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ORIGINAL RESEARCH ARTICLE

# Antimicrobial Susceptibility Patterns of *Enterococcus* Species and Molecular Detection of *Enterococcus faecalis* Isolated from Patients with Urinary Tract Infection in a Tertiary Care Hospital in Bangladesh

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**Abstract** One of the most prevalent infectious diseases identified in both communities and hospitalized patients is urinary tract infection (UTI). *Enterococcus* is evolved into a clinically pertinent uropathogen due to its evolving resistance to multiple antimicrobial agents.This study, detects antimicrobial susceptibility patterns of *Enterococcus* species and molecular detection of *Enterococcus faecalis* from patients with urinary tract infections. In this cross-sectional observational study, 165 urine samples were obtained from clinically diagnosed patients with UTIs of different ages and gender. *Enterococcus* species were identified by standard microbiological procedure and PCR (by using species-specific primers for *Enterococcus faecalis*). A modified Kirby Bauer Disc diffusion method was used to identify the antimicrobial susceptibility pattern following Clinical and Laboratory Standards Institute (CLSI) guidelines. Out of 165 urine samples, 134 samples yielded positive cultures. *Enterococcus* species were isolated from 23 (17.1%) urine samples. Among all *Enterococcus*, 16 (69.6%) isolates were *E. faecalis*, detected by PCR assay. A higher (30.4%) proportion of *Enterococcus*-positive patients were from the age group 48–57 years and female patients (78.2%) had a higher prevalence. Enterococcal infection was found in 56.5% of non-catheterized patients and 43.5% of catheterized patients. Vancomycin and linezolid (78.3%) and meropenem (73.9%) sensitivity was prevalent among all *Enterococcus* species. They showed 100% resistance towards ceftriaxone, cefixime 95.7%, cefuroxime 91.3%, azithromycin 82.6%. This research indicated the occurrence of *Enterococcus* species and the advent of multidrug-resistant *E. faecalis* in patients with UTIs. Routine speciation and antimicrobial susceptibility testing of *Enterococcus* in various clinical samples is encouraged.

**Keywords** Urinary tract infection · Uropathogen · *Enterococcus* species · PCR · Antimicrobial susceptibility · *E. faecalis*

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## Introduction

Over the past few decades, *Enterococcus* has emerged as an essential multidrug-resistant microorganism among all human pathogens. They are normal inhabitants of the gastrointestinal tract intestinal tract, oral cavity, and genitourinary tract of both humans and animals. *Enterococcus* was previously classified as group D streptococci because they have the group D cell wall antigen. Based on research using DNA hybridization and 16S rRNA sequencing, they were separated from the genus *Streptococcus* and reclassified as a distinct genus, "*Enterococcus*", in the 1980s [1]. They are facultative anaerobes, Gram-positive, oval cocci arranged in pairs or short chains. They can endure extreme conditions, such as high salt concentrations and a wide range of temperatures from 10 °C to 45 °C. Among different *Enterococcus* species, 90–95% of clinical enterococcal isolates were caused by *Enterococcus faecalis*. Distinctly more often than *E. faecalis*, *E. faecium* exhibits resistance to different antibiotics[2]. Before the 1990s, *Enterococcus* was first identified as a significant contributor to bacterial endocarditis [3]. Now it is considered an ascendant nosocomial pathogen, the second most frequent organisms found in catheter-associated bloodstream and urinary tract infections and from skin and soft-tissue infections [4]. Other infections caused by *Enterococcus* include meningitis, neonatal sepsis and intra-abdominal and pelvic infections [2]. The most prevalent type of enterococcal clinical disease affects the urinary tract and the *Enterococcus* species is regarded as a significant uropathogen [5].

Enterococcal infections occur through adhesion, colonization of the urinary tract, evasion of the host immune response and dissemination of the bacteria in the body. *Enterococcus* possess numerous virulence factors including enterococcal surface protein (Esp), gelatinase (GelE), collagen-binding protein (Ace), cytolysin (CylA) etc. These are crucial to the virulence potential of certain enterococcal species and contributes to bacterial adherence to host cells and in biofilm formation [6]. Additionally, the rapid acquisition of antibiotic resistance makes *Enterococcus* challenging to eradicate.

Urinary Tract Infection (UTI) is one of the most common infectious diseases and a substantial cause of morbidity worldwide. Elderly adults with bacteremia often have urinary origins in about 46.7% of cases [7]. In acute urinary tract infections, *E. faecalis* is frequently identified [8]. It also commonly contributes to catheter-associated and chronic urinary tract infections [9]. Enterococcal infections are more common in elderly patients [10]. In immunocompromised patients, *enterococcal* infection is associated with a higher mortality rate [11].

