Prevalence and Antimicrobial Susceptibility pattern of pathogens Isolated from different age groups with Urinary Tract Infection in Bangladesh

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Abstract

Background: Urinary tract infections (UTIs) are a common nosocomial infection in many hospitals and routine clinical practices. Contemporary knowledge of pathogens and their pattern of antibiotic susceptibility is essential for updating antibiotic policy.

Methods: This study was designed to determine the prevalence of microorganisms that cause urinary tract infections and to determine their antibiotic susceptibility pattern. A total of 1288 clinical samples were aseptically collected from UTI patients. After collection, pathogenic bacteria was isolated and identified with biochemical tests. Their antimicrobial susceptibility pattern was determined by Kirby–Bauer disc diffusion method using 14 commercial antibiotics.

Results: Out of 1288 samples, 398 samples showed positive growth with various pathogens. Further analysis of samples presented a high prevalence of UTI in female than male. Identified pathogens were mostly *E. coli* (82.41%) followed by *Enterococcus faecalis*, *Klebsiella sp*, *Pseudomonas aeruginosa* and *Proteus sp*. Most of the bacteria showed high (≥ 98.25%) sensitivity to Meropenem; moderate susceptibility to Amoxicillin, Azithromycin, Ciprofloxacin, Gentamicin, Levofloxacin, Ceftriaxone, Cefepime and Nitrofurantoin and least (<20%) sensitivity to Cefixime, Cephradine, Cefuroxime, Clindamycin, and Trimethoprim.

Conclusion: These results are of clinical and epidemiological significance, enhancing the studies to identify causative pathogens and sensitivity patterns of pathogens in UTIs, as well as improving clinicians’ knowledge of how to select the best antibiotics and ultimately contribute to the diagnosis and treatment of patients.

Background

Urinary Tract Infection (UTI) is one of the most frequently occurring nosocomial infections in routine clinical practices and hospital settings [1]. It refers to the presence of pathogenic organisms within the urinary tract and usually classified by the site of infection as bladder (cystitis), kidney (pyelonephritis) or urine (bacteriuria) [2]. The clinical manifestations depend on the part of the urinary tract involved, the etiologic organisms, the severity of the infection and the patient’s ability to mount an immune response to it. Chronic and acute infection of urinary tract leads to high blood pressure, kidney damage and ultimately, results in death [3].

UTI can occur in all age group of patients and both gender. Due to some clinical factors along with anatomical variation, hormonal effect and behavioral pattern, the prevalence of UTI is 14 times more common in female than male [4,5].

There are many types of antibiotics available for UTIs and the choice depends upon many factors including severity of infection and acute or recurrent infection [6]. Unfortunately, this infection may further be complicated due to indiscriminate use of antimicrobial agents that led to the emergence of antibiotic
resistant pathogens [7]. These resistance properties are easily transferred between bacteria of different genera through plasmids and other non-genetic basis [8]. The rate of resistance is high among uropathogens [9]. Often, frequency of resistance to antibiotics and drugs is directly linked to the consumption of antibiotics. Generally, treatment of UTI is started empirically and therapy is based on information determined from the antimicrobial sensitivity pattern of the urinary pathogens of a given community [10].

UTI can be caused by various types of microorganisms though the most common pathogens are *Escherichia coli* and other *Enterobacteriaceae*, which account for approximately 75% of the isolates. Also *Proteus, Klebsiella, Enterobacter, Serratia* and *Pseudomonas* isolated in recumbent, complicated and catheterized patients [11].

Because of the evolving and continuing antibiotic resistance phenomenon, regular monitoring of resistance pattern is necessary to update guidelines for empirical antibiotic therapy. Moreover, there is growing demand for more new drugs. However, constant monitoring of drug resistance is required because only limited data describing multidrug resistance among UTI isolates is available so far [12].

The present study was undertaken to determine the prevalence of microorganisms causing UTIs and to evaluate the current antibiotic susceptibility pattern of these bacterial pathogens. This will enable the clinicians to facilitate the effective treatment and management of patients with symptoms of UTIs.

**Methods**

**a. Study area and population**

This study was carried out at the Department of Microbiology, Khwaja Yunus Ali University and Lab Zone and Hormone center, Tangail for seven months from June 2020 to December 2020. The population studied was a heterogeneous population of different age group and sex type. Total 1288 urinary clinical samples were collected from patients who attended the Lab Zone and Hormone center from various regions of Bangladesh. Among them, 398 positive cases were enrolled in this study.

**b. Sample Collection**

Clinically suspected UTI patients (both male and female) presented to Lab zone and Hormone center, Tangail, were enrolled in this study. The specimens were collected aseptically in clean sterile screw capped bottles from patients with urinary tract infections. Then the samples were transported to the laboratory for further analysis.

