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RESISTANT PATTERN OF NALIDIXIC ACID AGAINST UROPATHOGENS IN SELECTED AREAS OF DHAKA CITY, BANGLADESH

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ABSTRACT
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. Our aim was to assess the susceptible pattern of Nalidixic Acid which is a synthetic drug of Quinolone antibiotic against uropathogens which cased Urinary Tract Infections. A total of 9178 urine samples were collected in 2016 (January-December) and out of which 890 (9.70%) were bacteriologically positive. Among the isolated uropathogens, 94.1% were gram negative and 5.9% gram positive organism. Male were found more prone to get UTI under 10 years and between 51-80 years of age and females were more affected in 10 to 50 years and over 80 years of age group. *E. coli* was the most prevalent (83.9%) isolate followed by *Klebsiella* spp. (7.1%), *Staphylococcus aureus* (3.1%), *Enterococci* spp. (2.6%), *Pseudomonas* spp. (2.2%), *Proteus spp.* (0.8%) and *Staphylococcus saprophyticus* (0.2%). The most predominant sensitive organisms to Nalidixic Acid were *Klebsiella* spp. (male 31.3% and female 40.4%) and resistant organisms *Pseudomonas* spp. (100%) and *Proteus spp*. (100%) in both male and female found. Around 87.6% male and 76.8% female were found resistant to *E. coli*.

KEYWORDS: Nalidixic Acid, Quinolone, UTI, Resistance, Uropathogen.

INTRODUCTION
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.[1] Urinary tract infections (UTIs) are serious health problem affecting 150 million people globally in each year.[2] 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.[3] They are the second most common types of infection in humans accounting for 8.3 million doctor's visit annually in USA.[4] They are the most common bacterial infection in patients of all ages with high risk in young women resulting in significant morbidity and health care costs.[5]

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.[6] Different factors like age, sex, immunosuppression and urological instruments may affect prevalence of UTIs.[7] 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.[8] 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.[9] Antibiotic resistance is an increasing threat to life and morbidity and mortality.[10]

Treatment of UTIs cases is often started empirically and therapy is based on information determined form the antimicrobial resistance pattern of the urinary pathogen.[11] However, a large proportion of uncontrolled antibiotic usage has contributed to the emergence of resistant bacterial infections.[12] Nalidixic acid was the first quinolone-based antibiotic.[13] It inhibits DNA gyrase and topoisomerase IV activity in Gram-negative bacteria with originally reported minimal bacteriostatic concentrations of 0.5-50.0 µg/ml.[14,13] Formulations

containing Nalidixic Acid were used for the treatment of urinary tract infections.[15] However, derivatives of Nalidixic Acid, comprising second, third and fourth generation quinolones, have improved antibacterial actions and fewer adverse effects, so it is no longer used clinically.

Over a period of a few decades, Nalidixic Acid have used as an important class of drugs used primarily to treat urinary tract infections in the world. Unfortunately, Nalidixic Acid usage is threatened by the rising occurrence of resistance, which has been observed in every species that is treated by this drug. The aim of our study was to see the pattern of Nalidixic Acid susceptibility against uropathogens in the selected areas (Doyagonj, Gandaria, Jatrabari, Sayedabad, Dhaka, Bangladesh).

MATERIALS AND METHODS

Materials

Study Design

Study Location

This was a retrospective analysis of laboratory data routinely collected from the microbiology department of IBN SINA Diagnostic and Consultation Center, Doyagonj, Dhaka from January 1, 2016 to December 31, 2016. The total sample volumes were 9178.

