Bacteria Isolated From Urinary Tract Infection among Patients and Determination of the Antibiotic Susceptibility Patterns of the Gram Negative Bacteria in Tertiary Care Hospital

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Abstract: Urinary tract infections (UTIs) are the most common bacterial infections encountered in clinical practice, which cause morbidity in both hospitalized and out patients. Increasing antimicrobial resistance among bacterial uro-pathogens is a significant health concern. Approximately 1 in 3 women will require antimicrobial treatment for a Urinary Tract Infection (UTI) before age 24, and 40% to 50% of women will have a UTI during their lifetime. UTIs in male patients are considered complicated. Escherichia coli are the most common cause of UTIs. To determine spectrum of microorganisms responsible for UTIs and their antimicrobial sensitive pattern. Clean-Catch midstream urine of the patients was collected. Urine samples received in microbiology laboratory for culture from 881 patients clinically suspected of having urinary tract infection during the study period were processed according to standard protocol. Isolates were identified by conventional phenotypic methods and antibiotic sensitivity determined by standard Kirby Bauer disc diffusion method and follow Clinical and Laboratory Standards Institute (CLSI) guidelines. In this study 191 (21.68 %) patients out of 881 were shown to be urine culture positive out of them 122 (63.87%) females and 69 (36.13%) males. The most isolated bacterium was E. coli with frequency rate of 126 (65.97%). The other bacteria were Klebsiella spp. 35(18.32%), Pseudomonas spp. 18 (9.43%), and Acinetobacter spp. 12 (6.28%). It is concluded that Gram-negative bacilli were responsible for UTI infections in our patients. The most common isolated bacteria from urinary tract infections were E. coli followed by Klebsiella pneumoniae. The most effective antimicrobial agent was Imipenam and the least effective one was cephalixin.

Keywords: Bacterial agents, Urinary Tract Infection, Antimicrobial susceptibility E. coli.

INTRODUCTION

Urinary tract infection (UTI) is one of the most common diseases in human societies which occur in women more than men [1, 3]. The UTI occurrence depends on several factors provide the presence of bacteria (more than 10⁵/ml) in urine [3]. These bacteria cause UTI and if not treated, the infection will spread and cause serious damage to the patient [2, 4, 5]. UTI treatment with antibiotics is carried out usually before receiving microbiology test results. This therapy, without rational drug prescription occasionally leads to antibiotic resistance and treatment failure is its result [3, 6].

Discovery of antibiotics was one of the greatest advances of modern medicine, but the availability and increased use of antibiotics gradually lead to microbial resistance to them [7]. Antimicrobial resistance is increasing around the world, especially in developing countries [8]. According to the World Health Organization in 2014, antimicrobial resistance is increasingly a global threat for public health and all countries have focused on this problem which is a serious threat to modern medicine [9].

The first important factor in increasing microbial resistance is improper use of antibiotics [10, 11]. The other is incorrect and unreasonable antibiotics prescription. Considering time, the appropriate dose and manner of administration are the most important aspects of rational drug prescription [11, 12]. Studies have shown that 30%–60% of the prescribing and use of antibiotics has been improper. Many hospitals have turned their supervision on the use of certain
antimicrobial agents to change this worrying trend in Iran and all over the world [13, 14].

Urinary tract infections (UTIs) are one of the most common bacterial infections, leading to increased morbidity in both hospitalized as well as outpatients [15, 16]. Although, a variety of organisms are responsible for UTI, Escherichia coli and other members of the family Enterobacteriaceae account for a large majority of UTIs [17].

After Escherichia coli, the most common pathogens causing UTIs include Klebsiella pneumoniae, Enterococcus spp., S. saprophyticus, Pseudomonas aeruginosa, Candida spp., Proteus spp. and Enterobacter spp [18]. Increasing prevalence, relapse and ramifications in eradication of UTI make it a cause for concern and present a real challenge to healthcare professionals. It is also a known fact that women are more vulnerable to UTI due to anatomical and physiological factors. Owing to its position, urogenital tract of women is more susceptible to bacterial infections caused by both internal and external flora.