**c. Isolation And Identification of Microorganisms**

Different types of selective media were used to identify the pathogens. All media were prepared according to the manufacturer’s guideline and sterilized at 121°C for 15 minutes at 15 lb PSI pressure. Inoculation of urine specimen was done using sterile inoculating wire loop onto nutrient agar and MacConkey
medium. The cultured media were then incubated at 37°C for 24 hours and observed for growth through the formation of colonies. For the media which had no growth after 24 hours, were further incubated up to 48 hours before declaring absence of bacterial growth. All the bacteria were isolated and identified through cultural, morphological, and microscopic tests following standard procedures. Further confirmation was carried out by biochemical tests including Catalase, Citrate, Oxidase, Indole, Motility and Urease test [13].

d. Antibiotic Susceptibility Testing

Mueller–Hinton agar medium was used for antimicrobial susceptibility testing following Kirby–Bauer disc diffusion method, against a panel of 14 commercial antibiotics (Himedia, India) including Amoxicillin (10 µg), Azithromycin (15 µg), Cefixime (5µg), Cephradine (30µg), Ceftriaxone (30µg), Cefuroxime (30µg), Ciprofloxacin (05µg), Clindamycin (2 µg), Cefepime(30µg), Gentamycin (10 µg), Meropenem (10µg), Nitrofurantoin (300µg), Levofloxacin (05µg), and Trimethoprim (5 µg).

In this technique, isolated colonies were taken from 24 hours culture plates into nutrient broth. A lawn of test pathogen (1ml of a 24-hour peptone broth culture) was prepared by evenly spreading 100µl inoculums on the Muller Hinton agar plate. Discs impregnated antibiotics were placed on plates firmly by means of sterile forcep aseptically, which were then left at room temperature for 1 hour to allow diffusion of the antibiotics into the agar medium. Following incubation at 37°C for 24 hours, susceptibility was noted as sensitive, resistant or intermediate based on the diameter of zone of inhibition. Standard strains of Escherichia coli ATCC 25922 and S. aureus 25923 were used as control during antimicrobial susceptibility testing [14].

e. Data Management and Analysis

Excel and SPSS were used to analyze the examined data. Descriptive statistics and chi-square tests were used to check the statistical evaluation. “< 0.5” was the significant value of the p-value considered in this study.

Results

During the research period, a total of 1288 urinary samples were collected of which 398 (30.9%) showed positive results and others appeared negative. Subsequent analysis of positive growth samples indicated that the ages of most infected people (50.5%) were between 21-40 years, followed by 41-60 years, <20 years, and >60 years (Fig. 1) where most of the infected people were female (72.6%) (Table 1).

**Table 1:** Prevalence of UTI based on gender
<table>
<thead>
<tr>
<th>Sex</th>
<th>Number of Sample</th>
<th>Percentage of sample (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>289</td>
<td>72.6 %</td>
</tr>
<tr>
<td>Male</td>
<td>109</td>
<td>27.4 %</td>
</tr>
<tr>
<td>Total</td>
<td>398</td>
<td>100 %</td>
</tr>
</tbody>
</table>

Organisms identified according to biochemical and microbiological tests were mostly *E. coli* (82.86%), followed by *Enterococcus faecalis, Klebsiella sp, Pseudomonas aeruginosa,* and *Proteus sp* (Fig. 2).

Our study presented that only Meropenem (97.8%) was highly sensitive while four antibiotics Clindamycin (98.7 %), Cefixime (98.4 %), Cefuroxime (84.5 %), and Cephradine (79.0 %) were highly resistant against *E. coli*. The remaining antibiotics, Amoxicillin, Azithromycin, Ceftriaxone, Ciprofloxacin, Cefepime, Gentamicin, Nitrofurantoin, Levofloxacin, and Trimethoprim, were moderately sensitive to *E. coli* (Fig. 3).

For *Enterococcus faecalis*, the most sensitive antibiotics were Meropenem (100%), whereas higher antibiotic resistance was found against Cephradine (100%), Clindamycin (100%), Cefixime (96.9%), Ceftriaxone (87.8%), Cefuroxime (78.8%), and Trimethoprim (75.7%). The remaining antibiotics were moderately sensitive against *Enterococcus faecalis* (Fig. 4).