Methods

Sample Collection and Bacteriological Assessment

Early morning midstream urine samples were collected aseptically from 9178 (male-2735 and female-6443) patients. The urine samples were collected into sterile wide container (China) with screw cap tops. On the label were the 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 diagnostic laboratory, each well mixed urine sample (1µL) was inoculated on MacConkey agar (Oxoid) and Blood agar (Oxoid) media plate under class-II laminar airflow (NUVO Sanaji Malzemelzeni, Imalat Vc Ticaret A.S, Turkey). The inoculum on the plate was streaked out for discrete colonies with a sterile wire loop sterilized by auto loop sterilizer (Germany) following standard procedures. The culture plates were incubated at 37°C by an incubator (Germany) for 48 hours and observed for the growth of bacteria through formation of colonies. All the bacteria were isolated and identified morphologically using microscopy (Japan) and biochemical tests like TSI (HiMedia), MIU (HiMedia) and Simmons Citrate (HiMedia) agar following standard procedures.[16]

Antibiotic Susceptibility Assessment

The disc diffusion technique was used for antibacterial susceptibility testing of the isolates using commercial antibiotics containing discs. We used the commercial antibiotic disc Nalidixic Acid (30µg, Oxoid). Interpretation of results was done using zone sizes. Zones of inhibition ≥19 mm were considered sensitive, 14-18 mm intermediate and ≤13 mm resistant. Isolates were classified as either sensitive or resistant based on the definition of the Clinical and Laboratory Standard Institute.[17] Some laboratory stains of known sensitivity of *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Streptococcus pneumoniae* ATCC 49619 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.

RESULTS

The total 9178 urine samples collected from patients, 890 (9.70%) samples were positive and 8288 (90.30%) samples were negative at 2016 (January-December) in selected areas (Doyagonj, Gandaria, Jatrabari, Sayedabad, Dhaka, Bangladesh).

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

Age	<10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	81-90	>90
Male	35	1	12	10	20	27	57	27	3	0
Female	72	64	125	76	116	92	85	48	12	8
Total	107	65	137	86	136	119	142	75	15	8

Table-1 showed the distribution of patients by age and gender. Highest of the study subjects belonged to the 61-70 years age group (142 patients=85 female + 57 male) and followed by 21-30 years age group (137 patients=125 female + 12 male), 41-50 years age group (136 patients=116 female + 20 male) and 51-60 years age group (119 patients= 92 females + 27 males) respectively. In our study we saw that mostly female patients are affected by uropathogens in all the age groups in contrast male patients. Most prevalent frequency of female patients affected by uropathogens was found in 21-30 years age group.

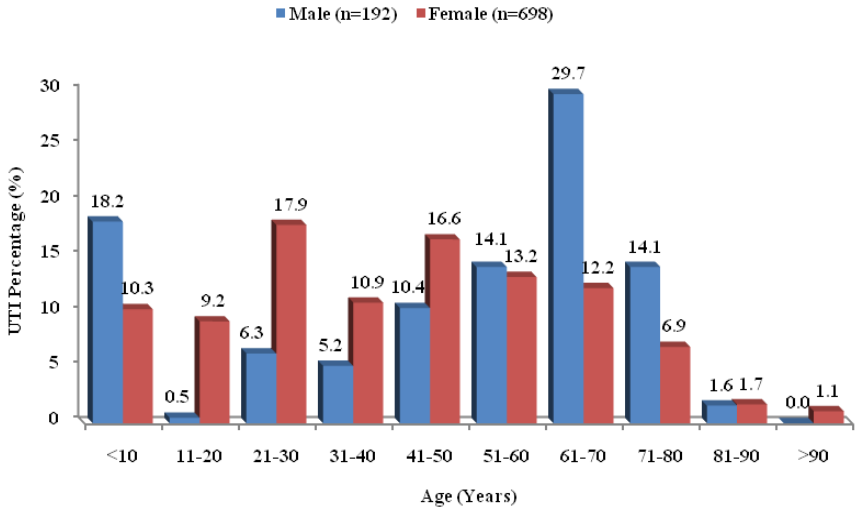

Fig.-1: UTI percentage among different age groups of male (N=192) and female (N=698).

In this study, percentage of male patients were more porne to female patients (18.20% > 10.30%) under 10 years age groups. In between 11-20, 21-30, 31-40 and 41-50 years of age group female UTI infection (9.2%, 17.9%, 10.9% and 16.6% respectively) is higher than male (0.50%, 6.3%, 5.2% and 10.4% respectively). In between 51-60, 61-70 and 71-80 years age male infection (14.1%, 29.7% and 14.1% respectively) is higher than female (13.2%, 12.2% and 6.9% respectively). In between 81-90 and above 90 years age female infection (1.7% and 1.1% respectively) is higher than male (1.6% and 0.0% respectively) but here number of patients were very few.

