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Resistance Pattern of Ciprofloxacin against common Uropathogens in Selected Area of Dhaka city, Bangladesh

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Abstract: *Resistance to antibiotics is a major public-health problem and antibiotic use is being increasingly recognized as the main selective pressure driving this resistance. Our aim was to assess the resistance pattern of ciprofloxacin antibiotic of quinolone group against potential uropathogens. A total of 3765 urine samples were collected in 2016 (Jan-Dec) and out of which 346 (9.19%) were bacteriologically positive. Among the isolated uropathogens, 97.9% were gram negative and 2.1% gram positive organism (Staphylococcus aureus, Streptococcus and Enterococcus). Male were found more prone to get UTI under 10 years and over 60 years of age and females were more affected in 10 to 60 years of age group. E.coli was the most predominant (83.8%) isolate followed by Klebsiella spp. (5.8%). All of the Klebsiella spp., other gram negative (Acinetobacter spp., Citrobacter spp. and Serretia spp.) 100% were found resistant to ciprofloxacin. And all the Pseudomonas spp. (100%) were found sensitive to ciprofloxacin. Around 43.5% males and 35.5% females were found resistant to E.coli.*

Keywords*: UTI, Quinolone, Ciprofloxacin, Resistance, Uropathogen, Gram Negative Bacteria, Gram Positive Bacteria.*

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I. Introduction

Antibiotic resistance is an increasing threat to life and morbidity and mortality. Urinary Tract Infection (UTI) is a very common infection all over the world but it is more prevalent in developing south Asian countries like Bangladesh. UTI can be nosocomially ubiquitous in clinical environment so that prevalence rate of uropathogens is being alarmingly accelerated. To prevent these pathogens, different types of antibiotics and their super generations are used irrespectively with different doses in misused and overused forms. So uropathogens are getting resistant to efficacious drugs adopting different mechanisms of mutations and genetic transformations [(1)].

Urinary Tract Infection (UTI) represents as one of the most common diseases encountered in medical practices these days and encompasses a broad range of clinical fields that are associated with a common finding of positive urine cultures [(2)]. Besides every year about 150 million people are affected by UTIs. Worldwide at a cost of about US$6 billion [(3)] and even UTIs have demonstrated significant morbidity and mortality.

Urinary tract infection is more common in female than male, because of the short length of the urethra and its proximity to anus. Pregnancy and sexual activity also make female more susceptible to UTI [(4)]. The etiology of UTIs and the antibiotic susceptibility of urinary pathogens, both in community and hospitals, have been changing over the past years and recently, the antibiotic resistance has become a major global problem [(2)].

A large proportion of uncontrolled antibiotic usage has contributed to the emergence of resistant bacterial infections [(5)]. The aim of our study was to see the pattern of ciprofloxacin (quinolone) susceptibility against uropathogens in the selected area (Badda, Baridhara, Basundhara, Dhaka city, Bangladesh).

II. Materials And Methods

Materials

Study Design:

Study Location: This retrospective study was carried out IBN SINA Diagnostic Center, Badda from 1st January, 2016 to 31st December, 2016. The total sample size was 3765.

Methods

Sample Collection and Bacteriological Assessment: Early morning midstream urine samples were collected aseptically from 3765 (Male-903 & female-2862) patients. The urine samples were collected into sterile

container with wide mouth & screw cap tops. Samples were labeled with name, age, sex and time of collection. All the patients were instructed on how to collect the urine samples aseptically and taken to the laboratory immediately for culture. In the laboratory, each well mixed urine sample 1µl was inoculated into MacConkey agar (Oxoid) and Blood agar (Oxoid) plate under class-II laminar airflow (NUVO Sanaji Malzemelzeni, Imalat Vc Ticaret A.S, Turkey). The inoculum on the plate was streaked for discrete colonies with a sterile wire loop sterilized by auto loop sterilizer (Germany). The culture plates were incubated at 37°C in the incubator (Germany) for 48 hours. Isolated colonies were identified by using morphological, microscopy (Japan) and biochemical tests like TSI (HiMedia), MIU (HiMedia) and Simmons Citrate (HiMedia) agar following standard procedures.

Antibiotic Susceptibility Assessment: The disc diffusion technique was used for antibacterial susceptibility testing of the isolates [6]. We used the commercial antibiotic disc ciprofloxacin (5µg, HiMedia) of Quinolone. Interpretation of results was done using zone sizes. Zones of inhibition ≥21 mm was considered sensitive, 16-20 mm intermediate and <15 mm resistant. Isolates were classified as either sensitive or resistant based on the definition of the Clinical and Laboratory Standard Institute [7]. Some laboratory strains of known sensitivity of *Staph. aureus*, *E.faecalis*, *E.coli* and *P.aeruginosa* were used as quality control strains for the antimicrobial discs.

