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**Research Article**

# ANTIMICROBIAL SENSITIVITY PATTERN OF BACTERIAL PATHOGENS FROM URINARY TRACT INFECTION PATIENTS IN SUBURB HOSPITAL OF DHAKA

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**ABSTRACT**

Urinary tract infections (UTI) caused by bacteria are very common in developed and developing counties. The etiological agents of these infections may vary with places along with their pattern of susceptibility and resistance. This study was conducted from October 2018 to December 2019 and aimed to assess the antibiotics susceptibility and resistance patterns. A total number of 1323 mid-stream urine samples from the UTI patients were tested microbiologically. The Kirby-Bauer disk diffusion method was used for antimicrobial susceptibility testing of the isolated uropathogens. Among 29.70% positive urine culture, 83.2% of the isolates were found Gram-negative in contrast to only 16.8% Gram-positive bacteria. Among Gram-negative organisms, *Escherichia coli* (50.2%), *Klebsiella pneumoniae* (33.9%), and *Pseudomonas* spp. (15.6%) were the most common uropathogens whereas *Staphylococcus aureus* (62.12%), and *Streptococcu*s spp. (37.88%) were found as common Gram-positive bacteria. UTI was detected more common in women (39.30%) than that in men (10.45%). Most of the isolates were found highly sensitive to imipenem, and amikacin. Almost all the isolates showed resistance against multiple antibiotics. A high level of resistance was found to cefadroxil (81.32%), nalidixic acid (69.16%), and cephradin (68.35%). Therefore, UTI should be treated with those antibiotics which are sensitive to the local infecting organisms.

**Keywords**: *UTI, antibiotic susceptibility, drug resistance, urinary pathogens*

## Introduction

Urinary tract infection (UTI), a frequent bacterial infection, is infecting 150 million people every year globally ranging from newborn to elderly people (Martha and Edgardo 2019). Approximately 30-40% of women, within the age of 24 years, experienced 1 episode of UTI that requiring antimicrobial treatment. Almost half of all women face one UTI during their life, 25%-50% require frequent hospital visits, antimicrobial usage, further morbidity, and costs due to repeated infections (Akram *et al*., 2007). At least one incident of UTI occurs in 20%-35% of females in their lives where the incidence of bacteriuria amongst pregnant women in Bangladesh has been described from 5% to 15.5%. (Ahmed 1996; Jhora *et al*., 2011). The presence of

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organisms >$10^5$ cells per ml in urine sample consider as serious bacteriuria and usually caused by normal flora *Escherichia coli*, causing over 75 % of incidents (Goddard 2006). *Staphylococcus saprophyticus* and *Enterococcus faecalis* from the Enterobacteriaceae family are also accountable for UTI.

The frequency of UTI is more in women than in men, because of anatomical predisposition or others host factors (Schaeffer *et al*., 2001). Besides the occurrence of uropathogens in the vagina, sexual activity, pregnancy are other factors that enhance incidence of UTI in female (Schaeffer *et al*., 2001). Due to ever changing pattern of susceptibility of uropathogens to antibiotics, updated knowledge on the diverse etiology of UTIs and the resistance against antibiotics of the causative organisms is important to clinicians for treating such patients. UTI is the most common infection among patients who have chronic in-dwelling bladder catheters, which may cause bacteriuria (Foxam 2002; Manges *et al*., 2008; Nahar *et al*., 2010).

In Bangladesh, antibiotic resistance is alarmingly increasing due to the arbitrary use of available antibiotics. The changes in the pattern of antibiotic susceptibility of uropathogens were due to extensive and random use of the broad spectrum of antibiotics. To manage these infections effectively, selection of antibiotics should be on the basis of antibiotics susceptibly pattern which is hindered by insufficient facilities for diagnostics and antimicrobial susceptibility testing (Ikeh 2003). Besides this, periodic evolutions of antimicrobial activity of different antibiotics is essential as the pattern of antibiotic sensitivity may vary over periods (Jones and Thornsberry 1982). There is a paucity of study addressing the etiologies, danger aspects, and outline of antibiotics resistance of UTI patients in Bangladesh. This study was conducted to address the lacunae.

## Materials and Methods

### *Study Design and Period*

The study was conducted from October 2018 to December 2019 in a suburb hospital located in periphery of Dhaka district. A total of 1323 samples were collected from those who visited physicians with complaints of burning or painful micturition, increased frequency of micturition or lower abdominal pain. Patients who were already taking antibiotics with in last 21 days, having menstruation, pregnancy period, catherization within 1 month were excluded from study.