Increasing drug resistance in bacterial uropathogens is an important and emerging public health problem. The distribution of antimicrobial susceptibility data of UTI causing microorganisms changes from place to place. Generally, the antimicrobial treatment is initiated before the culture sensitivity results are available which may lead to the frequent misuse of antibiotics [19]. The current knowledge of the organisms causing UTI and their antimicrobial susceptibility pattern is a necessity, in order to ensure appropriate empirical therapy. In view of the increasing antimicrobial resistance among bacterial isolates causing UTI, this study was undertaken at a tertiary care center to determine the spectrum of microorganisms responsible for UTI and their antimicrobial resistance pattern.

MATERIALS AND METHODS
Study population
This cross sectional study was carried out from February 2018 to July 2018 in Microbiology laboratory of a tertiary care center. A total of 881 patients clinically suspected of having urinary tract infections with complaints of fever, frequency or urgency of micturition were involved which included both inpatients and out-patients.

Collection of Sample
The patients were thoroughly instructed on how to collect the urine samples with strict asepsis. Clean-catch mid-stream samples were collected from each patient. In case of urination, the urine sample was collected by supra-pubic aspiration. In catheterized patients, the catheter was clamped and the area proximal to the clamp was disinfected. Then, using a 28 gauge needle and syringes, urine was withdrawn. The samples were immediately transported to microbiology laboratory for further processing.

Processing of Sample
The isolates were identified and characterized, based on the standard microbiological tests. For urine culture, midstream urine samples were collected. The samples were inoculated within 1 hour of sampling on blood agar and MacConkey agar. Depending on the microorganism type, the plates were incubated in both aerobic and anaerobic conditions at 37°C for 24-48 hours. Standard microbiological tests were conducted, depending on the type of isolated bacteria from various isolates. In order to identify Gram-negative bacteria, triple sugar iron (TSI) test, as well as Indole, citrate, urea, lysine decarboxylase, oxidase, and motility tests, was performed.

Interpretation of Growth and Identification of Isolates
A sample was considered positive for UTI if the colony count was more than 10^5CFU/ml by semi-quantitative technique and the direct microscopy revealed pus cells ≥ 8/cu mm. All the isolates were identified at the species level by standard biochemical tests. Samples growing more than two (02) organisms were not included in the study and were discarded as mixed growth. Urine samples with no growth after 48 hours of incubation were considered as negative.

Antimicrobial susceptibility testing
Antimicrobial sensitivity testing of all Gram negative and Gram-positive isolates was carried out by Kirby-Bauer disc diffusion method and the results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines. After identifying bacteria, antimicrobial susceptibility testing was performed by the Kirby-Bauer disc diffusion method. The results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines 2018. Susceptibility of gram-negative bacteria was tested against disks of Cephalexin (PR), Ampicillin-Sulbactum (AS), Ceftizoxime (CI), Nitrofurantoin (FD), Co-trimoxazole (BA), Tetracycline (TE), Chloramphenicol (CH), Ofloxacin (ZN), Norfloxacin (NX), Ciprofloxacin (RC), Sparfloxcin (DC), Gatifloxacin (GF) and Imipenem (IPM).

Results were recorded includes patient’s demographic profile, hospitalization status, previous treatment, microbiology laboratory data which includes type of organism and its antibiotic sensitivity.

RESULTS AND DISCUSSION
During the Six months period of study, a total of 881 urine samples were received in microbiology laboratory. Out of these urine samples, only 191 (21.68%) samples were considered as positive growth. Out of them 122 (63.87%) samples were from female
patients and 69 (36.13%) samples were from male patients.

Out of 191 isolated organisms, 126 (65.97%) were E. coli, 35 (18.32%) were Klebsiella pneumoniae, 18 (9.43%) were Pseudomonas aeruginosa and 12 (6.28%) were Acinetobacter baumannii (Table-1).

Table 1: Frequency of bacterial agents isolated from urine specimens in this study

<table>
<thead>
<tr>
<th>Organisms</th>
<th>No. of isolates (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>126 (65.97%)</td>
</tr>
<tr>
<td>Klebsiella pneumonia</td>
<td>35 (18.32%)</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>18 (9.43%)</td>
</tr>
<tr>
<td>Acinetobacter baumannii</td>
<td>12 (6.28%)</td>
</tr>
</tbody>
</table>

Urinary tract infections (UTIs) are one of the most common infections affecting all age groups and both sexes. They are the most frequent cause of morbidity in patients attending OPD and are also the most common healthcare-associated infection [20]. The most common uropathogens belong to the family Enterobacteriaceae such as Escherichia coli and Klebsiella pneumoniae followed by Pseudomonas aeruginosa and Acinetobacter baumannii.