Statistical Analysis: Data were assessed using the Statistical Package for Social Science (IBM SPSS Statistics, version 18, IBM Corporation, SPSS Inc. Chicago, III, USA). The Trend chi square test for statistical comparisons between the groups.

III. Results

The total 3765 urine samples collected from patients, 346 (9.19%) samples were positive and 3419 (90.81%) samples were negative at 2016 (January-December) in selected areas at Dhaka city.

Tabel-1: Distribution of Urinary Tract Infection (UTI) patients by age and gender

Age	**01-10**	**11-20**	**21-30**	**31-40**	**41-50**	**51-60**	**61-70**	**71-80**	**81-90**	**91-100**
Male	18	04	07	06	06	11	16	11	02	02
Female	31	19	56	37	33	38	39	08	02	00
Total	49	23	63	43	39	49	55	19	04	02

Table-1 showed the distribution of patients by age and gender. Highest of the study subjects belonged to the 21-30 years age group (63 patients) followed by 61-70 years age group (55 patients) and 01-10 years age group (49 patients) respectively. It was found that males were affected more under 10 years age than females and females in between 21 to 30 years age group than males.

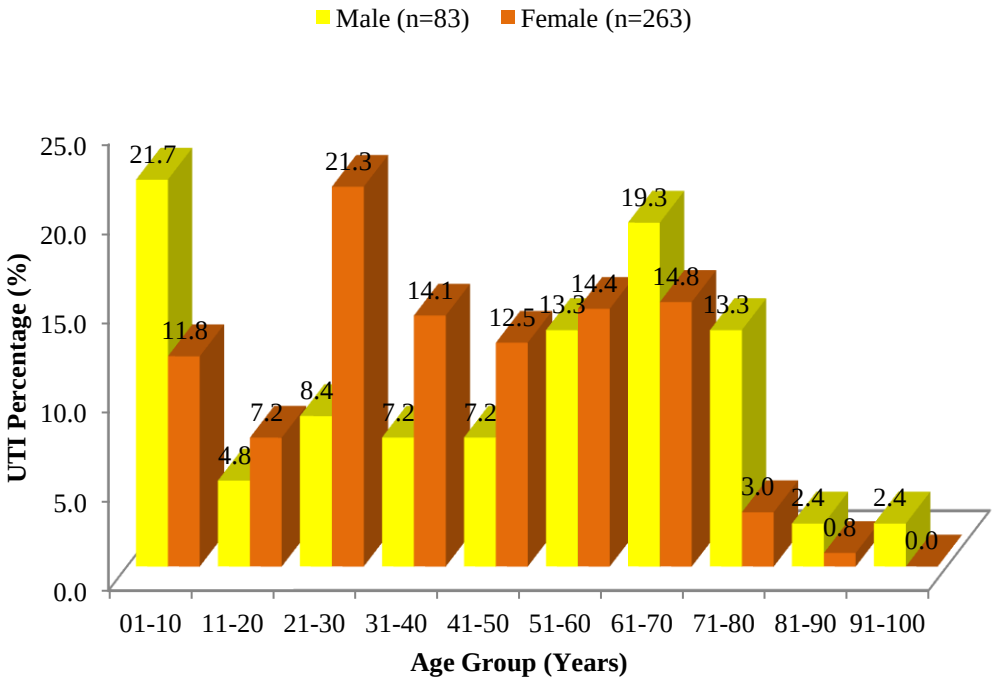

Figure-1: Dsitribution (in percentage) of UTI patients by age and sex (n=346)

Before 10 years age males were higher than females. In between 11-20, 21-30, 31-40, 41-50 and 51-60 years of age females UTI infection (7.2%, 21.3%, 14.1%, 12.5% and 14.4% respectively) were higher than males (4.8%, 8.4%, 7.2%, 7.2% and 13.3% respectively). In between 61-70 years age males infection were higher than females (19.3%>13.3%). In between 71-80 years age males infection were higher than females (13.3%>3.0%). In between 81-90 years age males infection were higher than females (2.4%>0.8%) but here number of patients were very few. In between 91-100 years age males infection were higher than females (2.4%>0.0%) but here number of patients are very few.