In public healthcare facilities worldwide, the prevalence of antibiotic-resistant *Enterococcus*, particularly Vancomycin-Resistant *Enterococcus* (VRE), is a persistent clinical concern [12]. Since this strain exhibits resistance to widely used antibiotics and transfers resistant genes to other bacteria, it is considered a superbug [13]. Vancomycin-Resistant *Enterococcus* was categorized as a high-priority pathogen and "serious threat" by the World Health Organization (WHO) and the Centers for Disease Control and Preventative (CDC), emphasizing the need for enhanced monitoring and prevention activities [14, 15]. At Bangabandhu Sheikh Mujib Medical University (BSMMU), enterococci identified from urine samples significantly increased over the previous five years (2003–2008). In 2003 and 2008, the prevalence of *Enterococcus* isolates was 11.38% and 13.29%, respectively [16]. Two different studies were conducted at BSMMU and Dhaka Medical College (DMC), where the prevalence of enterococcal urinary tract infection was noted at 8.44% and 14.47%, respectively [17, 18]. The phenotypic characterization is a critical component of the basic approach to *Enterococcus* identification. However, due to the phenotypic and biochemical similarities, species identification of *Enterococcus* is difficult and requires several days to complete [19]. The ddl gene, which codes for the D-alanine-D-alanine ligase enzyme (D-Ala: D-Ala), has been developed as one of many molecular techniques for identifying *Enterococcus* to species level [20]. The molecular epidemiology of clinical *enterococcal* isolates is easier and more precisely analyzed using the Polymerase Chain Reaction (PCR) technique. Different investigations have confirmed that species-specific PCR primers for primers ddl *E. faecalis* can identify *enterococcal* species.

Considering the diverse impact of *enterococcal* infection, this study is, therefore, intended to provide admissible data on antimicrobial susceptibility patterns of *Enterococcus* species and molecular detection of *Enterococcus faecalis* in urinary tract infection which would be beneficial to guide empirical treatment.

## Material and Methods

This laboratory-based cross-sectional study was conducted in the Department of Microbiology and Virology in collaboration with the Department of Medicine and Department of Obstetrics and Gynaecology of Sylhet M.A.G. Osmani Medical College, Sylhet, Bangladesh, from 1st July 2020 to 30th June 2021. Urine samples collected from 165 patients diagnosed with urinary tract infections.

### Data Collection

Patients clinically diagnosed with UTI were included in the study, regardless of their age and sex. Samples were collected from the outpatient and inpatient Department of Medicine and Department of Obstetrics and Gynaecology at

Sylhet M.A.G. Osmani Medical College, Bangladesh,using a non-probability consecutive sampling technique. A detailed history was taken after patients who met the inclusion criteria who provided written informed consent. The study excluded patients under 18 years and those on antibiotic for the previous seven days.

### Specimen Type and Collection Method

Patients were instructed to use a sterile wide mouth container or test tube and take all aseptic precautions while collecting 10–20 ml of freshly void clean, midstream urine. Prior to collection, instructions on how to collect the proper sample in a sterile container aseptically were provided.

In catheterized patient, after cleaning with an alcohol pad and clamp placement on the catheter with all aseptic precautions, about 10–20 ml of urine was aspirated with a syringe and needle directly from the part of the tubing proximal to the clamp. After correct labeling,the collected samples were transported as soon as possible to the microbiology laboratory.

### Microscopic Examination of Urine

All urine samples were examined microscopically to detect pyuria. In addition, the presence of pus cells of more than five cells/HPF in centrifuged urine was used to identify bacterial infection.

### Culture of Urine

The specimens were inoculated on the labeled Blood agar and Chromogenic agar media plates by using calibrated wire loop holding 0.004 ml of well mixed uncentrifuged urine.

### Identifications by Colony Characteristic

Bacterial colony growth was presumptively identified on Blood agar and Chromogenic agar with the following characteristics. In Blood Agar, colonies were small non-haemolytic; some are β –haemolytic. In Chromogenic agar, a blue colored colony was observed [21].

### Isolation and Identification of the Organism

Enterococcal isolates were confirmed by growth characteristics in the culture media, microscopic examination of stained smear and some biochemical tests.

*Microscopic Examination of Bacterial Morphology*

Oval Gram-positive cocci in pairs or short chains noted and selected for biochemical test.