### *Urine Sampling*

Patients who had given informed consent to participate in the study instructed verbally for the collection of a clean catch midstream urine in a sterile container. They were requested to give the early morning first void urine sample. The samples were then sent to the laboratory for urine culture examination within 6 hours.

### *Bacterial Isolation and Identification*

Maintaining the standard bacteriological technique, ccalibrated pplatinum loop was used to inoculate all samples on MacConkey agar and blood agar media and then incubated at $37^0$ C for 48 hours. Colony forming unit (CFU) of bacteria were manually counted from the resulting colonies to record in the urine sample. A specimen was considered positive for UTI where bacterial colony counting $10^5$ colony forming units (CFU)/ml or more was considered as

significant and was subjected to identification basis of colony characters and biochemical tests and preserved in glycerol stock at -20$^0$c for further culturing and characterization experiments.

***Characterization of the Isolated Bacteria***

For the identification of bacterial genera, strains were characterised through various morphological and biochemical tests as described by the methods of Cappuccino and Sherman. Simple staining and Gram staining was used to determine the bacterial morphology and differentiate between Gram-positive and Gram-negative by observing whether they were purple and pink stained respectively. Isolated strains from culture plates were further inoculated for biochemical tests analysis using standard microbiological methods according to the methods of Cappuccino and Sherman.

***Antimicrobial Susceptibility Testing***

The antimicrobial susceptibility assay of uropathogenic bacteria strains was performed using the disk diffusion method (Bauer *et al*., 1966). All the strains identified were tested for antimicrobial susceptibility testing. Mueller-Hinton (MH) agar plates were prepared and streaked heavily by a sterile cotton swab (Fig. 1). A total number of 11 commercially available paper antibiotic disks including Nalidixic acid (NAL) (30 µg), Cefadroxil (CFR) (30 µg), Ciprofloxacin (Cip) (5 µg) , Gentamycin (Gm) (10 µg), Nitrofurantoin (Nif) (300 µg) , Ceftriaxone (CRO) (30 µg) , Amoxicillin (Amx) (10 µg), Cephradin (Inn) (30 µg) , Cotrimoxazole (Sxt) (25 µg), Amikacin (An) (30 µg), Imipenem (Ipm) (10 µg) of fixed concentration (Oxoid, UK) placed on the inoculated agar surface and incubated at 37$^0$C for 24h. (Bauer *et al*., 1966) These antibiotics were chosen according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (Yusuf *et al*., 2015). After incubation, the diameter of zones of growth inhibition around the antibiotic disc were measure in millimeters. The diameter of the one of growth inhibition was interpreted as sensitive, intermediate, or resistant as reported by CLSI.

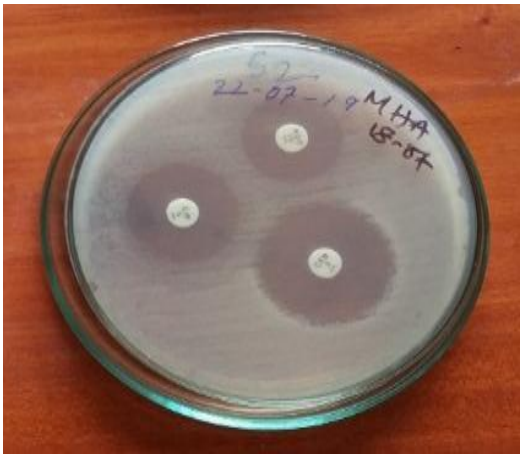


**Fig. 1.** Zone of inhibition (ZI) of *Escherichia coli* on Mueller Hinton agar plate due to the antimicrobial effect of diffused antibiotics impregnated in each disc.

**Results**

A total number of 1323 urine samples of different ages and sex were included in this study. Among the total samples, 440 (33.3%) were male and 883 (66.6%) were female. Out of the 1323 samples 393 (29.70%) samples showed significant bacteriuria. The percentage of isolation of pathogens in males and females was 10.45% and 39.30 % respectively (Table 1).

**Table 1. Gender distribution for rate of isolation in urine culture (n=910).**

| Sex | No. of sample | Growth for Uropathogens | Percentage of Growth (%) |
|---|---|---|---|
| Male | 440 | 46 | 10.45 |
| Female | 883 | 347 | 39.30 |
| Total | 1323 | 393 | 29.70 |

The maximum number of pathogens isolated from the group to 26 to 35 years age group, though there were significant percentages of positive cases from 16-25 and 36-45 age groups. On the other hand, there were nominal percentages of patients <15 years and >45 years age group found too (Table 2).