Table-2: Percentage distribution of uropathogens among UTI patients

Organisms	Percentage (n=346)		
	Male	Female	Total
E.coli	19.9%	63.9%	83.8 %
Klebsiella spp.	0.3%	5.5%	5.8 %
Enterobacter spp.	0.3%	2.6%	2.9 %
Pseudomonas spp.	1.7%	0.3%	2.0 %
Proteus spp.	0.9%	1.1%	2.0 %
Gram positive organisms	0.6 %	1.5 %	2.1 %
Other gram negative organisms	0.3 %	1.1 %	1.4 %
Total	24 %	76 %	100 %

Table 2 revealed percentage distribution of organisms in UTI. Majority of the organisms (97.9%) were gram negative organisms amongst which most of the organisms were (83.8%) *E.coli* followed by *Klebsiella* spp. & *Enterobacter* spp., *Pseudomonas* spp. and *Proteus* spp. (5.8%, 2.9%, 2%, 2% respectively).

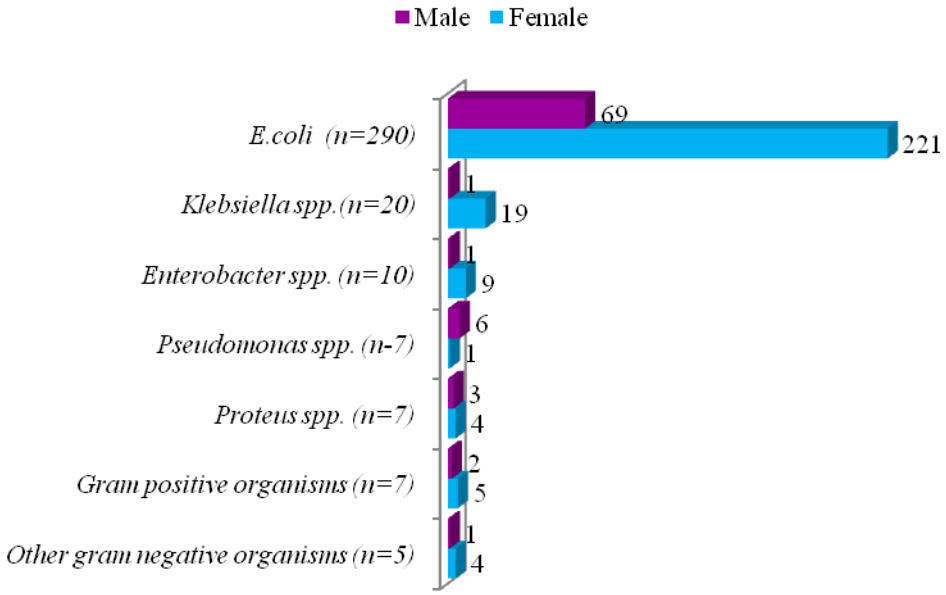

Figure-2: Frequency distribution of specific uropathogens among UTI patients

Out of 290 *E.coli* infection 69 males 221 females. *Klebsiella* spp. was the second prevalent organism and here male patient was 1 and female patients were 19. Other isolated uropathogen in male and female were *Pseudomonas* spp., *Proteus* spp, gram positive organisms (*Staphylococcus aureus*, *Streptococcus* and *Enterococcus*), *Enterobacter* spp. and other gram negative organisms (*Acinetobacter* spp., *Citrobacter* spp. and *Serretia* spp.).

Table-3: Prevalence of different uropathogens among male and female patients

Organisms	Male (n=83)		Female (n=263)	
	Number	Percentage	Number	Percentage
E.coli	69	83.1%	221	84.0%
Klebsiella spp.	1	1.2%	19	7.2%
Enterobacter spp.	1	1.2%	9	3.4%
Pseudomonas spp.	6	7.2%	1	0.4%
Proteus spp.	3	3.6%	4	1.5%
Gram positive organisms	2	2.4 %	5	1.9 %
Other gram negative organisms	1	1.2 %	4	1.5 %

Table-3 showed that the prevalence of the uropathogens among male and female patients. 83 samples (24%) were obtained from male subjects while the remaining 263 (76%) were from female. Among the male and female patients *E.coli* was 83.1% and 84.0%, *Klebsiella* spp. 1.2% and 7.2%, *Enterobacter* spp. 1.2% and 3.4%, *Pseudomonas* spp. 7.2% and 0.4%, *Proteus* spp. 3.6% and 1.5%, gram positive organisms 2.4% and 1.9%; and other gram negative organisms were 1.2% and 1.5% respectively.