*Biochemical Test*

- Catalase test–*Enterococcus* were catalase negative.
- Bile Esculin Test- Diffuse blackening of the slant within 24–48 h indicate esculin hydrolysis in the presence of 40% bile salts.
- Salt Tolerance Test (6.5% NaCl Broth)- Medium was observed for growth by the turbidity seen after dispersing any sediment. *Enterococcus* species was salt-tolerant.

### Antimicrobial Susceptibility Test

Samples that showed significant colony count were considered in this study. In addition, the sensitivity pattern of the isolated *Enterococcus* species was tested for Antimicrobial Susceptibility by the modified Kirby–Bauer Disk Diffusion method as described by the Clinical and Laboratory Standards Institute [22]. All the isolates were tested for antimicrobial susceptibility for the following antibiotics: Amikacin (30 µg), Gentamycin (10 µg), Nitrofurantoin (300 µg), Meropenem(10 µg), Azithromycin (15 µg), Cotrimoxazole (25 µg), Ciprofloxacin (1 µg), Ceftriaxone(30 µg), Cefuroxime (30 µg), Cefixime (5 µg), Vancomycin (30 µg), Linezolid (10 µg).

### Molecular Detection of *Enterococcus faecalis* Specific ddl (D-alanine-D-alanine ligase) Gene

For the PCR technique, at first, DNA was extracted using Monarch Genomic DNA Purification Kit (New England Biolabs, USA) according to the manufacturer's instructions. Next, extracted DNA was used for thermal cycling to amplify the target gene using a PCR mixture composed of master mix, forward primer, reverse primer, and template DNA.

### Thermal Cycling to Amplify *Enterococcus faecalis* Specific ddl (D-alanine-D-alanine ligase) Gene and Visualization by Gel Electrophoresis

The ddl gene was amplified using the following primers obtained from Macrogen Incorporation, Korea (Table 1).

*Amplification through thermal cycler*

A DNA thermal cycler was used to carry out the PCR study. Each PCR run included a 10 min preheating step at 94 °C for 10 min followed by 36 cycles of denaturation at 94 °C

**Table 1** PCR Primer sequence, amplicon size, and PCR condition [23]

| Gene | Primer sequence | Size of amplified product (base pair) | Annealing temperature | Cycle |
|---|---|---|---|---|
| ddl | 5'-ATCAAG TACAGTTAG TCTTTATTA G-3' | 941 bp | 58 °C | 36 |
| | 5'- ACGATT CAAAGC TAACTGAAT CAGT-3' | | | |

for 1 min, annealing at 58 °C for 45 s, extension at 72 °C for 2 min with final extension at 72 °C for 10 min [23].

*Gel electrophoresis and Visualization*

Gel electrophoresis in 1.5% agarose gel electrophoresis was used to evaluate the PCR results. The Digigel gel documentation system was then used for viewing and photographed the target gene (Figs. 1, 2).

*Ethical Considerations*

The authors have entirely observed all ethical aspects of this research. Approval was obtained from the Ethical Review Committee of Sylhet MAG Osmani Medical College. All experiments were performed following relevant guidelines and regulations. Informed consent was obtained from all participants or their legal guardians before collecting urine samples. The patient's demographic characteristics were recorded in a questionnaire and their information was kept confidential.

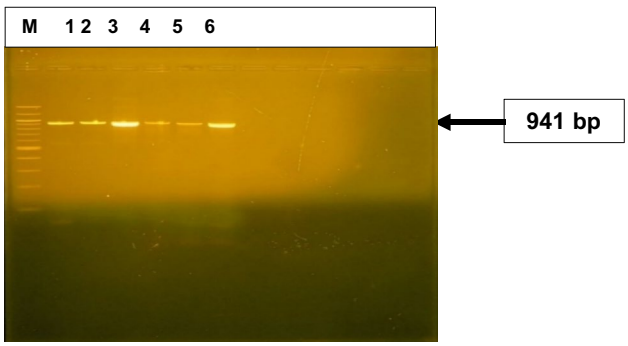


**Fig. 1** Agarose Gel Electrophoresis of PCR products for detection of ddl E. *faecalis* gene in enterococcal isolates.; Lanes: (M) is the marker, 100-bp DNA ladder (New England Biolabs); lanes 1–6 show bands of amplification products in specific 941 bp for the ddl gene