Highly sensitive antibiotics against *Klebsiella* sp. found in our study were Meropenem (100%), Cefepime (95.4%), Gentamicin (95.4%), Nitrofurantoin (95.4%), and Ciprofloxacin (81.8%). In this case, highly resistant antibiotics were Cefixime (100.0%), Cephradine (100.0%), Cefuroxime (100.0%), Clindamycin (100.0%), Ceftriaxone (95.4%), Amoxicillin (86.3%), and Levofloxacin (86.3%). Trimethoprim (90.9%) and Azithromycin (86.3%) were moderately sensitive against *Klebsiella* sp (Fig. 5).

For *Pseudomonas aeruginosa*, higher sensitivity was found in Meropenem (100%). On the other hand, most of the used antibiotics Cefixime (100%), Cephradine (100%), Cefuroxime (100%), Clindamycin (100%), Nitrofurantoin (100%), Ceftriaxone (90.9%), Trimethoprim (81.8%) and Cefepime (63.6%) were resistant against *Pseudomonas aeruginosa*. Amoxicillin, Azithromycin, Ciprofloxacin, Gentamicin, and Levofloxacin were moderately susceptible against *Pseudomonas aeruginosa* (Fig. 6).

For *Proteus* sp, only Meropenem (100%) and Nitrofurantoin (75%) were highly sensitive; most of the used antibiotics Cefixime (100%), Cephradine (100%), Ceftriaxone (100%), Cefuroxime (100%), Clindamycin (100%), Cefepime (100%), Trimethoprim (100%), and Levofloxacin (75.0%) were highly resistant and remaining antibiotics Gentamicin (100%), Azithromycin (75%), and Ciprofloxacin (75%) were moderately susceptible against *Proteus* sp (Fig. 7).

Overall antimicrobial susceptibility study of different isolates from urinary tract infections (UTIs) revealed that most of the antibiotics Clindamycin (98.9%), Cefixime (98.50%), Cefuroxime (87.43%), Cephradine (83.1%), Trimethoprim (64.9%), and Ceftriaxone (63.1%) were highly resistant against *E. coli, Enterococcus faecalis, Pseudomonas aeruginosa, Klebsiella* sp, and *Proteus* sp. The remaining
experimental antibiotics were moderately sensitive to all isolated pathogens while only Meropenem showed the highest sensitivity (98.25%) to all tested pathogens (Table 2).

**Table 2: Overall antibiotic sensitivity pattern against UTI pathogens**

<table>
<thead>
<tr>
<th>Antibiotics Name</th>
<th>Intermediate (I) No.</th>
<th>Percentage</th>
<th>Resistance (R) No.</th>
<th>Percentage</th>
<th>Sensitive (S) No.</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin</td>
<td>120</td>
<td>30.15%</td>
<td>170</td>
<td>42.71%</td>
<td>108</td>
<td>27.14%</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>128</td>
<td>32.17%</td>
<td>141</td>
<td>35.43%</td>
<td>129</td>
<td>32.42%</td>
</tr>
<tr>
<td>Cefixime</td>
<td>06</td>
<td>1.50%</td>
<td>392</td>
<td>98.50%</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>Cephradine</td>
<td>37</td>
<td>9.30%</td>
<td>330</td>
<td>83.1%</td>
<td>31</td>
<td>7.78%</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>102</td>
<td>25.7%</td>
<td>251</td>
<td>63.1%</td>
<td>45</td>
<td>11.30%</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>45</td>
<td>11.31%</td>
<td>348</td>
<td>87.43%</td>
<td>05</td>
<td>1.26%</td>
</tr>
<tr>
<td>Ciprofl oxacin</td>
<td>168</td>
<td>42.2%</td>
<td>108</td>
<td>27.2%</td>
<td>122</td>
<td>30.65%</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>02</td>
<td>0.51%</td>
<td>394</td>
<td>98.9%</td>
<td>02</td>
<td>0.51%</td>
</tr>
<tr>
<td>Cefepime</td>
<td>106</td>
<td>26.64%</td>
<td>182</td>
<td>45.8%</td>
<td>110</td>
<td>27.64%</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>130</td>
<td>32.67%</td>
<td>117</td>
<td>29.4%</td>
<td>151</td>
<td>37.94%</td>
</tr>
<tr>
<td>Meropenem</td>
<td>07</td>
<td>1.76%</td>
<td>00</td>
<td>00%</td>
<td>391</td>
<td>98.25%</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>95</td>
<td>23.9%</td>
<td>168</td>
<td>42.3%</td>
<td>135</td>
<td>33.92%</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>75</td>
<td>18.85%</td>
<td>108</td>
<td>27.13%</td>
<td>215</td>
<td>54.0%</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>84</td>
<td>21.1%</td>
<td>258</td>
<td>64.9%</td>
<td>56</td>
<td>14.0%</td>
</tr>
</tbody>
</table>

