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Evaluation of antimicrobial resistance pattern of uropathogens in a tertiary care hospital in Dhaka city, Bangladesh

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Abstract

Urinary tract infection (UTI) is one of the most common bacterial infections seen in developing countries like Bangladesh. This study is, therefore, designed to determine the bacterial uropathogens and their antibiotic resistance pattern among patients with complaints of UTIs in Dhaka city. This study was carried out in the laboratory of the Department of Microbiology, Bangladesh Medical College, Dhaka, Bangladesh from January to June 2015. A total of 2541 urine samples were collected in sterile containers from suspected urinary tract infected cases. A specimen was considered positive for UTI if an organism was cultured at a concentration of $\geq 10^5$CFU/ml or when an organism was cultured at a concentration of 10^4CFU/ml and >5 pus cells per high power field. Antimicrobial susceptibility testing of the isolated bacterial species was performed by disc diffusion method following the National Committee for Clinical laboratory Standards (NCCLS) guidelines. A total of 303 (11.92%) bacterial uropathogens were isolated from 2541 urine samples. Among the 303 isolates, majority of the isolates 197 (65.02%) were from females. Both the age groups up to 18 years and above 18 years the highest prevalence was found in females 34(11.22%) and 163 (53.79%) respectively. Most predominant organism was *Escherichia coli* 262(86.46%)followed by *Pseudomonas* 12(3.96%), *Enterococci* 12(3.96%), *Klebsiella*11(3.63%). *Escherichiacoli* showed very high resistance to amoxycillin 95.41%, cefradin 90.45%, nalidixic acid and *Klebsiella* to amoxycillin 90.90%, nitrofurantoin 90.90%. Again *Pseudomonas* was highly resistant to cefuroxime 100%, cefexime 100%and ceftriaxone 83.33%. *Enterococci* were found highly resistance to cefexime 91.66%, cloxacillin 83.33%, and erythromycin 83.33%.Due to wide scale resistance of the drugs used to treat UTI, choice of drugs in the treatment of UTI is quite narrow. In country like ours awareness for prevention of UTI should be encouraged among the community level as it affects all age groups.

Keywords: Urinary tract infection, Uropathogens, Antibiogram, Antimicrobial resistance, Bangladesh.

Introduction

Urinary tract infection (UTI) is one of the most common bacterial infections seen in clinical practice particularly in developing countries with a high rate of morbidity and financial cost.[1] It is the second most common infections in community practice with approximately 150 million diagnosed cases each year.[2]

UTI affect patients in all age groups and both sexes.[3] Neonates, girls, young women and older men are most susceptible to UTIs. In women, bacterial cystitis is the most common bacterial infection. Every woman has a 60% lifetime risk of developing bacterial cystitis, which develops mostly before the age of 24.[4]The most common pathogenic organism of UTI is *Escherichia coli*, which is responsible for more than 80% cases.[5,6] Other pathogenic organisms of UTI are *Staphylococcus saprophyticus*, *Staphylococcus aureus*, *Proteus* sp., *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Enterococci*.[7-9]

The resistance pattern of uropathogens is changing drastically, specifically in developing countries, such as Bangladesh because of uncontrolled and widespread use of antibiotics. Antibiotics are usually given empirically before the laboratory results of urine culture are available. To ensure appropriate therapy, current knowledge of the organisms that cause UTI and their antibiotic susceptibility is mandatory.[10] Due to rising antibiotic resistance among uropathogens, it is important to have local hospital based knowledge of the organisms causing UTI and their antibiotic sensitivity patterns. The spectrums of etiologic agents causing UTIs and their antimicrobial resistance pattern have been continuously changing over the years, both in community and in hospitals.[11]

Increasing multidrug resistance in bacterial

Practice Points

- Urinary tract infection (UTI) is one of the most common bacterial infections in Bangladesh. Highest prevalence of UTI was found among the females having age 18 years and above.
- Among the pathogens *Escherichia coli* was found to be most common uropathogen.
- Culture sensitivity & antibiogram is crucial for identification of pathogen and to institute proper treatment.
- Due to inappropriate use of antibiotics and lack of awareness among the patients are considered to be increased antimicrobial resistance.
- Antibiotic policy should be formulated by the government and should be strictly implemented by the physicians.