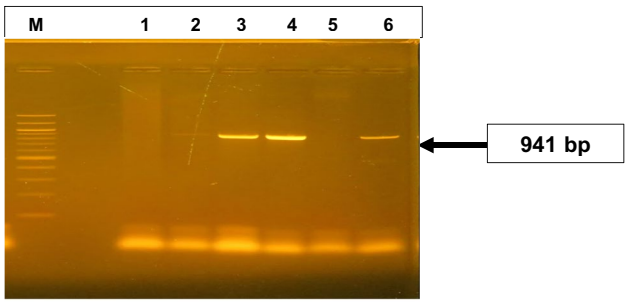


**Fig. 2** Agarose Gel Electrophoresis of PCR products for detecting ddl *E. faecalis* gene in enterococcal isolates.; Lanes: (M) is the marker, 100-bp DNA ladder (New England Biolabs); lanes 3, 4,6 show bands of amplification products in specific 941 bp of ddl gene

*Statistical Analysis*

Demographic and clinical data were extracted from study questionnaires, and quantitative data were analyzed and displayed in an Excel spreadsheet. The association between demographic factors was assessed using Chi-square test with a $p$ value $< 0.005$ considered significant. All data were processed and analyzed with the help of SPSS (Statistical Package for Social Sciences) version 26.

## Results

### Demographic Characteristics

From Table 2 illustrating the demographic data of the patients selected for this study, it is found that the patients' ages (n = 165) ranged from 18 to 85 years, with a mean age of 51.4 years (SD ± 16.6 years). Interestingly, most of the patients (23.1%) were between the ages of 48 and 57. In addition, more than two-third of the patients were female (72.8 percent), while the remainder were male (27.2 percent). Regarding the place of residence, the majority of patients (74.5%) were from rural areas, while the remaining patients (25.5%) were from urban areas. It emerged that 74% of the patients belonged to the lowest socioeconomic class, while 26% were from the middle class. Notably, no patients from the upper socioeconomic class were identified in this study. In relation to UTI patients specifically, nearly half (47.3%) had undergone catheterization, and the vast majority (81.3%) of tested urine samples contained evidence of growth. Finally, 17.1% of UTI patients who tested positive (n = 134) for different bacterial pathogens belonged to the *Enterococcus* species, while the overwhelming majority (82.9%) of them were infected with other bacterial organism (Fig. 3).

**Table 2** Frequency distribution of different demographic variables

| Demographic variables | Category | Frequency | Percentage (%) |
|---|---|---|---|
| Age group(years) | 18 – 27 | 20 | 12.1 |
| | 28 – 37 | 20 | 12.1 |
| | 38 – 47 | 19 | 11.5 |
| | 48 – 57 | 38 | 23.1 |
| | 58 – 67 | 36 | 21.8 |
| | ≥ 68 | 32 | 19.4 |
| Sex | Male | 45 | 27.2 |
| | Female | 120 | 72.8 |
| Area of residence | Rural | 123 | 74.5 |
| | Urban | 42 | 25.5 |
| Socio-economic condition | Lower | 122 | 74 |
| | Middle | 43 | 26 |
| | Higher | 0 | 0 |
| Catheterization | Catheterized | 78 | 47.3 |
| | Non-catheterized | 87 | 52.7 |
| Urine samples with growth | Growth | 134 | 81.3 |
| | Non-growth | 31 | 18.7 |
| Patients with different bacterial pathogens | *Enterococcus* | 23 | 17.1 |
| | Others | 111 | 82.9 |

**Fig. 3** *Enterococcus* species isolated in chromogenic agar media

### Distribution of *Enterococcus* species Positive Patients Over Different Age Groups

Table 4 shows the distribution of the patients who tested positive for *Enterococcus* species over different age groups. According to the table, more than 70% of patients who tested positive for *Enterococcus* were aged 48 or older. Besides, this pathogen appears to be more prevalent among elderly and late middle-aged patients, as opposed to young adults and those in their early middle ages.

### Distribution of *Enterococcus* species and Other Organism Positive Patients Over Gender

The distribution of urinary tract infection (UTI) patients infected with *Enterococcus* species and other organisms over gender and their association have been presented in Table 5. The data reveals that the majority (more than 80%) of both male and female UTI patients were affected by other bacterial organisms as opposed to *Enterococcus* species. Additionally, it is notable that regardless of the organisms causing the UTI, over two-thirds of the patients who tested positive for UTI were female, with the remaining third being male. However, Pearson's Chi-square test's $p$ value ($p = 0.615$) indicates that the association between the gender of the patients tested positive and the type of bacterial organism that affected them is not statistically significant.