Table-2: Distribution of specific uropathogen mediated UTI among UTI patients (n=890).

Organisms	Male	Female	Total
E. coli	153(17.2%)	594(66.7%)	747(83.9%)
Klebsiella spp.	16(1.8%)	47(5.3%)	63(7.1%)
Staphylococcus aureus	4(0.4%)	24(2.7%)	28(3.1%)
Enterococci spp.	7(0.8%)	16(1.8%)	23(2.6%)
Pseudomonas spp.	9(1.0%)	11(1.2%)	20(2.2%)
Proteus spp.	3(0.3%)	4(0.4%)	7(0.8%)
Staphylococcus saprophyticus	0(0.0%)	2(0.2%)	2(0.2%)
Total	192(21.6%)	698(78.4%)	890(100.0%)

Table 2 showed that the most predominant organism *E.coli* 747(male 153 and female 594) found in UTI patients. As per number distribution, the second prevalent organism was *Klebsiella* spp. 63 (male 16 and female 47). Other isolated organism found as follows *Staphylococcus aureus* 28 (male 4 and female 24), *Enterococci* spp. 23 (male 7 and female 16), *Pseudomonas* spp. 20 (male 9 and female 11), *Proteus* spp. 7 (male 3 and female 4) and *Staphylococcus saprophyticus* 2 (female 2). On the other hand the study showed that the gram positive organism 5.9% and gram negative organism 94.1%. 192 samples (21.6%) were obtained from male subjects while the remaining 698 (78.4%) were from female.

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

Organisms	Male (n=192)		Female (n=698)	
	Number	Percentage	Number	Percentage
E. coli	153	79.7%	594	85.1%
Klebsiella spp.	16	8.3%	47	6.7%
Staphylococcus aureus	4	2.1%	24	3.4%
Enterococci spp.	7	3.6%	16	2.3%
Pseudomonas spp.	9	4.7%	11	1.6%
Proteus spp.	3	1.6%	4	0.6%
Staphylococcus saprophyticus	0	0.0%	2	0.3%
Total	192	100.0%	698	100.0%

In our study table-3 showed that the prevalence of the uropathogens among male and female UTI patients. Among the male and female patients the most prone uropathogen *E.coli* was found 79.7% and 85.1% followed by *Klebsiella* spp. 8.3% and 6.7%, *Staphylococcusaureus* 2.1% and 3.4%, *Enterococci* spp. 3.6% and 2.3%, *Pseudomonas* spp. 4.7% and 1.6%, *Proteus* spp. 1.6% and 0.6% and *Staphylococcus saprophyticus* 0.0% and 0.3% respectively.

Table-4: Susceptibility pattern of Nalidixic Acid against uropathogens among male UTI patients.

Name of organisms	Sensitive		Resistant	
	Number	Percentage	Number	Percentage
E. coli	19	12.4%	134	87.6%
Klebsiella spp.	5	31.3%	11	68.8%
Staphylococcus aureus	0	0.0%	4	100.0%
Enterococci spp.	1	14.3%	6	85.7%
Pseudomonas spp.	0	0.0%	9	100.0%
Proteus spp.	0	0.0%	3	100.0%
Total	25	13.0%	167	87.0%

Table-4 showed that Nalidixic Acid were sensitive against isolated uropathogenic bacteria in male 13.0% and rest of resistant 87.0%. The most sensitive organism to Nalidixic Acid was *Klebsiella* spp. (31.3%) found but here numbers were very few. On the other hand the most prevalent resistant organisms (100%) were *Staphylococcus aureus*, *Pseudomonas* spp. and *Proteus* spp found but here the number were very few. In contrast of frequency, *E.coli* was the most significant organism which was 12.4% sensitive and 87.9% resistant to Nalidixic Acid. The other sensitivity patterns were *Enterococci* spp. 14.3%, sensitive and resistant *Klebsiella* spp.(68.8%) and *Enterococci* spp.(85.7%).