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uropathogens is an important and evolving public health challenge.[1,12] The prevalence of antimicrobial resistance in urinary pathogens is increasing worldwide.[13]Accurate bacteriological records of culture results provide guidance on empirical therapy before sensitivity patterns are available.[13,14] Since most UTIs are treated empirically, the criteria for the selection of antimicrobial agents should be determined on the basis of the most likely pathogens and its expected resistance pattern in a geographic area.[1,15] Thus there is a need for periodic monitoring of causative agents of UTI and their resistance pattern in a given locality.[1]

This study is, therefore, designed to determine the bacterial uropathogens and their antibiotic sensitivity patterns among patients with complaints of UTIs in Dhaka city, Bangladesh.

Materials and methods

This cross-sectional study was carried out in the laboratory of the Department of Microbiology, Bangladesh Medical College, Dhaka, Bangladesh. The duration of the study was 6 months (January to June 2015). Patients admitted in inpatient department and visited the outpatient department of Bangladesh Medical College and Hospital Dhaka with suspected UTI cases was included in this study. Written consent was taken from the concerned authority.

Clean catch midstream and/or catheter catch urine sample was collected into a sterile container/test tube aseptically. The samples were processed according to a previously described methodology.[16]A sterile platinum wired calibrated loop was used which delivered 0.001 ml of urine. A loopful urine sample was plated on Cystine-lactose-Electrolyte Deficient (CLED) agar media (Hi Media Laboratories, India). The inoculated plates were incubated at 37^0C for 24 hours and extended to 48 hours in culture negative cases. The plates were then examined macroscopically for bacterial growth. A specimen was considered positive for UTI if an organism was cultured at a concentration of $\geq 10^5$CFU/ml or when an organism was cultured at a concentration of 10^4CFU/ml and >5 pus cells per high power field were observed on microscopic examination of the urine.[17-19]

Identification of bacterial pathogens was made on the basis of Gram reactions, morphology, motility test, biochemical and cultural characteristics.[20] Antimicrobial susceptibility testing of the isolated bacterial species was performed by disc diffusion method following the National Committee for Clinical laboratory Standards (NCCLS) guidelines.[21]

All discs were obtained from Oxoid Ltd. Antibiotics used for uropathogens were amoxycillin (10µg), ciprofloxacin (5µg), gentamicin (10µg), cefradine (30µg), cefuroxime (30µg), cephalexin (30µg), cefexime (5µg), ceftriaxone (30µg), ceftazidime (30µg), cefepime (30µg), cotrimoxazole (25µg), micellinum (25µg), imipenem (10µg), meropenem (10µg), amikacin (30µg), nitrofurantoin (300µg), netilmicin (30µg), nalidixic acid (30µg), levofloxacin (5µg), piperacillin/tazobactam (110µg), carbenicillin (100µg), aztreonam (30µg), colistin (10µg), cloxacillin (5µg), erythromycin (15µg), methicillin (5µg), vancomycin (30µg), linezolid (30µg).

Results

Table 1 showed the age and sex distribution of the isolated pathogens. Among the 303 isolates, majority of the isolates 197 (65.02%) were from females while the remaining were from male 106 (34.98%). In both the age groups up to 18 years and above 18 years the highest prevalence was found in females 34 (11.22%) and 163 (53.79%) respectively.

Out of 2541 urine samples 303 (11.92%) were bacterial isolates. Among them most predominant organism was *Escherichia coli* 262 (86.46%) followed by *Pseudomonas* 12 (3.96%), *Enterococci* 12 (3.96%), *Klebsiella* 11 (3.63%), *Enterobacter* 4 (1.32%), *Proteus* 1 (0.33%) and *Staphylococcus aureus* 1 (0.33%) as shown in Table 2.