Table-4: Frequency distribution of sensitivity/resistant pattern of ciprofloxacin against the isolated uropathogens among male patients (n=83)

Name of organisms	Sensitive		Resistant	
	Number	Percentage	Number	Percentage
E.coli	39	56.5%	30	43.5%
Klebsiella spp.	00	0.0%	01	100%
Enterobacter spp.	01	100%	00	0.0%
Pseudomonas spp.	06	100%	00	0.0%
Proteus spp.	03	100%	00	0.0%
Gram positive organism	02	100%	00	0.0%
Other gram negative organism	00	0.0%	01	100%

Table-4 showed the susceptibility of the isolated uropathogens to the ciprofloxacin antibiotic in male patients. All of them (100%) *Pseudomonas* spp., *Enterobacter* spp., *Proteus* spp. and gram positive organisms were found sensitive to ciprofloxacin. On the other hand all of the *Klebsiella* spp. and other gram negative organisms were found resistant to ciprofloxacin. 56.5% of the *E.coli* was found sensitive to ciprofloxacin.

Table-5: Sensitivity/resistance pattern of ciprofloxacin against uropathogens among female patients (n=263)

Name of organisms	Sensitive		Resistant	
	Number	Percentage	Number	Percentage
E.coli	142	64.5%	79	35.5%
Klebsiella spp.	19	100.0%	00	0.0%
Enterobacter spp.	08	88.8%	01	11.2%
Pseudomonas spp.	01	100.0%	00	0.0%
Proteus spp.	03	75.0%	01	25.0%
Gram positive organism	04	80.0%	01	20.0%
Other gram negative organism	04	100.0%	00	0.0%

Table-5 showed the susceptibility of the isolated uropathogens to ciprofloxacin among female patients. All of the isolated organisms were sensitive to ciprofloxacin & many of the *E.coli* (35.5%) was found resistant to ciprofloxacin. Sensitivity pattern of *E.coli, Proteus* spp., gram positive organisms, *Enterobacter* spp. were 64.5%, 75%, 80%, 88.8% respectively; and resistant pattern of *Proteus* spp., *gram positive organisms, Enterobacter* spp. were 25%, *20%,* 11.2% respectively.

IV. Discussion

Bacterial resistance to antibiotics is increased may be due to overuse, underuse and misuse of antibiotics. This study aimed to evaluate the pattern of antimicrobial susceptibility of bacteria isolated from patients with UTI to ciprofloxacin. It is important that clinicians are aware of the regional antibiotic resistance rates before initiating experimental antimicrobial therapy for UTI treatment, as it is well-described that urinary infection with a resistant pathogen is more likely to lead to bacteriological/clinical failures [(8)]. In this study, 3765 urine samples were tested, out of which 346 (9.19%) were bacteriologically positive and 3419 (90.81%) were negative. Female suffered more with UTI and most of UTIs were caused by *E.coli* (83.8%). This may explain the highest frequency of UTIs observed in women when compared to men, which is often attributed to a shorter urethra that facilitates colonization by these microorganisms [(9)]. Furthermore, another mechanism that could explain the lower frequency of UTI in men would be the prostatic fluid, which has antibacterial substances [(10)].

UTIs are particularly common among the female population with an incidence of about 1% of school-aged girls and 4% of women through child-bearing years. Incidence of infection in females increases directly with sexual activity and child-bearing. In the women, 25-30% of women between 21-40 years of age get UTIs

[11]. The anatomical relationship of the female urethra and the vagina makes it liable to trauma during sexual intercourse as well as bacteria been massaged up the urethra into the bladder during pregnancy and child birth [12]. It has been reported in several studies that women who are sexually active, and especially if they use contraceptives, foams, gels, diaphragm and spermicides which are known to promote greater colonization of the vagina are at higher risk of developing UTIs [13]. In the present study it was found that most predominant age group was 21-30 years than other age groups.

The present study revealed that mostly male patients are affected by uropathogens under 10 years age than female. Our finding is supported by the fact that uncircumcised male infants appear to be at increased risk of UTIs in the first three months of life. In a study of 100 otherwise healthy infants ranging in age from five days to eight months and admitted to the hospital because of a first known UTI [14] most of the UTIs in infants younger than three months of age were in males, but female infants predominated thereafter.