### Distribution of *Enterococcus* species and Other Organism Positive Patients Over Their Socio-Economic Condition

Table 6 depicts distribution of patients who were tested positive for *Enterococcus* species and other organisms based on their socioeconomic status and how they are interconnected. According to the data presented in the table, it is clear that fewer than 25% of UTI patients in the lower and middle socio-economic categories tested positive for *Enterococcus* species, while more than 75 percent were infected with other organism. In addition, for both types of organism causing urinary tract infection, over two-third of the patients belonged to the lower socio-economic category, while the remainder were from the middle socio-economic class. Nonetheless, test result ($p = 0.6$) reveals no significant correlation between the type of infectious organism and the socioeconomic status of the patients.

### Distribution of *Enterococcus* species and Other Organism Positive Patients According to Catheterization

Table 7 illustrates how patients tested positive for *Enterococcus* species and other organisms and catheterization are distributed and associated with one another. From the table, it is evident that regardless of whether the UTI patients were catheterized or non-catheterized, the vast majority (over 80%) of the patients had contracted infections caused by other organisms, while fewer than 20% were infected with *Enterococcus* species. Remarkably, UTI patients without catheters were found more susceptible to *Enterococcus* species and other organisms

than those with catheters. Nevertheless, the test result ($p = 0.821$) shows no significant relationship between the type of infectious organism and the catheterization status of the UTI patients.

### Antimicrobial Susceptibility Patterns of Identified *Enterococcus* species

The antimicrobial susceptibility patterns of 23 distinct *Enterococcus* species are shown in Table 8. It displays the percentage of isolates that were sensitive, intermediate, or resistant to the antimicrobial agents mentioned. Of the 23 species tested, 43.5% were susceptible to amikacin, while a staggering 56.5% were resistant. With 52.2% of the species being sensitive and only 47.8% being resistant to gentamycin, it was found more efficacious than amikacin. Vancomycin and linezolid had the highest efficacy among all the enumerated antimicrobial agents, with 78.3% of the species showing sensitivity, while 21.7% were still resistant. Moreover, meropenem was effective against 73.9% of the tested species, and 26.1% exhibited resistance. Finally, the efficacy of ciprofloxacin was similar to that of azithromycin, with 17.4% of species being sensitive and 69.6% being resistant. Ceftriaxone and cefixime demonstrated no efficacy against any of the tested species.

## Discussion

Urinary tract infection as a community-acquired bacterial infection is increasing in prevalence. Among numerous bacterial pathogens, *Enterococcus* continue to emerge as significant uropathogens that are difficult to treat due to the increasing prevalence of antibiotic resistance. It is one of the most important pathogens affecting individuals of all ages. E. *faecalis* is the most common species of the *Enterococcus* genus that is associated with human enterococcal infection. Due to their significance in causing urinary tract infections and the increasing antibiotic resistance, it was essential to investigate the antimicrobial susceptibility patterns of *Enterococcus* species isolated from patients with urinary tract infections. Hence, a cross-sectional observational study was conducted in Sylhet MAG Osmani Medical College, Sylhet. Total 165 individuals,clinically diagnosed with urinary tract infections were included in this study. All patients' urine samples were collected and tested using standard microbiological procedures. From the 134 growth-positive samples, 23 cases (17.1%) of urinary tract infections were determined to be caused by *Enterococcus* species. This result is consistent with a study conducted in Bangladesh by Ahsan et al. (2020) where the proportion was 14.47% [18]. Another study by Raj et al. (2019) also reported a prevalence of *Enterococcus* spp. of 13.77% among patients with UTI [24]. The higher rate of *enterococcal* infection in this study may be attributable to the fact that the patients were from a tertiary care hospital, and many had catheters.

Moreover, 16 (69.6%) of the 23 *Enterococcus* species detected phenotypically in this study tested positive for the *Enterococcus faecalis* specific ddl (D-Alanine-D-Alanine ligase) gene when confirmed by PCR. Interestingly, these findings agree with a study performed in Bangladesh, where PCR detected 71.43% of *E. faecalis*-specific ddl genes [18]. Furthermore, two other studies in Iran and Bangladesh discovered *E. faecalis*-specific ddl genes at 62.5% and 71.42%, respectively [23, 25]. Moreover, another study found that only 56.12% of the ddl gene was specific to *E. faecalis* [26].

