ORIGINAL ARTICLE

OCCURRENCE OF URINARY TRACT INFECTION IN ADOLESCENT AND ADULT WOMEN OF SHANTY TOWN IN DHAKA CITY, BANGLADESH

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ABSTRACT

BACKGROUND: *Urinary tract infection (UTI) is commonly experienced by women of various age groups especially elderly ones. We planned to find out the prevalent microbial strains causing UTI in slum inhabitant adolescent and adult women in Dhaka City, Bangladesh.*

METHODS AND MATERIALS: *Urine sample was collected from 462 UTI suspected female subjects. Pathogenic bacteria were identified using standard microbiological tests, and antimicrobial sensitivity profiles of the pathogens were determined.*

RESULTS: *Bacteriuria was present in 9% of the subjects. A higher incidence (16.8%) of UTI was noted among adult women aged above 19 years. Escherichia coli (69%), Streptococcus spp. (15%) and Pseudomonas aeruginosa (7%) were more frequently isolated from the urine samples compared to Enterococcus faecalis (3%), Staphylococcus aureus (2%), Klebsiella pneumoniae (2%) and Hafnia alvei (2%). The E. coli isolates showed complete resistance to commonly used drugs, and 58% of these isolates were multidrug resistant (MDR). Minimum Inhibitory Concentration (MIC) values for ciprofloxacin ranged between 64µg/ml and 512µg/ml, and the Minimum Bactericidal Concentration (MBC) values against the isolates were 128µg/ml or above. Isolated strains of E. coli exhibited equal extent of ciprofloxacin resistance irrespective of the presence or absence of plasmid in them.*

CONCLUSION: *The extent of drug resistance among the uropathogens if ignored may render them uncontrollable. This study suggests regular monitoring of drug resistance phenotype of the UTI pathogens to reduce the morbidity of female UTI patients and offer better treatment strategy in the healthcare sectors of Bangladesh.*

KEYWORDS: *Urinary tract infection (UTI), Multidrug resistance (MDR), Adolescent women*

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INTRODUCTION

Acute UTI occurs in many women each year, and the annual costs of caring for those women are invariably very high in Bangladesh. Approximately, 60% of all women experience at least one UTI within their lifetime and roughly 20-30% women suffer from repeated infections (1, 2). Sexual activities of women have been considered as important risk factors for UTI infections and recurrences (3), and with as frequently early marriage occurs in the slum women in Bangladesh, UTI may be promoted. In post-menopausal women, UTI risk factors may also comprise urinary incontinence (4). Bacterial virulence properties may affect the risk of recurrence of infection as well (5).

UTI is commonly caused by *Escherichia coli, Proteus, Klebsiella, Enterococcus, and Enterobacter* spp. (6). However, *Pseudomonas,*

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Staphylococcus aureus, Group B *Streptococcus are* usually reported with increased rates in patients with urological disorders and following repetitive courses with antibiotic treatments (7). Uropathogenic *E. coli* utilize a number of virulence factors to adherence the uroepithelial cells; however, the strains fit to a limited number of serogroups mainly to O1, O2, O4, O6, O7, O14, O15, O18, O22 and O75 (8, 9). Anti-microbial resistance among uropathogenic *E. coli* may be increased with temporal and geographic fluctuations which may introduce multidrug resistant *E. coli* into the community (10). In recent years, an increased number of Extended-Spectrum-Beta-Lactamases (ESBL) producing pathogens have been observed in outpatient settings, especially related to urinary tract infections (UTI), narrowing the treatment option with antibiotics (11).

In addition to age, sex, marital and social status, other risk factors include nature and type of strains associated with UTI. For example, Shiga toxin-producing *E. coli* may contribute to hemolytic uremic syndrome, elevated white blood cell count and C-reactive protein levels in infected patients (12). Little is known about the pathogenesis, natural history, risk factors, and epidemiology of the multi-drug resistant UTI pathogens of slum adolescent and adult women origin in Bangladesh. Therefore, we designed our study to determine the prevalence, drug resistance phenotype and plasmid profile of the uropathogens.