Table-5: Susceptibility pattern of Nalidixic Acid against uropathogens among female UTI patients.

Name of organisms	Sensitive		Resistant	
	Number	Percentage	Number	Percentage
E. coli	138	23.2%	456	76.8%
Klebsiella spp.	19	40.4%	28	59.6%
Staphylococcus aureus	3	12.5%	21	87.5%
Enterococci spp.	1	6.3%	15	93.8%
Pseudomonas spp.	0	0.0%	11	100.0%
Proteus spp.	0	0.0%	4	100.0%
Staphylococcus saprophyticus	0	0.0%	2	100.0%
Total	161	23.1%	537	76.9%

Table-5 showed that Nalidixic Acid were sensitive against isolated uropathogenic bacteria in female 23.1% and rest of resistant 76.9%. All of them (100%) *Pseudomonas* spp., *Proteus* spp. and *Staphylococcus saprophyticus* were resistant to Nalidixic Acid but here numbers were very few. On the other hand the most prevalent sensitive organism was *Klebsiella* spp. (40.4%). In contrast *E. coli* was the most significant organism which was 23.2% sensitive and 76.8% resistant to Nalidixic Acid. The other sensitivity patterns were found *Staphylococcus aureus* 12.5% and *Enterococci* spp. (6.3%) sensitive and resistant *Klebsiella* spp. (59.6%), *Staphylococcus aureus* (87.5%) and *Enterococci* spp. (93.8%).

DISCUSSION

Due to overuse, underuse and misuse of antibiotics, followed by an increase in the bacterial resistance rates, this study aimed to evaluate the pattern of antimicrobial susceptibility of bacteria isolated from patients with UTI seen at the IBN SINA diagnostic center, Doyagonj, Dhaka, Bangladesh. Moreover, we identified the major bacterial species associated with UTI and described the susceptibility profile to Nalidixic Acid.

In this study, we tested total 9178 urine samples and 890 (9.7%) were bacteriological positive and 8288 (90.3%) were bacteriological negative found. In table-1 showed the distribution of patients by age and gender. Highest of the study subjects belonged to the 61-70 years age group (142 patients=85 female + 57 male) and followed by 21-30 years age group (137 patients=125 female + 12 male), 41-50 years age group (136 patients=116 female + 20 male) and 51-60 years age group (119 patients= 92 females + 27 males) respectively. In our study we saw that mostly female patients are affected by uropathogens in all the age groups in contrast male patients. Most prevalent frequency of female patients affected by uropathogens was found in 61-70 years age group. There is a significant difference between gender and age group at 5% ($P<0.05$).

In addition figure-1 also demonstrated the UTI percentage among different age groups of male (N=192)

and female (N=698). The percentage of male patients were more porne to female patients (18.20% > 10.30%) under 10 years age groups. In between 11-20, 21-30, 31-40 and 41-50 years of age group female UTI infection (9.2%, 17.9%, 10.9% and 16.6% respectively) is higher than male (0.5%, 6.3%, 5.2% and 10.4% respectively). In between 51-60, 61-70 and 71-80 years age male infection (14.1%, 29.7% and 14.1% respectively) is higher than female (13.2%, 12.2% and 6.9% respectively). In between 81-90 and above 90 years age female infection (1.7% and 1.1% respectively) is higher than male (1.6% and 0.0% respectively) but here number of patients were very few. Females were more suffered with UTI and it caused by *E.coli* (83.9%). 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. Furthermore, another mechanism that could explain the lower frequency of UTI in men would be the prostatic fluid, which has antibacterial substances.[3] There is a significant difference between gender and age group at 5% (P<0.05).

