Original Article

Prevalence and Antimicrobial susceptibility pattern of Bacterial Uropathogens in 250 Bedded General Hospital, Pabna, Bangladesh

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UTI, Uropathogens, MDR

Abstract

Background*: Urinary tract infections (UTIs) are one of the most common infectious diseases encountered in the medical practices and often caused by multi-drug resistant (MDR) organisms. This study was aimed to determine the prevalence of bacterial uropathogens and their antimicrobial susceptibility pattern. Midstream clean catch urine samples were collected from 875 patients of both sexes and different age groups attending Medicine outpatient department of 250 Bedded General Hospital, Pabna, Bangladesh from July 2021 to December 2022. Uropathogens were identified by standard and specific microbiological techniques and antimicrobial susceptibility pattern was determined by Kirby Bauer Disc diffusion method following Clinical and Laboratory Standards Institute (CLSI) guidelines.*

Results*: Culture yielded a total of 316 (36.11%) significant growth of uropathogens. Females were found to be much more prone to UTIs than males (2.89:1).* E. coli *was the most predominant (77.53%), followed by* Klebsiella spp. *(10.13%) and* S. saprophyticus *(7.91%). E coli was highly sensitive to amikacin (97.96%) and meropenem (97.55%), followed by gentamycin (76.73%) and nitrofurantoin (76.33%). Maximum resistance was shown to cloxacillin and penicillin-G while ampicillin, linezolid and co-trimoxazole were found to be largely ineffective.* Klebsiella spp. *demonstrated maximum sensitivity towards amikacin and meropenem, and good sensitivity to gentamicin but showed total resistance to penicillin-G, linezolid and cloxacillin and high resistance to third- and fourth-generation cephalosporins.* S. saprophyticus *showed high sensitivity to amikacin and meropenem, but absolute resistance to cefixime and ceftazidime, and high resistance to ceftriaxone and co-trimoxazole.*

Conclusion*: Uropathogens in the present study showed maximum susceptibility to amikacin and meropenem but increasing resistance to oral antibiotics is very alarming. Commonly prescribed antimicrobials need to be continuously evaluated and empirical therapy must be considered accordingly.*

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Introduction:

Urinary tract infection (UTI) refers to the presence of micro-organism in the urinary tract including urinary bladder, prostate, collecting system or kidney. The syndrome ranges from asymptomatic bacteriuria to perinephric abscess with sepsis[1]. UTI is the second most common infection in community practice[2]. About 35% of healthy women suffer from symptoms of UTI at some point in their life. The incidence of UTI is greater in women as compared to men, which may be

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due to either anatomical predisposition or other host factors[3]. About 150 million people suffer from UTIs each year globally which results in greater than 6 billion dollars in direct health care[4]. The prevalence increases among patients from lower socio-economical group[5].

Indiscriminate use of antimicrobial agents is a common practice in underdeveloped and many developing countries that often leads to emergence of resistant microorganisms[6]. As a common practice, empirical antimicrobial treatment is initiated before the laboratory results of urine culture are available which may lead to emergence and spread of antimicrobial resistant strains[7]. The prevalence and pattern of antimicrobial susceptibility of uropathogens are constantly changing with the ever-increasing use of antimicrobials[8]. For the selection of appropriate drugs as well as for rational choice of empirical therapy proper knowledge and continuous monitoring of the susceptibility pattern is of paramount importance. The present study was carried out to determine the prevalence of bacterial pathogens responsible for UTIs and their antimicrobial susceptibility pattern with the aim to disseminate information about choice of empirical antibiotics.