MATERIALS AND METHODS

Study population and sample*:* The study included UTI suspected female subjects living in slums located around the Dhaka City, Bangladesh. Urine sample was collected from February 2011 to December 2011. Individuals were requested to fill out a questionnaire regarding their consent, morbidity and recent history of medication. Subjects receiving antimicrobial treatment for existing complications were excluded from this study. One midstream-urine sample per female subject was collected and examined by standard quantitative culture methods (13). Positive culture was defined as the culture of a single microorganism at a concentration of $>10^5$ colony-forming units (CFU)/ml (14). In cases of delay in processing, the samples were stored at 4^oC.

Identification of the uropathogens: Nutrient agar plates were used for total bacterial count of the urine samples and uropathogens were isolated on Blood agar and MacConkey agar media. All the plates were incubated aerobically at 37^oC for 24-48 hours and the colonies were enumerated. For confirmation of specific bacterial spp., standard biochemical tests were performed.

Serogrouping: The uropathogenic *E. coli* isolates were serotyped using different monovalent O-antisera (Eurobio, France) which have association with human infection. Strains that did not display agglutination with any of the 13 used O-antisera were defined as O non-typed (5, 8).

Antimicrobial susceptibility testing: Antimicrobial susceptibility testing of the isolated bacterial spp. was performed by disc diffusion method following the National Committee for Clinical Laboratory Standards (NCCLS) guidelines (15). Standard strains of *Escherichia coli* ATCC 25922, *Enterococcus faecalis* 29212, *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 25923 were used for quality control. For this study, we presented susceptibility data for ampicillin (10μg), azithromycin (15μg), doxycycline (30μg), nalidixic acid (30μg), amoxicillin (10μg), tetracycline (30μg), cephalexin (30μg), ciprofloxacin (5μg), levofloxacin (5μg), imipenem (10μg), meropenem (10μg), amikacin (30μg), nitrofurantoin (300μg), netilmicine (30μg), gentamicin (10μg) and ceftriaxone (30μg).

Defining multidrug resistant (MDR) strains: MDR in *Enterobacteriaceae* is defined as resistance to at least one drug from three or more of the following antimicrobial categories: aminoglycosides (e.g., gentamicin, tobramycin, amikacin or netilmicin), carbapenems (imipenem, meropenem, ertapenem or doripenem), fluoroquinolones (e.g., ciprofloxacin), penicillins (e.g., ampicillin) and tetracyclines (e.g., tetracycline, doxycycline or minocycline) etc. (16).

Minimum inhibitory concentration (MIC): MIC is the lowest concentration of antimicrobial drug that inhibits visible growth of the test organism as outlined by the NCCLS (15). Minimum inhibitory concentrations (MICs) of the

selected ciprofloxacin resistant isolates were determined in Mueller-Hinton broth by macro-broth dilution technique (17). The lowest concentration of antibiotic that killed 100% of the test organism on solid media was taken as minimum bactericidal concentration (MBC) for the bacterium.

Plasmid profiling: Alkaline lysis method was followed to extract plasmid DNA from the isolated pathogens (18) and the molecular weight of the unkwon plasmid was determined by agarose (0.8%) gel electrophoresis method comparing the pattern of migration with that of known plasmid markers.

Plasmid curing and plasmid profile analysis: Plasmid curing for the selected isolates was performed according to Tomeda, *et al.* (1968) (19), and then the isolates were transferred onto 30µg of ciprofloxacin containing plates. After overnight incubation, colonies which appeared on ciprofloxacin plates were assumed as strains bearing drug resistance genes in their chromosome. To confirm the absence of plasmid in the cured isolates, plasmid DNA extraction procedure was performed by the technique mentioned in the previous section.

RESULTS

A total of 462 female subjects suspected of having urinary tract infection were included in this study; of them 42 (9%) had significant bacteriuria. Among the 42 positive cases, 29 were adult women (aged >19 years), 11 were adolescents (aged between 10-19 years) and the remaining two were children (aged <10 years). UTI prevalence in adult subjects (n=173) was 16.8%, but 6.6% in adolescents (n=167). Therefore, UTI prevalence between adult and adolescent women was significantly different (p=0.001). A complex microbial population as shown in figure 1 was identified from the urine sample. *Escherichia coli* (69%), *Streptococcus* spp. (15%) and *Pseudomonas aeruginosa* (7%) were most common, while *Enterococcus faecalis* (3%), *Staphylococcus aureus* (2%), *Klebsiella pneumoniae* (2%) and *Hafnia alvei* (2%) were less frequent in the urine samples.