As indicated in Table 3, the highest percentage (30.4%) of patients who tested positive for *Enterococcus* were in the age group of 58–67 years. This result is consistent with the findings of an investigation conducted in Bangladesh by Islam and Shamsuzzaman [27], who noticed 37.50% of *Enterococcus*-positive patients within the age range of 41–60 years. Similarly, a study in India found a prevalence of 29.3% of *enterococcal* infections in patients aged 61 and older [28]. Another study found a higher incidence of *enterococcal* infections among those aged 51–75 [29]. This may be because immunity declines with age, leading to increased bacterial colonization. In addition, elderly patients may have a history of obtaining care from multiple facilities, which could serve as a source of the infection's spread. Moreover, postmenopausal women are more prone to UTIs. Several factors, including bladder or uterine prolapse resulting in insufficient bladder emptying, lower estrogen levels, and altered vaginal flora, enable diverse uropathogens, such as *Enterococcus*, to colonize the periurethral region [30].

As shown in Table 4, females (78.2%) had a higher prevalence of *Enterococcal* infection than males (21.8%). This finding is consistent with another study by Srivastava et al. (2013), who reported a prevalence of *Enterococcal* infection among females 72% than males 28% [31]. This study's findings may be explained by the fact that female anatomy, with its short urethra and close proximity to the anus, renders females more susceptible to UTIs than males.

In addition, 78.2% of the UTI patients chosen for this study were from rural areas, with the remaining patients

**Table 3** Molecular detection of *Enterococcus faecalis* specific ddl (D-Alanine- D-Alanine ligase) gene among *Enterococcus* species by PCR (N = 23)

| Species | No. of isolates | Percentage (%) |
|---|---|---|
| *E.faecalis* | 16 | 69.6 |
| Other species | 7 | 30.4 |

**Table 4** Distribution of *Enterococcus* species positive patients over different age groups

| Age group (years) | Frequency | Percentage (%) |
|---|---|---|
| 18–27 | 2 | 8.7 |
| 28–37 | 3 | 13.0 |
| 38–47 | 1 | 4.3 |
| 48–57 | 4 | 17.4 |
| 58–67 | 7 | 30.4 |
| ≥ 68 | 6 | 26.2 |

coming from urban settings. This result is in agreement with a prior study by Majumder et al. (2018), which also observed a higher percentage of culture-positive cases in rural areas (67%) than in urban areas (33%) [32]. In addition, according to this study's finding in Table 5, over two-thirds (69.6%) of the *Enterococcus*-positive patients belonged to a lower socioeconomic background, while fewer than a third (30.45%) were from the lower middle class. No patient was from a more affluent socioeconomic stratum. UTI is related with malnutrition, poor sanitation, and low socioeconomic position, and these conditions are prevalent in rural areas. No participants from higher socioeconomic backgrounds were found because this study was conducted in a hospital offering tertiary care.

Besides, the results of this research, as presented in Table 6, revealed that the prevalence of enterococcal urinary tract infection was higher among the non-catheterized patients (56.5%) than the catheterized patients (43.5%). This result is similar to Raj et al.'s [24] study, which reported a 67.7% prevalence of enterococcal infection among non-catheterized UTI patients and a 32.3% prevalence among catheterized UTI patients. Furthermore, in another investigation conducted by Shrestha et al. [33], 54.11% of patients without catheters had an *Enterococcus faecalis* mediated UTI, compared to 34.28% of catheterized patients. Transmission of *Enterococcus* from a hands of a doctor or other health care provider to a patient may involve direct inoculation to urinary catheters should be attributed for the greater prevalence of enterococcal infection more likely explanation, however, is that healthcare-associated strains invade patients' GI tracts who have low colonization resistance and multiply. Thus, patient's endogenous flora acquires new strains, raising the chance of infection [34]. UTIs are significantly influenced by bacterial biofilms. They develop into catheters causing their blockage and causes both acute and persistent infections [35]. Samples were gathered for this study from newly catheterized patients. A number of factors, including infection prevention measures like aseptic precautions, catheter care, and catheterization time, could explain the variation in the frequency of catheter-associated Urinary Tract infections.

**Table 5** Gender distribution of *Enterococcus* species and other organism positive UTI patients

| Sex | *Enterococcus* | Other Organism | Total | *P* value*($\chi^2$ *test*) |
|---|---|---|---|---|
| Male | 5 (13.8%) | 31 (86.2%) | 36 | 0.615 |
| Female | 18 (18.4%) | 80 (81.6%) | 98 | |
| Total | 23 | 111 | 134 | |

**Table 6** Distribution of *Enterococcus* species and other organism positive UTI patients according to socio-economic status

| Socio-economic condition | *Enterococcus* | Other organism | Total | *P* value*($\chi^2$ *test*) |
|---|---|---|---|---|
| Lower | 16 (16%) | 84 (84%) | 100 | 0.600 |
| Middle | 7 (20.6%) | 27 (79.4%) | 34 | |
| Total | 23 | 111 | 134 | |