Materials and Methods:

This study was done at the Department of Medicine, 250 Bedded General Hospital, Pabna for a period of eighteen months from July 2021 to December 2022. A total of 875 urine samples were collected from patients who visited Medicine Out Patient Department of 250 Bedded General Hospital, Pabna, Bangladesh during the study period. Patients of both sexes and all age groups from 15 years and above were included. Midstream clean catch urine samples were collected in two sterile containers by standard procedures. Semiquantitative culture was done on blood agar and MacConkey's agar media. The plates were incubated aerobically at 37°C overnight. Isolated organisms were identified by colony morphology, Gram staining and relevant biochemical tests. Samples showing significant colony count were taken into consideration. Sensitivity patterns of the organisms were determined by modified Kirby-Bauer Disc diffusion method using Mueller-Hinton agar according to Clinical and Laboratory Standards Institute (CLSI) guidelines[9,10]. The following antibiotic discs were used in antibiogram: amikacin, amoxycillin, ampicillin, azithromycin, cefepime, cefixime, cefotaxime, cefradine, ceftazidime, ceftriaxone,cefuroxime, ciprofloxacin, cloxacillin, colistin, co-amoxiclav, co-trimoxazole, doxycycline, gentamicin, levofloxacin, linezolid, meropenem, nalidixic acid, nitrofurantoin, penicillin-G and tetracycline.

The protocol was approved by the Ethical Review Committee of 250 Bedded General Hospital, Pabna, Bangladesh and informed written consent was taken from patients before collection of their samples.

Statistical analysis was done with Statistical Package for Social Sciences (SPSS) version 23.0.

Results:

225 (25.71%) of the population under study were males and 650 (74.29%) females with a male to female ratio of 1:2.89. Out of the total 875 samples, 316 (36.11%) showed significant growth of bacteria, of which 50 (15.82%) isolates were from male and 266 (84.18%) were from female patients. Bacterial growth was found in 22.22% of the urine samples (50 out of 225) in men compared to40.92% of the samples (266 out of 650) in women.

Table 1. Distribution of specimens with significant growth

	Number of samples	Percentage	Number of positive cultures	Percentage of positive cultures
Female	650	84.18	266	40.92
Male	225	15.82	50	22.22
Total	875	100	316	36.11

Three age groups were considered to determine the distribution of uropathogens according to age; highest significant bacterial growth (38.35%) was observed in the 15-29 age group, followed by 45+ age group (35.71%) in females and in the 45+ age group (48%) in males.

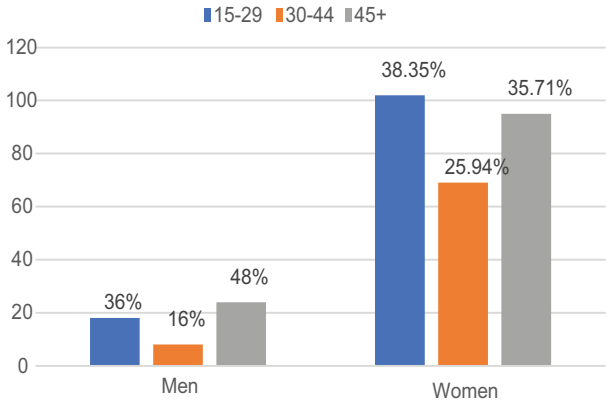

Fig.-1: *Distribution of positive growth of uropathogens in age groups*

Table II. *Frequency of isolated uropathogens*

Isolated uropathogens	Number	Percentage
Escherichia coli	245	77.53
Klebsiella spp.	32	10.13
Staphylococcussaprophyticus	25	7.91
Staphylococcus aureus	6	1.89
Pseudomonas spp.	4	1.27
Enterococci	2	0.63
Proteus spp.	1	0.32
Staphylococcus epidermidis	1	0.32
Total	316	100

Gram negative bacteria overwhelmingly outnumbered (89.24% versus 10.76%) Gram positive ones in frequency of uropathogens. *Escherichia coli* was the most predominant (77.53%), followed by *Klebsiella* spp. (10.13%), *Pseudomonas* spp. (1.27%) and *Proteus* spp. (0.32%).