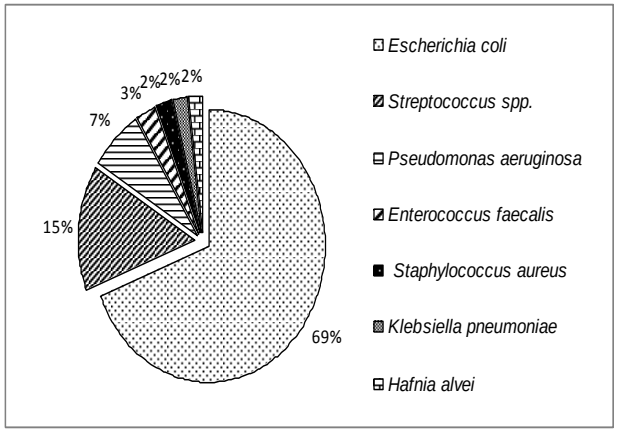

Figure 1: *Distribution of uropathogens (n=42) among the slum women in Dhaka city*, Bangladesh

All the 29 *E. coli* isolates were grouped with O-antisera where six strains (20.69%) were non-typable. Serogroup O6 was most frequently identified (24.14%) that was followed by serogroup O20 (13.79%) and O18 (10.34%) (Table 1). In total, these three serogroups (O6, O20 and O18) consisted of 48.28% of all *E. coli* isolates.

Table 1: Frequency of *E. coli* serogroups in UTI patients living in the slum of Dhaka city, Bangladesh

Serogroup	Number	Percent
O1	2	6.90
O6	7	24.14
O8	2	6.90
O15	2	6.90
O18	3	10.34
O20	4	13.79
O25	1	3.45
O55	1	3.45
O78	0	0.00
O86	0	0.00
O111	0	0.00
O119	0	0.00
O125	1	3.45
Non-typed	6	20.69
Total	29	100.00

The *E. coli* isolates of this study, as represented in figure 2, were 100% resistant to ampicillin, azithromycin, doxycycline, 90% to nalidixic acid and amoxicillin, 70% to levofloxacin, 68% to ciprofloxacin and 65% to tetracycline and cephalexin. Conversely, lower resistance was recorded for imipenem (20%), meropenem (15%), amikacin (12%), and nitrofurantoin (10%). The *E. coli* isolates were completely (100%) sensitive to netilmycine, gentamicin and ceftriaxone.

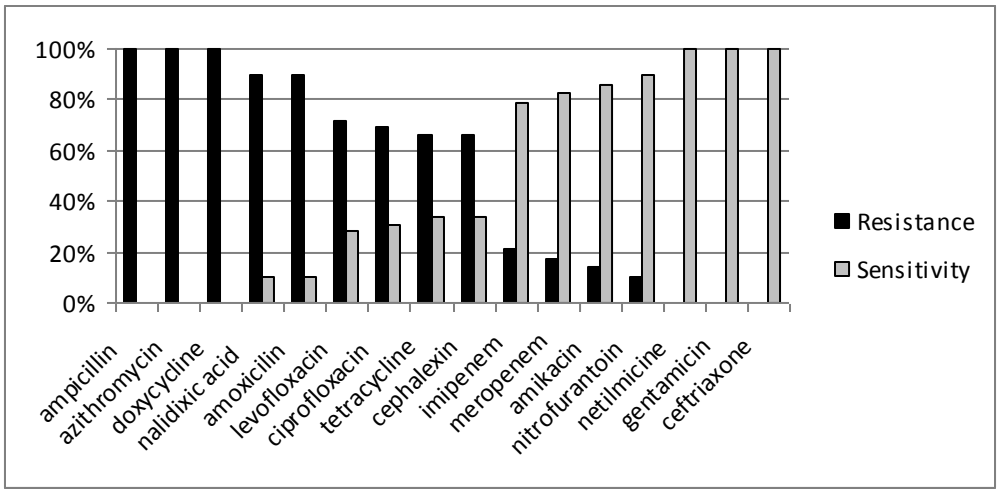

Figure 2: *Antibiotic resistance/sensitivity pattern of the Escherichia coli isolates (n = 29)*