**Table 7** Distribution of *Enterococcus* species and other organism positive patients according to catheterization

| | *Enterococcus* | Other organism | Total | *P* value*($\chi^2$ *test*) |
|---|---|---|---|---|
| Non-catheterized | 13 (18.1%) | 59 (81.94%) | 72 | 0.821 |
| Catheterized | 10 (16.1%) | 52 (83.9%) | 62 | |
| Total | 23 | 111 | 134 | |

**Table 8** Antimicrobial susceptibility patterns of identified *Enterococcus* species

| Antimicrobial agents | Sensitive | Intermediate | Resistant |
|---|---|---|---|
| Amikacin | 10 (43.5%) | 0 (0%) | 13 (56.5%) |
| Gentamycin | 12 (52.2%) | 0 (0%) | 11 (47.8%) |
| Nitrofurantoin | 14 (60.9%) | 2 (8.7%) | 7 (30.4%) |
| Meropenem | 17 (73.9%) | 0 (0%) | 6 (26.1%) |
| Azithromycin | 4 (17.4%) | 0 (0%) | 19 (82.6%) |
| Cotrimoxazole | 7 (30.4%) | 0 (0%) | 16 (69.6%) |
| Ciprofloxacin | 4 (17.4%) | 3 (13%) | 16 (69.6%) |
| Ceftriaxone | 0(0%) | 0 (0%) | 23(100%) |
| Cefuroxime | 0(0%) | 2 (8.7%) | 21 (91.3%) |
| Cefixime | 0 (0%) | 0 (0%) | 22 (95.7%) |
| Vancomycin | 18 (78.3%) | 0 (0%) | 5 (21.7%) |
| Linezolid | 18 (78.3%) | 0 (0%) | 5 (21.7%) |

Table 7 illustrates the susceptibility patterns of *Enterococcus* spp. that were isolated urinary tract infections in this study. The table suggests that *Enterococcus* was resistant to most antimicrobial agents. Notably, a high rate of antimicrobial resistance of isolated *Enterococcus* was observed towards ceftriaxone, cefixime, cefuroxime, and

azithromycin. This high level of resistance is probably due to the widespread and injudicious use of these drugs, which are readily available over the counter in Bangladesh. Moreover, due to a lack of access to health care services, unqualified practitioners and untrained pharmacists may be administering antimicrobials extensively, contributing to this alarming situation.

Moreover, resistance to ceftriaxone was discovered to be extremely high (100%) in isolated *Enterococcus* species in this particular study. This outcome corresponds to those of earlier studies by Suchi et al. [23], which reported 92.86% resistance to ceftriaxone in *Enterococcus*. In addition, *Enterococcus* species exhibited a high level of resistance to cefixime, with 22 (95.7%) out of 23 demonstrating high resistance and 1 (4.3%) showing intermediate sensitivity. A previous study by Ali et al. [36] also reported that 88% of *Enterococcus faecalis* were immune to cefixime. Extensive usage of cefixime in hospitals in recent years may have contributed to the drug's ineffectiveness. It is worth noting that local drug prescription practices can also influence the resistance rate. Besides, this study revealed that cefuroxime was less effective against *Enterococcus*. Total 21 (91,3%) of 23 *Enterococcus* species exhibited resistance to cefuroxime, while 2 (8.7%) showed intermediate sensitivity. This result is in agreement with the study conducted in Bangladesh by Akhter et al. (2014), who reported that 80.95% of *Enterococcus* species were resistant to cefuroxime [17].

In our study, 82.6% of the *Enterococcus* species tested positive for azithromycin resistance. The findings agree with Suchi et al. (2017), who observed that 85.71% of *Enterococcus* species were resistant to azithromycin [23]. Besides, thirteen (81.3%) of the sixteen *Enterococcus faecalis* strains were resistant to azithromycin. However, in their study, Abdelkareem et al. [37] found that 57.9% of *E. faecalis* were resistant to azithromycin, which was lower than ours. The increased resistance could be attributed to widespread, indiscriminate use and ubiquitous availability of the drug.

Again, in this study, cotrimoxazole resistance was detected in 69.6% of *Enterococcus* species. This finding resembles Haque et al.'s [38] study, in which 73.91 percent of *Enterococcus* species resisted cotrimoxazole. Also, this study revealed that *Enterococcus* species showed 69.6% resistance and 13.0% intermediate sensitivity to ciprofloxacin. This result is lower than that of Haque et al. [38], who found 82.6% of *Enterococcus* species being resistant to ciprofloxacin.