**Table 2. Age distribution for rate of positive cultures in urine culture.**

| Age Group | Growth for Uropathogens | Growth (%) |
|---|---|---|
| <15 yrs | 18 | 4.58 |
| 16-25 | 103 | 26.2 |
| 26-35 | 150 | 38.17 |
| 36-45 | 90 | 22.9 |
| 46-60 | 22 | 5.6 |
| 61-75 | 10 | 2.54 |
| Total | 393 | 100 |

***Characterisation of Isolated Bacterial Strains***

For the identification of bacterial genera, strains were characterised through various morphological and biochemical tests. There were 347 (39.3%) female and 46 (10.45%) male patients with positive culture of pathogens of UTI. Gram-negative bacterial isolates reported for 327 (83.2%) of the positive cultures, while Gram-positive cocci were reported 66 (16.8%). Regardless of the male-female category among the Gram-negative bacilli, the predominant isolate was *E. coli* (50.15%) followed by *Klebsiella* spp. (33.94%), *Pseudomonas* spp. (15.59%) and *Proteus* spp. (0.3%). *Staphylococcus aureus* (62.12%) and *Streptococcus* spp. (37.88%) were the most predominant in the Gram-positive bacteria (Table 3).

**Table 3. Distribution of positive pathogens isolated from urine samples (n=393) with percentage.**

| Name of the Isolates | | Number of Isolated Organisms | | Percentage (%) |
|---|---|---|---|---|
| Gram-negative organisms (n=327) | Male | *E. coli* | 19 | 5.8 |
| | | *Klebsiella* spp. | 8 | 2.44 |
| | | *Pseudomonas* spp. | 4 | 1.22 |
| | | *Proteus* spp | 1 | 0.3 |
| | Female | *E. coli* | 145 | 44.34 |
| | | *Klebsiella* spp. | 99 | 30.28 |
| | | *Pseudomonas* spp. | 51 | 15.59 |
| Gram-positive Organisms (n=66) | Male | *Staphylococcus aureus* | 7 | 10.6 |
| | | *Streptococcus* spp. | 7 | 10.6 |
| | Female | *Staphylococcus aureus* | 34 | 51.51 |
| | | *Streptococcus* spp. | 18 | 27.27 |

***Prevalence of Drug Resistance Isolated Bacterial Strains***

The antimicrobial resistance patterns of isolates are shown in Table 4. For all the isolates a varying percentage of resistance were observed to different antibiotics. Very high frequency of resistance observed ranging from 57.05% to 81.32%. The higher resistance to cefadroxil (81.32%), Nalidixic acid (69.16%), cephardin (68.35%), cotrimoxazole (65.32%), amoxicillin (57.05%) moderate resistance to ceftriaxone (44.79%), and gentamicin (36.25 %), and Ciprofloxacin (34.59%), nitrofurantoin (32.9%) and low resistance to Amikacin (11.40%) and Imipenem (5.9%) were found in UTI samples in low percentages.

**Table 4. Percentage of resistance isolates to the different antimicrobial agents.**

| Antimicrobial Agents (Amount per disc) | Number of Isolate Sensitive | Resistance | |
|---|---|---|---|
| | | Number of resistant Isolates | Percentage (%) |
| Nalidixic acid (NAL) (30µg) | 111 | 249 | 69.16 |
| Cefadroxil (CFR) (30µg) | 59 | 257 | 81.32 |
| Ciprofloxacin (Cip) (5µg) | 191 | 101 | 34.59 |
| Gentamycin (Gm) (10µg) | 190 | 108 | 36.25 |
| Nitrofurantoin (Nif) (300µg) | 182 | 88 | 32.90 |
| Ceftriaxone (CRO) (30µg) | 140 | 186 | 44.79 |
| Amoxicillin (Amx) (10µg) | 48 | 190 | 57.05 |
| Cephradin (Inn) (30µg) | 88 | 190 | 68.35 |
| Cotrimoxazole (Sxt) (25µg) | 86 | 162 | 65.32 |
| Amikacin (An) (30µg) | 233 | 30 | 11.40 |
| Imipenem (Ipm) (10µg) | 252 | 16 | 5.90 |

**Discussion**

Urinary tract infections are second and most common infections after respiratory tract infections among the human beings while may affects urethra, bladder of kidney (Mobley and Alteri 2015). *E. coli*, a Gram-negative bacillus which is responsible for more than 70% cases of urinary tract infections worldwide (Nicolle 2008). It is well postulated that UTI is very common among the women due to closer proximity of urethra to the anus and shorter urethra (O'Brien *et al.*, 2015). It is documented that UTI is more common in females than in males and finding our investigation are also in agreement with this generalization and rightly coincided with a recent study done by Deshpande *et al*. (2011). Likewise, regarding prevalence of uropathogens, our observation which was found in this study at a ratio of 7.5:1 in good agreement with several previous reports (Al-Badr and Al-Shaikh 2013; Keah *et al*., 2007). Our study showed further analogous results with some previous studies in the country in the case of patient's sex distribution that females (83.1%) suffer predominantly from UTI (Shaqra 2000; Yusuf *et al*., 2015).