Antimicrobial resistance pattern of uropathogens (Gram negative rods) had been shown in Table 3. *Escherichia coli* showed very high frequency of resistance to amoxycillin 95.41%, cefradine 90.45%, nalidixic acid 88.16%, moderately high resistance to cefexime 61.83%, cefuroxime 53.81%, ciprofloxacin 52.29%, cotrimoxazole 51.14%, levofloxacin 50%, ceftriaxone 44.65%, cefepime 41.98% and low resistance to amikacin 7.63%; imipenem 0.38%; meropenem 1.14%. *Klebsiella* was 90.90% resistant to amoxycillin as well as nitrofurantoin,63.63% to nalidixic acid, 54.854% to cotrimoxazole, 45.45% to micellinum. *Enterobacter* was 100% resistant to cefradine, 75% resistant to micellinum, nalidixic acid, ciprofloxacin, 50% resistant to amoxicillin, cefexime, ceftriaxone, cotrimoxazole, gentamicin, 25% resistant to cefuroxime, nitrofurantoin, levofloxacin and netilmicin. *Proteus* was 100% resistant to amoxicillin, nalidixic acid, micellinum, nitrofurantoin, cotrimoxazole, ciprofloxacin and levofloxacin.

Antimicrobial resistance pattern of *Pseudomonas* was shown in Table 4. *Pseudomonas* was 100% resistant to cefuroxime and cefexime, 83.33% to ceftriaxone, 75% to netilmicin, 66.66% to carbenicillin, 58.33% to aztreonam, 41.66% to ceftazidime; amikacin; levofloxacin, 50% to colistin, 33.33% to ciprofloxacin. Low resistance showed in piperacillin/tazobactam 25%, imipenem 25% and meropenem 25%.

Enterococci showed 91.66% resistance to cefexime, 83.33% to cloxacillin and erythromycin, 75% to ciprofloxacin and cotrimoxazole, 66.66% to cephalexin, 50% to cefradine; cefuroxime and methicillin, 25% to amoxicillin and vancomycin. *Staphylococcus aureus*

Table 1: Age and sex distribution of patients with positive UTI (n=303)

Age (in years)	**Sex**		**Total** (%)
	Female (%)	**Male (%)**	
Up to 18	34 (11.22%)	28 (9.24%)	62 (20.46%)
Above 18	163 (53.79%)	78 (25.74%)	241 (79.54%)
Total	197 (65.02%)	106 (34.98%)	303 (100%)

Table 2: Pattern of bacteria isolated from urine culture (n=303)

Isolated organisms	Number of isolates (%)
Escherichia coli	262 (86.46%)
Pseudomonas	12 (3.96%)
Enterococci	12 (3.96%)
Klebsiella	11 (3.63%)
Enterobacter	4 (1.32%)
Proteus	1 (0.33%)
Staphylococcus aureus	1 (0.33%)
Total	303

were 100% resistant to amoxycillin, cefradine, cefuroxime, cephalexin, cefexime, cloxacillin, erythromycin and methicillin (Table 5).

Discussion

Urinary tract infection (UTI) is commonly experienced by women of various age groups especially elderly ones. They are mostly treated empirically and the criteria for the selection of antimicrobial agents should be determined on the basis of the most likely pathogen and its expected resistance pattern in the locality.[1,15] Thus, there is a need for periodic monitoring of the causative agents of UTI and their resistance/ susceptibility pattern in a locality.

UTI is more common in females than in males at different age groups and these findings of our study (Table 1) correlates with a study done in Bangladesh by Haque *et al.*[22] In India, Prakash and Saxena found high prevalence of UTI in females (73.57%) than in males (35.14%) which also correlates with our study.[1] The reason behind this high prevalence of UTI in females is due to close proximity of the urethral meatus to the anus, shorter urethra, sexual intercourse, incontinence and bad toilet.[23-25]