Moreover, in this study, 13 (56.5%) *Enterococcus* isolates were observed to be resistant to amikacin, which is similar to the finding of Suchi et al.'s [23] study, showing a resistance rate of 66.67%. Out of total 23 urinary isolates of *Enterococcus* in the present study 11 (47.8%) showed resistance to gentamycin. This finding differs from that of Ahsan et al. [18] where they observed 84.78 isolates of *Enterococcus* showed resistance to gentamycin.

Notably, *Enterococcus* species exhibited low nitrofurantoin resistance in this study. Seven (30.4%) of 23 *Enterococcus* were resistant to nitrofurantoin, while two (8.7%) displayed intermediate sensitivity. This is marginally higher than the 21.74% resistance to nitrofurantoin found in a previous study conducted by Haque et al. [38] in Bangladesh. Due to its localized action on the urinary tract, nitrofurantoin's resistance rate remained low despite its use for many years. Therefore, nitrofurantoin can be considered a first-line, cost-effective, and efficacious oral therapy for urinary tract infections.

Our study also aimed to examine *Enterococcus* resistance to meropenem, an antibiotic commonly used to treat urinary tract infections. Six (26.1%) out of 23 isolates of *Enterococcus* showed resistance to meropenem. To the best of our knowledge, no prior study has examined the susceptibility pattern of meropenem in *Enterococcus*, making our study a significant contribution to the field. However, several studies have used imipenem, a drug that belongs to the same group as meropenem. Interestingly, our result aligns with a previous study conducted by Suchi et al. [23], in which 28.57% of *Enterococcus* species were imipenem resistant. This suggests that the resistance patterns of these two drugs may be similar, although further studies are needed to confirm this.

Been increasingly observed worldwide, with this study revealing that five (21.7%) of the 23 *Enterococcus* species examined were resistant to vancomycin as determined by the disc diffusion method. This result is similar to the study of Nasaj et al. [25], in which 24% of *Enterococcus* showed resistance to vancomycin. Furthermore, Parameswarappa et al. (2013) also reported 34%of *enterococcal* isolates as vancomycin-resistant, whereas Shahi et al. (2020) found that 27.7% of *Enterococcus* species showed resistance to vancomycin [28, 39].

Finally, linezolid has so far demonstrated good anti-*enterococcal* activity. However, the emergence of linezolid resistance in *Enterococcus* is an alarming problem for treating VRE infections. Using the disk diffusion method, five (21.7%) *Enterococcus* urinary isolates These findings are comparable to those of Abdelkareem et al. [37], who observed a high linezolid resistance rate of 14% and an intermediate resistance rate of 17.5%.

## Conclusions

Globally, particularly in underdeveloped nations, antimicrobial resistance to bacteria that cause urinary tract infections is a serious concern. This study showed that urinary tract

infections caused by *Enterococcus* species are widespread in our hospital, especially among older female patients. The study also revealed a significant prevalence of *E. faecalis* with high resistance rates to nearly every tested antibiotic, posing a considerable treatment challenge in hospital settings.

The emergence of resistance to vancomycin or linezolid highlights the necessity of considering alternative therapeutic options in such circumstances. Utilizing molecular techniques to screen for the drug-resistant gene may aid in selecting an appropriate antibiotic therapy strategy. Research identifying factors facilitating the transmission of *enterococcal* infection within the hospital environment and effective clinical management is urgently needed to address this alarming health problem. In addition, public health initiatives should raise awareness of the risks of improper antimicrobial usage, which will help keep dangerous illnesses at bay.

**Author Contributions** All authors contributed to the study conception and design. Material preparation, data collection, methodology and analysis were performed by [Nahla Islam Neeva]. The manuscript was written by [Nahla Islam Neeva] [Nahida Zafrin] and review and editing by [Nahida Zafrin]. Supervised by [Nahida Zafrin] [Azima Aktar Jhuma]. All authors read and approved the final manuscript.

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**Data Availability** The datasets generated during and/or analysed during the current study are not publicly available due to these are classified data concerning patients confidential infoemations but are available from the corresponding author on reasonable request.

**Declarations**

**Conflict of interest** The authors have no relevant financial or non-financial interests to disclose.

**Ethical Approval** The above research project has been granted Ethical permission based research guide's forwarding and reviewers subsequent favourable observation and comments. Approval was granted by the Ethical Committee and Principal of Sylhet MAG Osmani Medical College (Date 29.05.2021/No/SOMC/2021/33).

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**Consent to Publish** Manuscript does not contain any individual person's data in any form (including any individual details, images or videos),

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