Most of the isolated *E. coli* was found to be resistant to more than four test drugs (Table 2). Among the *E. coli* isolates, 17 (58%) were Multidrug Resistant, exhibiting their insensitiveness against ampicillin, doxycycline and ciprofloxacin, each belonging to different antibiotic groups. However, 11 (37.93%) of the *E. coli* isolates were resistant to five while two (6.89%) of them were resistant to seven different groups of antibiotic.

Table 2: Drug resistant phenotypes of selected E. coli in UTI patients living in the slum of Dhaka city, Bangladesh

Percentage (%) of resistant isolates	Resistant phenotype†
100%	AMP + DO
58.62%	AMP + DO + CIP
37.93%	AMP + DO + CIP + TET + CL
6.89%	AMP + DO + CIP + TET + CL + IM + AMK

†AMP=Ampicillin;DO=Doxycycline; CIP=Ciprofloxacin; TET= Tetracycline; CL= Cephalexin; IM= Imipenem; AMK= Amikacin

Among the multidrug resistant isolates, ten highly resistant strains were chosen for Minimum Inhibitory Concentration (MIC) determination against ciprofloxacin (Table 3). MIC values were lower with the range of 64-128 µg/ml. The Minimum Bactericidal Concentration (MBC) values against the isolates were 128 µg/ml or more.

Table 3: MIC and MBC values for ciprofloxacin resistant *E. coli* in UTI patients living in the slum of Dhaka city, Bangladesh

Isolate number	MIC (µg/ml)	MBC (µg/ml)
3	64	256
5	128	256
8	128	256
9	64	>128
11	128	256
16	64	128
18	128	256
21	64	128
23	64	>128
27	128	256

Plasmid profile of highly ciprofloxacin resistant *E. coli* isolates showed plasmids with variable size ranging from 140 to < 2 MDa (Figure 3), among them, four isolates were found to pose large plasmid of 140 MDa size.

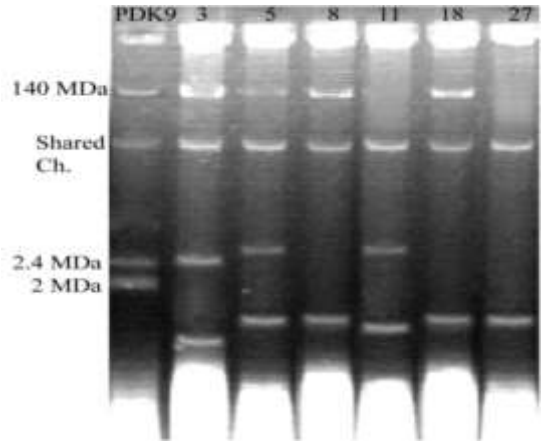

Figure 3: *Electrophoretic patterns of plasmid DNA from uropathogenic isolates of E. coli electrophoresed in 0.8% agarose gel; first lane showing Marker plasmid pDK9 and rests indicating isolate number*.

Plasmid was cured from the six selected isolates (isolate no. 3, 5, 8, 11, 18 and 27) and was grown on nutrient agar. These isolates showed no bands on agarose gel when plasmid DNA extraction procedure was performed. It emphasizes that the resistance of genes might be carried by the chromosomal DNA of the isolates.