The genitourinary structures of females and some other host factors such as alterations in normal vaginal flora are the cause of predominant UTI among females (Strom *et al*., 1987). In this study, the most common microorganisms related to UTI were Gram-negative (83.2%). Similar findings were reported by other studies (Mansour *et al*., 2009; Olafsson, *et al*., 2000). *E. coli* (50.17%), and *Klebsiella* spp. (33.94%) were found as the-predominant uropathogens in our study and indicated that *E. coli* is the key etiological agent (60.02%) of UTI (Saleh *et al*., 2010). The study reports correlate of other study of *Escherichia coli* (69%), *Streptococcus spp.* (15%), *Pseudomonas aeruginosa* (7%), *Enterococcus faecalis* (3%), *Staphylococcus aureus* (2%), *Klebsiella pneumoniae* (2%) in UTI. Other investigators also reported higher association of *E. coli* (66.67%, 77.8% cases respectively) (Basar *et al*., 2009; Saber *et al*., 2010).

However, Gram-positive organisms reported for 16.8% (66 out of 393) of UTI is similar to the findings of Shetty *et al*. (2013). In Bangladesh, a major public health concern is the increasing antibiotic resistance among UTI-related pathogens (Rahman *et al*., 2004). The prevalence of micrograms associated with UTI was evaluated by this study to determine their pattern of antimicrobial resistance. In our study, drug resistance was high against Cefadroxil (81.83%), Nalidixic acid (69.97%), Cotrimoxazole (65.86%), Cephradin (63.22%), and has similarity with the previous finding Saleh *et al*. (2010).

Ciprofloxacin resistance was found 34.17% in our study which is similar to other studies previously conducted (Sorlozano *et al*., 2007; Yasmeen *et al*., 2015). Recently in the hospitals, the extensive use of Cefuroxime may be the cause of lesser activity (46.21%) of this antibiotic against uropathogens found in this study. The resistance rate depends on local antibiotic prescription practice. Nitrofurantoin was found resistant only in 32.59 % proving as a moderate oral drug for treating UTI. In the United States and some southern European countries, uncomplicated cystitis was treated with Nitrofurantoin, where fluoroquinolones and co-trimoxazole showed high resistance to isolates from urine (Warren *et al*., 1999).

This study found a resistance rate of 44.79% to Ceftriaxone, which indicated the analogous result of other studies (Maraki *et al*., 2013). Imipenem and amikacin were found as the most effective

against uropathogens (Table 4) resembles the previous report of Mortazavi and Shahin (2009). Another study showed 93-100% susceptibility of uropathogens to amikacin and imipenem (Schaeffer *et al*., 2001). As bacterial resistance increased in recent decades, (Gupta *et al*., 2001; Levy and Marshall 2004) the isolates of the present study recovered from UTI showed high resistance. Hence, 82.5% of them demonstrated resistance to at least one antibiotic similar to other studies were the most prevalent Gram-negative and Gram-positive bacteria, respectively (Khoshbakht *et al*., 2013). One of the more alarming issues on the perspective for bacterial infections, with urinary tract infection (UTI), is the rise of antibiotic-resistant organisms, although the antimicrobial resistance pattern differs in various regions.

Common risk factors causing UTIs can be sexually active premenopausal women, age of the first UTI, maternal history of UTI, pregnancy, deferred voiding habit, wearing tight undergarments, change of sexual partners, DM and immunosuppression due to any cause (Mohsin and Siddiqui 2010). So, reproductive age does play a vital role as a risk factor for developing UTI in both sexes. To reduce UTI in the community one should drink lots of water, wipe from front to back when one goes to bathroom and when one goes for medication one should complete the full course of antibiotic. Even in the event of UTI, antibiotic resistance to bacterial infections can be a significant barrier to successful treatment alternatives.

## Conclusion

Urinary tract infection is significantly prevailing, does not differ nevertheless changed settings but increasing antimicrobial resistance UTI pathogens is a huge concern all over the world both especially in underdeveloped countries. Constant monitoring of predominant organisms and their pattern of antibiotic susceptibility is very essential for proper selection of antimicrobials for the effective and pragmatic treatment regimen of urinary tract infection in that region. Short study period, tiny sample size, no control group, and single centered study are the limitations of this study.

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