In this study, a total of 303 (11.92%) bacterial uropathogens were isolated from 2541 urine samples. In our country 42.66% bacterial growth was isolated in other study and in India 53.81% bacterial growth was isolated which were dissimilar with our study.[22,1] *Escherichia coli* was found to be the predominant isolates 86.46% causing UTI, followed by *Pseudomonas* 3.96%, *Enterococci* 3.96%, *Klebsiella* 3.63%, *Enterobacter* 1.32%, *Proteus* 0.33% and *Staphylococcus aureus* 0.33% in our study (Table 2). Similar study in Bangladesh was previously reported by Jhora *et al.*, who found the predominant isolated uropathogen was *Escherichia coli* 82.61% and others were *Staphylococcuss saprophyticus* 7.01%, *Klebsiella* 3.86%, *Pseudomonas* 3.14%, *Proteus* 1.45%, *Staphylococcus aureus* 0.24%.[26] A study from Kathmandu, Nepal showed that *Escherichia coli* was the most prevailing organism (81.3%).[27] Another study done in India where *Escherichia coli* was 31.25%, *Pseudomonas* 15.62%, *Proteus* 15.62%, *Klebsiella* 6.25% which did not correlate with our findings.[28] This variation further supports the fact that the distribution of UTI-causing pathogens, including their antimicrobial susceptibility pattern, varies from place to place and changes from time to time.[29]

The studies on UTI in other places of the world also showed that *Escherichia coli* and *Klebsiella* spp. are the commonest uropathogens in UTI.[30-34] Higher incidence of Gram negative bacteria, related to *Enterobacteriaceae*, in causing UTI has many factors which are responsible for their attachment to the uroepithelium. In addition, they are able to colonize in the urogenital mucosa with adhesions, pili, fimbriae and P-1 blood group phenotype receptor.[35]

The present study showed the uropathogens as in *Escherichia coli*, *Klebsiella*, *Proteus* and *Enterobacter* were resistant to amoxicillin (Table 3) which correlates with a study done in Bangladesh by Haque *et al.*[22] The increasing level of abuse of drugs by the public, where patients indulge in antibiotic self-medication, commonly to treat all kinds of infections, has been recorded as one significant way of promoting antibiotic resistance.[36,37]

In case of cephalosporin group, cefradine showed highest resistance to *Escherichia coli* 90.45%; *Enterobacter* 100%, cefuroxime showed resistance to *Escherichia coli* 53.81%; *Klebsiella* 36.36%; *Enterobacter* 25%, cefexime showed resistance to *Escherichia coli* 61.83%; *Enterobacter* 50%; *Klebsiella* 27.27%,

Table 3: Antimicrobial resistance pattern of Uropathogens (Gram negative rods)

Drugs	*Esch. coli* (n=262)	*Klebsiella* (n=11)	*Enterobacter* (n=4)	*Proteus* (n=1)
Amoxycillin	250 (95.41%)	10 (90.9%)	2 (50%)	1 (100%)
Cefradine	237 (90.45%)	4 (36.36%)	4 (100%)	0
Cefuroxime	141 (53. 81%)	4 (36.36%)	1 (25%)	0
Cefexime	162 (61.83%)	3 (27.27%)	2 (50%)	0
Ceftriaxone	117 (44.65%)	4 (36.36%)	2 (50%)	0
Cefepime	110 (41.98%)	4 (36.36%)	0	0
Nitrofurantoin	67 (25.57%)	10 (90.9%)	1 (25%)	1 (100%)
Micellinum	65 (24.80%)	5 (45.45%)	3 (75%)	1 (100%)
Cotrimoxazole	134 (51.14%)	6 (54.54%)	2 (50%)	1 (100%)
Nalidixic acid	231 (88.16%)	7 (63.63%)	3 (75%)	1 (100%)
Ciprofloxacin	137 (52.29%)	3 (27.27%)	3 (75%)	1 (100%)
Levofloxacin	131 (50%)	3 (27.27%)	1 (25%)	1 (100%)
Gentamicin	78 (29.77%)	3 (27.27%)	2 (50%)	0
Amikacin	20 (7.63%)	0	0	0
Netilmicin	51 (19.46%)	1 (9.09%)	1 (25%)	0
Imipenem	1 (0.38%)	0	0	0
Meropenem	3 (1.14%)	0	0	0

Table 4: Antimicrobial resistance pattern of Uropathogen (*Pseudomonas*; n=12)

