sensitive antibiotic (86.96%). This result is in agreement with study results where vancomycin sensitivities range from 70% to 98.3%^{12,14,20}. In our study, 11.30% enterococci isolates were resistant to vancomycin. However, confirmation of VRE was not possible due to non-availability of E-strips and Vitek AST cards. Nitrofurantoin was another antibiotic with good sensitivity (82.61%) after linezolid and vancomycin. Similar sensitivities of nitrofurantoin (87.60% and 88.57%) were reported in studies^{3,20}. on the contrary, lower rate of sensitivity (60%-62%) was also observed by researchers^{14,19}.

The current study showed high resistance to tetracycline (60.87%), ciprofloxacin (53.04%) and levofloxacin (53.04%), which is consistent with other reports in Bangladesh^{3,7,10,20}. Ciprofloxacin resistance rate was also found higher (60-92%) in India^{1,2}. Some of the studies found high ampicillin and penicillin resistant enterococci isolates¹⁻³. Lower rate of resistance to ampicillin and penicillin was reported by other studies^{7, 12}, which is in agreement with present study. Changing resistance pattern could be due to uncontrolled and widespread use of

antibiotics. Antibiogram may also vary in different places and even in same place from time to time or even institution to institution¹⁹.

LIMITATION

Lacking adequate clinical information of patients, unavailability of species and drug resistance gene identification were the limitations of the study.

CONCLUSION

Pattern of uropathogens vary in different settings. It is shown that enterococci are the second leading cause of UTI and resistant to multiple antibiotics. Linezolid, vancomycin and nitrofurantoin are the promising drugs to treat this infection. It is highly recommended to do urine culture and sensitivity routinely. Proper measures and regular monitoring can reduce spread of nosocomial enterococcal infection in hospitals.

CONFLICT OF INTEREST

There is no conflict of interest.

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