DISCUSSION

In Bangladesh, slums are population-dense residences with poor facilities of the basic requirements of life. Slum inhabitants reside in a highly polluted environment comprising of numerous pathogenic microorganisms. Thus, the lives of slum populations are always at risk of getting infected with various pathogenic microbes. Urinary tract infections (UTIs) are common in women, often associated with significant morbidity and mortality (20), and may affect women of all age groups especially sexually active ones (21). We studied 462 urine samples and found UTI in about 9% cases where a higher incidence (16.8%) of UTI was noticed for adult women aged over 19 years. The frequency is close to the incidence reported by Ahmed and Avasarala (2008), i.e. 12.7% (22), but is higher than the study of Singh MM *et al.* (2001) (23) who reported 4.2% UTI in a community based study. In Bangladesh, Begum *et al.* (2006) (24) reported 16.4% UTI in the female garments workers of Dhaka City. Patients in Bangladesh usually see a doctor after experiencing severe health complications for a particular disease condition. Therefore, Bashar *et al.* (2009) and Rahman *et al.* (2009) reported higher frequency of UTI i.e., 27% and 24.14% respectively in hospital or clinic based study (25, 26).

This study reports association of *Escherichia coli* (69%), *Streptococcus* spp. (15%), *Pseudomonas aeruginosa* (7%), *Enterococcus faecalis* (3%), *Staphylococcus aureus* (2%), *Klebsiella pneumoniae* (2%) and *Hafnia alvei* (2%) in UTI. Other investigators (Basar *et al.* 2009, Saber *et al.* 2010, and Jan *et al.(*2009) also reported higher association of *E. coli* (66.67%, 77.8% and 52.65% cases respectively) in UTI patients (25, 27-28). A major part of O-groupable *E. coli* strains mainly O1, O2, O4, O6, O7, O18 and O75 have been reported to account for UTI in different parts of the world (29). The uropathogenic *E. coli* isolates of this study mainly belong to one of 3 serogroups O6, O15 and O18. Strains belonging to these serogroups have been reported to pose specific virulence factors for their invasive ability (29). The finding of this study nearly supports this theory.

Antibiotic resistance among uropathogens has become a public health concern in Bangladesh (30). Under individual predisposing conditions, *E. coli* can multiply rapidly in the urinary tract of an UTI patient. However, if the patient doesn't maintain antibiotic dose regimen properly, the organism may emerge as drug resistant variant and eventually result in both community-and nosocomially acquired UTIs (31, 32). The present study found the *E. coli* isolates to be completely resistant to the commonly used drugs such as penicillin group and also to a higher extent to broad spectrum quinolone group. A similar frequency among the UTI related *E. coli* isolates was recorded in some developing countries (33-34). However, a lower frequency for the same pathogen was reported from the isolates of developed countries (35). Antibiotic abuse and practicing incomplete antibiotic regimen has considerably

promoted the dissemination of multidrug resistant bacteria (36-37). This study reports lower resistance for less commonly used drugs like imipenem, meropenem, amikacin and nitrofurantoin, and complete sensitivity to netilmycine, gentamicin and ceftriaxone among the *E. coli* isolates. This finding is supported by the study of Sharmin *et al.* (2009) which reported a good sensitivity for imipenem, ceftazidime and amikacin against UTI-isolates of *E. coli* in Bangladesh (38). Encarnacion AR (2012) also reported sensitivity of the UTI-isolates of *E. coli* for amikacin in Philippines (39). This finding suggests the use of drugs that are less commonly prescribed by practitioners for arresting the pathogens in UTI patients may be beneficial.

Multidrug resistance among the pathogenic microbes is a common problem which may be either plasmid-borne or chromosomal or sometimes both (40, 41). At least 17 of all the *Escherichia coli* isolates examined in this study exhibited multidrug resistance. Such multidrug resistance complicates empiric treatment of *E. coli* infections and may contribute plasmid mediated multidrug resistance transfer (42). The present study demonstrated chromosome-borne resistance for ciprofloxacin since the *E. coli* isolates were able to colonize on the ciprofloxacin containing plates even after curing off their plasmids. A similar mode of resistance has been studied in detail by Lindgren *et al.* (2003) (43). On the other hand, Paiva *et al.* (2012) (44) studied ciprofloxacin resistance gene in plasmid DNA of uropathogenic *E. coli.* The evolution of fluoroquinolone resistance has been found to be involved in the accumulation of multiple mutations in several genes, and it is ultimately reflected in the phenotype of the resistant organism. Therefore, the use of ciprofloxacin to treat patients should be restricted. Healthcare practitioners must think critically before prescribing the commonly used drugs to a patient. They should suggest a drug only after having the antibiogram of the associated pathogens during a disease.

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