Drugs	***Pseudomonas* (%)**
Piperacillin/tazobactam	3 (25%)
Carbenicillin	8 (66.66%)
Cefuroxime	12 (100%)
Cefexime	12 (100%)
Ceftriaxone	10 (83.33%)
Ceftazidime	5 (41.66%)
Ciprofloxacin	4 (33.33%)
Levofloxacin	5 (41.66%)
Amikacin	5 (41.66%)
Netilmicin	9 (75%)
Aztreonam	7 (58.33%)
Imipenem	3 (25%)
Meropenem	3 (25%)
Colistin	6 (50%)

ceftriaxone showed resistance to *Escherichia coli* 44.65%; *Enterobacter* 50%; *Klebsiella* 36.36% and cefepime showed resistance to *Escherichia coli* 41.98%; *Klebsiella* 36.36% in this study, similar picture is also noted in a study in case of cephalosporin group.[22] The high rate of resistance against third generation cephalosporin (*Klebsiella* 79.31% and *Proteus* 92.86%) was observed by other study done in India.[1]

It is reasonable to speculate that there were ESBL-producing uropathogens especially from Gram negative isolates that couldn't be separated in the present investigation due to limitation are thought to be responsible for resistance to different generations of cephalosporin.[38,31]

Nitrofurantoin was found to be reasonably high efficacious agent among all antimicrobials used to almost all uropathogens in a study in Bangladesh which does not correlates with our study.[22] In Nigeria greater percentage of the UTI isolates were sensitive to nitrofurantoin, it would be an excellent choice for UTI empiric therapy while awaiting the result of culture and sensitivity tests.[39]

Ciprofloxacin was once considered to be the drug of choice for uncomplicated and complicated UTI but due to lack of rational use, this broad spectrum molecule has entirely lost its efficacy not only in UTI but to other common infections too.[38,31] So, empiric use of fluoroquinolones should be restricted.

Regarding fluoroquinolone group, this study showed the higher resistance to nalidixic acid among uropathogens as in *Proteus* 100% and *Escherichia coli* 88.16%; resistance to ciprofloxacin were 100% in *Proteus* and 75% in *Enterobacter* and resistance to levofloxacin was 100% in Proteus. This finding is alarming for the clinician to treat UTI. It is a great concern for them to choose effective drugs against organisms causing UTI. This high rate of resistance against fluoroquinilones was also suggested by other studies done in India, Spain, Iran and Europe.[1,40,41] Our finding did not correlate with others where organisms showed higher sensitivity.[16,42-45]

Gram negative rods were highly resistant to cotrimoxazole in this study which co-relate with another finding done in Bangladesh.[22] Regarding aminoglycosides, Gram negative rods showed low resistance in our study which was similar with a study done in Bangladesh.[22] It might

Table 5: Antimicrobial resistance pattern of Uropathogens (Gram positive cocci)

Drugs	***Enterococci* (n=12)**	***Staphylococcus Aureus* (n=1)**
Amoxycillin	3 (25%)	1 (100%)
Cefradine	6 (50%)	1 (100%)
Cefuroxime	6 (50%)	1 (100%)
Cephalexin	8 (66.66%)	1 (100%)
Cefexime	11 (91.66%)	1 (100%)
Cloxacillin	10 (83.33%)	1(100%)
Ciprofloxacin	9 (75%)	0
Cotrimoxazole	9 (75%)	0
Imipenem	0	0
Meropenem	0	0
Erythromycin	10 (83.33%)	1 (100%)
Methicillin	6 (50%)	1 (100%)
Vancomycin	3 (25%)	0
Linezolid	0	0

be due to their limited use because of parenteral use.

Imipenem and meropenem were used in this study and found to be most sensitive drugs against all isolated uropathogens. Both the drugs were 100% sensitive to *Klebsiella*, *Enterobacter*, *Proteus*, but *Escherichia coli* was0.38% and 1.14% resistant to imipenem and meropenem respectively. Imipenem and meropenem were found to be 98% and 100% sensitive against highly resistant Gram negative bacilli, found in another study.[46] In King Fahd Hospital, Saudi Arabia showed that meropenem and imipenem were 95.8% and 91.71% sensitive respectively against Gram negative rods.[47]