Table III. *Antimicrobial sensitivity and resistance pattern of E. coli(n=245)*

Antibiotic	Sensitive	Antibiotic	Resistance
Amikacin	240 (97.96%)	Cloxacillin	228 (93.04%)
Meropenem	239 (97.55%)	Penicillin-G	226 (92.24%)
Tobramycin	211 (86.12%)	Ampicillin	217 (88.57%)
Gentamicin	188 (76.73%)	Linezolid	200 (81.63%)
Nitrofurantoin	187 (76.33%)	Cefradine	184 (75.10%)
Levofloxacin	166 (67.76%)	Amoxycillin	180 (73.47%)
Ciprofloxacin	152 (62.04%)	Co-trimoxazole	180 (73.47%)
Doxycycline	133 (52.29%)	Cefixime	167 (68.16%)
Ceftriaxone	99 (40.41%)	Co-amoxiclav	166 (66.12%)
Cefepime	91 (37.14%)	Cefuroxime	160 (65.31%)

*Klebsiella spp.*showed maximum sensitivity to amikacin and meropenem (96.88% each) followed by gentamicin (75%).

Table IV. *Antimicrobial sensitivity and resistance pattern of Klebsiella spp.(n=32)*

Antibiotic	Sensitive	Antibiotic	Resistance
Amikacin	31 (96.88%)	Cloxacillin	32 (100.00%)
Meropenem	31 (96.88%)	Penicillin-G	32 (100.00%)
Gentamicin	24 (75.00%)	Linezolid	32 (100.00%)
Tobramycin	20 (62.50%)	Ampicillin	29 (90.63%)
Nitrofurantoin	18 (56.25%)	Amoxycillin	29 (90.63%)
Cefotaxime	16 (50.00%)	Co-trimoxazole	29 (90.63%)
Levofloxacin	13 (40.63%)	Co-amoxiclav	24 (75.00%)
Ciprofloxacin	13 (40.63%)	Cefradine	23 (68.16%)
Ceftazidime	13 (40.63%)	Tetracycline	21 (66.12%)
Azithromycin	12 (37.50%)	Cefuroxime	20 (62.50%)

Most predominant Gram-positive bacteria found in this study, *S. saprophyticus*, showed high sensitivity to amikacin (88%), meropenem (80%), nitrofurantoin (72%) and gentamicin (72%).

Table V. *Antimicrobial sensitivity and resistance pattern of staphylococcus saprophyticus (n=25)*

Antibiotic	Sensitive	Antibiotic	Resistance
Amikacin	22 (88.00%)	Cefixime	25 (100.00%)
Meropenem	20 (80.00%)	Ceftazidime	25 (100.00%)
Tobramycin	19 (76.00%)	Cefepime	24 (96.00%)
Gentamicin	18 (72.00%)	Colistin	23 (92.00%)
Nitrofurantoin	18 (72.00%)	Ampicillin	21 (84.00%)
Ciprofloxacin	16 (64.00%)	Nalidixic acid	21 (84.00%)
Cefradine	16 (64.00%)	Cefotaxime	20 (80.00%)
Tetracycline	15 (60.00%)	Ceftriaxone	20 (80.00%)
Doxycycline	13 (52.00%)	Co-trimoxazole	20 (80.00%)
Levofloxacin	13 (52.00%)	Cefuroxime	17 (68.00%)

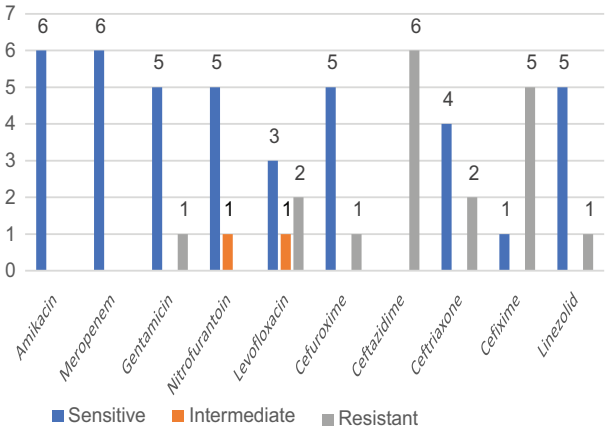

Fig.-2. Antibiogram profile of *S. aureus* (n=6)