In our study the most predominant organism *Escherichia coli* (83.8%), as gram negative bacteria that caused urinary tract infection. There was a statistically significant difference in favor of *E.coli* ($P<0.05$) than others. *E.coli* causes more than 80% of urinary tract infections in previously healthy women. There is a chance of fecal contamination of periurethral area, then the bacteria spreads on ascending through the bladder and causes cystitis. These infections of the lower urinary tract, in some cases, can affect the kidneys and cause acute pyelonephritis, which consequently may result in bacteremia and sepsis [15]. The other organisms isolated were *Klebsiella* spp. (5.8%), *Enterobacter* spp (2.9%), *Pseudomonas* spp (2.0%), *Proteus* spp (2.0%), gram positive organisms (2.1%) and other gram negative organisms (1.4%). and majority of the isolates were from females. The most frequent microorganisms in UTIs are gram-negative bacilli, accounting for 90% of the reported cases, whereas gram-positive cocci are responsible for only 6% of all UTIs [16]. Several studies have shown that *Escherichia coli* is the major bacterial species associated with UTIs, and *Klebsiella pneumoniae* was the second most important bacteria in this type of infection [16]. The early introduction of effective drugs against bacterial infections in the last century has changed the medical behavior and significantly reduced the mortality rates due to these agents. However, the widespread use of antibiotics has induced different mechanisms of bacteria resistance to these drugs [17]. Bacterial resistance is naturally developed, being a consequence of bacterial adaptation to the environment. The exposure of microorganisms to different antibiotics increases the selective pressure and favors the development of resistance [18]. The most frequently prescribed antibiotics to treat UTIs are sulfamethoxazole+trimethoprim, fluoroquinolones (ciprofloxacin or norfloxacin), 1st and 2nd generations of cephalosporins, amoxicillin + clavulanate and nitrofurantion [19]. The knowledge on the regional pattern of bacterial resistance is critical to guide the medical staff to choose an appropriate antibiotic for the treatment of UTI patients [20].

Treatment of urinary tract infections is becoming more complicated with an increase of the number of resistant strains to antibiotics and prevalence of antibiotic resistance mechanisms. In Spain, for instance there were reports that some 22-27% of *E.coli* strains were resistant to ciprofloxacin [21, 22]. It was observed that horizontal gene transfer is a factor in the emergence and spread of antimicrobial resistance in clinical isolates [23]. Consequently, it has been suggested that the high prevalence of resistance to a particular antibiotic does not always reflect antibiotic consumption in a given environment [24].

In the present study 83(24%) male patients were found with UTI. *E.coli* mediated UTI (83.1%) was more prevalent than other organisms. *E.coli* showed sensitivity against ciprofloxacin in 56.5% cases. All of the *Pseudomonas* spp., *Proteus* spp., *Enterobacter* spp. and Gram positive organisms (*Staphylococcus aureus*, *Streptococcus* and *Enterococcus*) were sensitive to ciprofloxacin. All of the *Klebsiella* spp. and other gram negative organisms were resistant to ciprofloxacin. Significant difference between resistant and sensitivity of ciprofloxacin against these bacteria statistically were found at 5% level ($P<0.05$).

Out of 263 female patients with UTI, *E.coli* was more prevalent (84%) organisms. *E.coli* was sensitive against ciprofloxacin in (64.5%) cases. All of the *Pseudomonas* spp., *Klebsiella* spp., other gram negative organism(*Acinetobacter* spp., *Citrobacter* spp. and *Serretia* spp.) were sensitive to ciprofloxacin. 75%, *Proteus* spp, 80% Gram positive organism (*Staphylococcus aureus*, *Streptococcus* and *Enterococcus*), 88.8% *Enterobacter* spp. were sensitive to ciprofloxacin. Most prevalent resistance to ciprofloxacin was found against *Proteus spp. 25%, Gram positive organism (Staphylococcus aureus*, *Streptococcus* and *Enterococcus*) *20%, Enterobacter* spp. *11.2%*. There was significant difference between resistance and sensitivity of ciprofloxacin at 5 % ($P<0.05$).

Antimicrobial therapy for treatment of UTIs especially when using ciprofloxacin should be based on local experience of sensitivity, tolerability and resistance patterns. Bacterial resistance has become a public health issue and has increasingly been associated with risk factors that put life in danger [25]. Awareness is needed of both the population and health professionals about the importance for the correct use of antibiotics, and it is mandatory to take into account the result of antibiotics susceptibility tests. The ciprofloxacin use should be performed only after the microbial susceptibility confirmation, and it is necessary to find other alternatives

for the empirical treatment. The bacterial resistance prevention can be performed through control measures that limit the spread of resistant bacteria and the rational use of antimicrobial therapy.

V. Conclusion

In conclusion, the present results showed that there is a high prevalence of occurrence of urinary tract infection among patients in selected area of Dhaka city. Most of the bacteria were susceptible to ciprofloxacin. The prescribed ciprofloxacin antibiotic was still effective against the uropathogens, but should be reserved for only complicated UTIs and should be used to follow the antibiotic guidelines in order to prevent emergence of multi drug resistant organisms.

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