According to our study, *Pseudomonas* in UTI patients showed higher resistance to cefuroxime, cefexime, ceftriaxone and netilmicin (Table 4). Lowest resistance showed in pipercillin/tazobactam as well as in Carbapenem group. Low resistance to pepercillin/tazobactam possibly due to the bata-lactamase inhibitor in addition to the extended-spectrum nature and rare use of the drug.[48] In Bangladesh, another study done in Square hospital, Dhaka (November 2011 to February 2013) showed resistance to imipenem 90%, meropenem 90%, gentamicin 60%, amikacin 60%, ciprofloxacin 50%, cefepime 60%, co-trimoxazole 10% but 100% sensitive to cefixime and ceftriaxone in *Pseudomonas* which does not correlate with our study.[49] In India, Prakash and Suxena found resistant to ceftriaxone 95%, amikacin 95%, nitrofurantoin 90%, nalidixic acid 85%, ceftazidime 65%, levofloxacin 40%, netilmicin 20%, gentamicin 10%, cotrimoxazole 5%, imipenem 5% but meropenem were 100% sensitive in case of *Pseudomonas*.[1] In Pakistan, Shah found that, *Pseudomonas* was resistant to cefuroxime 99.2%, nalidixic acid 98.8%, cefepime 63.9%, ceftriaxone 61.7%, ceftazidime 56.1%, ciprofloxacin 50%, gentamicin 35.3%, amikacin 25.3%, piperacillin/tazobactam 19.6% & imipenem 10.4%.[48]

Though some antibiotic sensitivity discs are not used in our laboratory against *Pseudomonas* but

some studies showed higher sensitivity to cotrimoxazole, gentamicin and ofloxacin to this organism.[1,48]

Over the last decade there has been a substantial increase in resistance of uropathogens to antibiotics. Resistance rates among *Staphylococcus aureus* strains are increasing and a major part of this species has become resistant to bata-lactamase resistant penicillin, for such resistant species, vancomycin is the effective choice of drug.[50,51] *Staphylococcus aureus* and *Enterococcus* both were resistant to amoxycillin, cloxacillin, cephalosporins, erythromycin in a variable percentage in this study (Table 5).

Resistance to vancomycin is reported among *Enterococci*, but this resistance has also begun to develop among *Staphylococci*.[52-54] We concentrated on resistance to vancomycin and resistant strains to vancomycin were observed in case of *Enterococci* 25%. *Staphylococcus aureus* was 100% sensitive to ciprofloxacin and cotrimoxazole but *Enterococcus* was 75% resistant to these drugs in this study. Haque *et al*., found similar findings like us in case of *Enterococcus*.[22] Our finding does not match with another study done in India by Shalini *et al*., who found *Staphylococcus aureus* was resistant to cotrimoxazole 46.15% and ciprofloxacin 22.2%.[55] Another study in India by Prakash & Saxena found *Staphylococcus aureus* was sensitive to gentamicin 80%, levofloxacin 73.33%, netilmicin 93.33% and ceftriaxone 93.33%.[1] In Bangladesh, Saha showed *Staphylococcus aureus*100% sensitive to amikacin, ceftriaxone and ciprofloxacin.[56]

Staphylococcus aureus and *Enterococci* both were 100% sensitive to imipenem, meropenem and linezolid in our study. Another study in Square Hospital, Dhaka Bangladesh (November 2011 to February 2013) found 93.3% resistant to imipenem and meropenem which does not correlate with our study.[49] So, UTI caused by Gram positive cocci may be treated by linezolid, vancomycin, imipenem and meropenem according to the finding of this study.

The higher antibiotic resistance in the present study might be due to the fact that common antibiotics are sold over the counter in Bangladesh and people of any age can buy them without doctor's prescription. Antimicrobial drug resistance is a burning issue in national and global perspective. Highest incidence and prevalence of UTI is observed in developing countries like us. So, government should formulate drug policy especially regarding antibiotic among the chemist and the users.

Limitations

ESBL producing bacteria was not detected in our study leading to inappropriate use of antibiotic and treatment failure.

Conclusion

Due to wide scale resistance of the drugs used to treat UTI, choice of drugs in the treatment of UTI is quite narrow. In country like Bangladesh, awareness for prevention of UTI should be encouraged among the community level as it affects all age groups. A strong antimicrobial stewardship program is needed which is followed by the physicians. An infection control measure is must to control infection and to prevent the spread of these notorious drug resistant organisms.

Competing interest

The authors declare that they have no competing interests.

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