**Discussion**

Urinary tract infection is one of the major community acquired infections throughout the world as well as Bangladesh due to current emergence of antibiotic resistant uropathogens. This study showed a high prevalence of UTI in female (72.6 %) than male (27.4%) which correlates with Akhtar *et al.* (2016) [15]; who reported UTI in female (59.3%) than in male (40.7%) which is almost similar to our study. Age groups of 21-40 years were found more susceptible to UTIs. This is similar to other study like Pondei *et al.*, 2017 [16]. In their study, the highest prevalence was among the 21-30 years of age group. The main reasons behind this increasing incidence of UTI with advancing age is due to prostate enlargement in male and neurogenic bladder in female [15].
Of the 398 positive samples, 324 samples comprised bacteria among which the most prevalent was *E. coli* followed by *Enterococcus faecalis*. Other isolated bacteria from UTI cases were *Klebsiella* sp, *Proteus* sp, and *Pseudomonas aeruginosa*. These findings are agreed to other study like Abedin *et al.* 2021 [11].

In our study, we found maximum sensitivity (98.25%) in Meropenem, no sensitivity was found in Cefixime. Maximum resistance (98.9%) was found in Clindamycin, no resistance in Meropenem, Highest intermediate resistance (42.2%) showed in Ciprofloxacin and lowest sensitivity (0.51%) was found in Clindamycin. Majumder *et al.* in Bangladesh found nearly similar results [17]. The higher bacterial resistance to antibiotics in our region may be due to a higher rate of antibiotic usage, even used as OTC products and in absence of a prescription [18].

Highest sensitivity (98.25%) was found in Meropenem and no resistance was found against it. This result is found similar to the study of Abedin *et al.*, 2020 who reported highest sensitivity of Meropenem and Imipenem against uropathogens [6].

The overall study showed that, most of the organisms had higher susceptibility to Meropenem, moderate susceptibility to Amoxicillin, Azithromycin, Ciprofloxacin, Gentamicin, Levofloxacin, Ceftriaxone, Cefepime and Nitrofurantoin and least effectivity to Cefixime, Cephradine, Cefuroxime, Clindamycin, and Trimethoprim; which is also comparable to susceptibility patterns reported from previous studies.

**Conclusion**

The aim of this study was to determine bacterial etiological agents responsible for UTIs and to evaluate their in vitro susceptibility pattern to commonly used antimicrobial agents. A total 398 positive cases of different age and sex were enrolled in this study. Majority (50.5%) patients were 21-40 years of age. Among them (72.6%) patients were female and 27.4% patients were male. We found *E. coli, Enterococcus faecalis, Klebsiella* sp, *Proteus* sp and *Pseudomonas aeruginosa* as causative agents of UTIs. Meropenem was found to be the most sensitive (98.25%) antibiotic, whereas no sensitivity was found in Cefixime and intermediate resistance was in Ciprofloxacin (42.2%).

**Abbreviations**

1. UTI- Urinary tract Infection
2. Psi-Pound per square inch
3. ATCC-American type culture collection

**Declarations**
Ethical Approval and Consent to the participate: Institutional ethical approval was taken before initiation of the project (Reg. No.: KYAU/EC/2020/008) from the respective authority of Khwaja Yunus Ali University. All patients provided written and informed consent.

Consent for Publication: Not Applicable

Availability of Data and Material: Supporting data which make the findings of the study are not openly available but are accessible from corresponding author upon reasonable request.

Competing interests: The authors declare there are no conflicts regarding publication of this article.

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Author’s contribution:

AMZ planned and supervised the experiments, and reviewed the manuscript.

KFA prepared the manuscript and interpreted the data.

QMO co-supervised and reviewed the manuscript.

AMB supervised the experiments and reviewed the manuscript.

IMI carried out the study, revised and reviewed the manuscript.

RMA carried out the study.

FNEK carried out the study.

EMA carried out the study.

BMS reviewed the manuscript.

HR reviewed the manuscript.

SJH reviewed the manuscript.

AAA co-supervised and reviewed the manuscript.

All the authors read and approved the entire manuscript including their contributions mentioned in the respective section of title page. All of the authors agreed with the statement.

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**Figures**

**Figure 1**

Distribution of infected persons based on age
**Figure 2**

Prevalence of bacterial pathogens in UTIs

**Figure 3**

Antibiotic susceptibility pattern of *E. coli*
Figure 4

Antibiotic sensitivity pattern of *Enterococcus faecalis*

Figure 5

Antibiotic sensitivity pattern of *Klebsiella sp*
Figure 6

Antibiotic sensitivity pattern of *Pseudomonas aeruginosa*

Figure 7

Antibiotic sensitivity pattern of *Proteus sp*