UTI is a common, yet serious ailment in humans due to its recurrence and complexity in eradication. This problem is compounded by the declining number of newer antimicrobial agents entering the clinical practice and increasing antimicrobial resistance.

Table 2: Antimicrobial sensitivity pattern of isolated organisms

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Sensitivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imipenem (IPM)</td>
<td>93.72%</td>
</tr>
<tr>
<td>Chloramphenicol (CH)</td>
<td>69.11%</td>
</tr>
<tr>
<td>Nitrofurantoin (FD)</td>
<td>61.26%</td>
</tr>
<tr>
<td>Gatifloxacin (GF)</td>
<td>56.00%</td>
</tr>
<tr>
<td>Tetracycline (TE)</td>
<td>44.50%</td>
</tr>
<tr>
<td>Co-trimoxazole (BA)</td>
<td>36.64%</td>
</tr>
<tr>
<td>Ampicillin-Sulbactum (AS)</td>
<td>34.55%</td>
</tr>
<tr>
<td>Cefitoxime (CI)</td>
<td>27.75%</td>
</tr>
<tr>
<td>Sparfloxcin (DC)</td>
<td>22.51%</td>
</tr>
<tr>
<td>Ciprofloxacin (RC)</td>
<td>20.94%</td>
</tr>
<tr>
<td>Norfloxacin (NX)</td>
<td>17.80%</td>
</tr>
<tr>
<td>Ofloxacin (ZN)</td>
<td>17.28%</td>
</tr>
<tr>
<td>Cephalexin (PR)</td>
<td>13.61%</td>
</tr>
</tbody>
</table>
Our study revealed a positivity rate of 21.68% (191/881) which is much lower as compared to other studies of Kibret et al., [20], (22.7%) Banerjee [17] (24.5%), Prakash et al., [19], (53.8%).

In the present study, out of 881 samples which yielded bacterial growth and were considered positive for UTI, 122 (63.87%) samples were from female patients and 69 (36.13%) samples were from male patients. Similar findings regarding the sex distribution of positive urine samples have been reported in a study by Puneet bhatt et al., and Angoti et al., (Table-3) [21, 22].

In our study, Escherichia coli (65.97%) was the found to be the predominant isolate followed by Klebsiella pneumoniae (18.32%), Pseudomonas aeruginosa (9.43%), Acinetobacter baumannii (6.28%). These findings are consistent with other studies which also reveal that E. coli and K. pneumoniae are the commonest pathogens responsible for urinary tract infections [21, 22].

### Table-3: Comparison of other studies

<table>
<thead>
<tr>
<th>Studies</th>
<th>Positive (%)</th>
<th>Female (%)</th>
<th>Male (%)</th>
<th>E. coli (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Puneet bhatt et al., [21]</td>
<td>2731 (15.9%)</td>
<td>63%</td>
<td>37%</td>
<td>60.9%</td>
</tr>
<tr>
<td>Angoti et al., [22]</td>
<td>2035 (24.96%)</td>
<td>62.47%</td>
<td>37.53%</td>
<td>55.38%</td>
</tr>
<tr>
<td>Present Study</td>
<td>191 (21.68%)</td>
<td>63.87%</td>
<td>36.13%</td>
<td>65.97%</td>
</tr>
</tbody>
</table>

### CONCLUSION

In conclusion, the isolation of bacterial Urinary tract infection with a higher resistance rates for commonly used antimicrobials leaves the clinicians with very few options to choose drug used for empirical treatment of UTIs. Therefore, it is important to urge physician and other health worker in the field on the need of reevaluation of empiric treatment of UTI.

As drug resistance among pathogens is an evolving process, so there is a need to improve the effectiveness of integrated infection control programs to control and manage nosocomial infections caused by highly resistant organisms.

### REFERENCES


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