In Table 2 showed that the most predominant organism *E. coli* 747 (male 153 and female 594) found in UTI patients. As per number distribution, the second prevalent organism was *Klebsiella* spp. 63 (male 16 and female 47). Other isolated organism found as follows *Staphylococcus aureus* 28 (male 4 and female 24), *Enterococci* spp. 23 (male 7 and female 16), *Pseudomonas* spp. 20 (male 9 and female 11), *Proteus* spp. 7 (male 3 and female 4) and *Staphylococcus saprophyticus* 2 (female 2). On the other hand the study showed that the gram positive organism 5.9% and gram negative organism 94.1%. 192 samples (21.6%) were obtained from male subjects while the remaining 698 (78.4%) were from female. In contrast of frequency, female UTI patients were higher than male in all the causeting agent of UTI. There is no significant difference between gender and causeting agent of UTI at 5% (P>0.05).

In our study table-3 showed that the prevalence of the uropathogens among male and female UTI patients. Among the male and female patients the most prone uropathogens *E. coli* was found 79.7% and 85.1% followed by *Klebsiella* spp. 8.3% and 6.7%, *Staphylococcus aureus* 2.1% and 3.4%, *Enterococci* spp. 3.6% and 2.3%, *Pseudomonas* spp. 4.7% and 1.6%, *Proteus* spp. 1.6% and 0.6% and *Staphylococcus saprophyticus* 0.0% and 0.3% respectively. The study noted that male patients were more infected by all of the organism (*Klebsiella* spp., *Pseudomonas* spp, *Enterococci* spp. and *Proteus* spp.) except some organisms (*E. coli, Staphylococcus aureus* and *Staphylococcus saprophyticus)* which were more infected female patients than male patients. Several studies have shown that *Escherichia coli* is the major bacterial species associated with UTIs and *Klebsiella pneumoniae* is the second most important bacteria in this type of infection.[18] There is no significant difference between gender and causeting agent of UTI at 5% (P>0.05).

Moreover, the early introduction of effective drugs against bacterial infections in the last century has changed the medical behavior and has 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.[19] Table-4 showed that Nalidixic Acid was sensitive against isolated uropathogenic bacteria in male 13.0% and rest of resistant 87.0%. The most sensitive organism to Nalidixic Acid was *Klebsiella* spp. (31.3%) found but here numbers were very few. On the other hand the most prevalent resistant organisms (100%) were *Staphylococcus aureus*, *Pseudomonas* spp. and *Proteus* spp found but here the number were very few. In contrast of frequency, *E. coli* was the most significant organism which was 12.4% sensitive and 87.9% resistant to Nalidixic Acid. The other sensitivity patterns were *Enterococci* spp. 14.3%, sensitive and resistant *Klebsiella* spp. (68.8%) and *Enterococci* spp.(85.7%). There is no significant difference among gender, causeting agent and susceptibility of Nalidixic Acid at 5% (P>0.05).

However, Table-5 showed that Nalidixic Acid was sensitive against isolated uropathogenic bacteria in female 23.1% and rest of resistant 76.9%. All of them (100%) *Pseudomonas* spp., *Proteus* spp. and *Staphylococcus saprophyticus* were resistant to Nalidixic Acid but here numbers were very few. On the other hand the most prevalent sensitive organism was *Klebsiella* spp. (40.4%). In contrast *E. coli* was the most significant organism which was 23.2% sensitive and 76.8% resistant to Nalidixic Acid. The other sensitivity patterns were found *Staphylococcus aureus* 12.5% and *Enterococci* spp. (6.3%) sensitive and resistant *Klebsiella* spp. (59.6%), *Staphylococcus aureus* (87.5%) and *Enterococci* spp. (93.8%) There is a significant difference among gender, causeting agent and susceptibility of Nalidixic Acid at 5% (P<0.05). 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. It had observed that horizontal gene transfer is a factor in the emergence and spread of antimicrobial resistance in clinical isolates. 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.[20] 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.[21]

CONCLUSION

In a nutshell, the results showed that there was a high prevalence of occurrence of urinary tract infection among patients of areas (Doyagonj, Gandaria, Jatrabari, Sayedabad, Dhaka, Bangladesh). Most of the bacteria were resistant to Nalidixic Acid which is very alarming news for human being. Awareness is needed of both the population and health professionals about the importance for the correct use of antibiotics. The Nalidixic Acid 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 policy.

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