Pseudomonas spp. were fully susceptible to amikacin and meropenem, and 75% susceptible to gentamicin; but showed total resistance to cefuroxime, cefixime, ceftriaxone, co-trimoxazole and linezolid, and 75% resistance to both nitrofurantoin and ciprofloxacin.

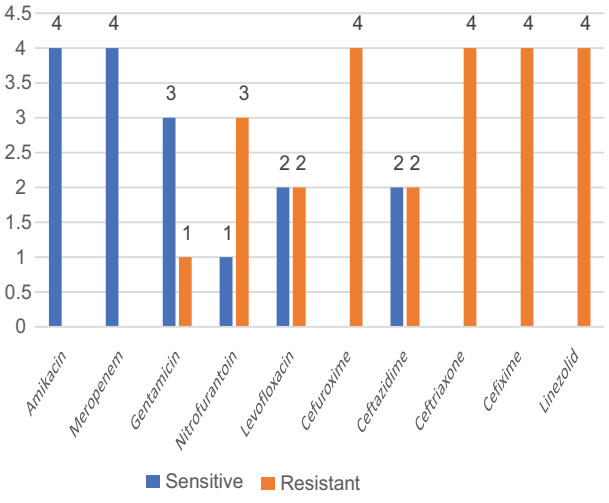

Figure 3. *Antibiogram profile of Pseudomonas spp. (n=4)*

Discussion:

Urinary tract infections are one of the most common infectious diseases encountered in the medical practices and only second to respiratory tract infections as a cause of hospital visit[11]. Antimicrobial resistance to various classes of antimicrobials to uropathogens continues to be a major health problem in different parts of the world[12,13]. Consequently, the empirical treatment of UTIs becomes difficult and unpredictable due to the lack of alternative effective antibiotics[14]. As urinary pathogens vary considerably in different geographic areas, antibiogram profile of the isolates is a must for the physicians to combat drug-resistant organisms. This study has been carried out to determine the prevalence of bacterial pathogens causing uncomplicated UTIs and their antibiotic susceptibility pattern.

It is well established that UTI is more common in females than in males and our findings have rightly coincided with those in other studies[5, 14-16]. Regarding prevalence of the uropathogens, our observation is in good agreement with several previous reports[15-18].

In the present study, 15-29 years age group had the greatest number of positive bacterial growth, followed by 45+ age group in females and in the 45+ age group in males, which corelates with the observations by some other researchers[14,19]. The variation of prevalence within different age groups may be attributed to the hormonal changes affecting the mucosal adherence of bacteria, frequent sexual activity, use of spermicidal agents, menopause for women and prostate gland enlargement of men[20].

E.coli was found to be the predominant organism with a prevalence rate of 77.53%, followed by Klebsiella spp. and S. saprophyticus. Again, Gram-negative bacteria greatly outnumbered Gram-positive ones. These findings are remarkably consistent with previous studies conducted home and abroad[14, 21-25]. The prevalence of *E. coli* as the most common organism causing UTIs is due to the fact that they are the normal fecal flora and possess several adhesion factors such as adhesin, pili, P-fimbriae and P1-blood group phenotype receptor responsible for their attachment to the uroepithelial cells[26,27].

The most common bacterial agent of UTI, *E. coli*, showed maximum sensitivity to amikacin and meropenem. Gentamycin and nitrofurantoin were found to be sensitive in 76.73% and 76.33% cases respectively. Maximum resistance was shown by *E. coli* to cloxacillin and penicillin-G while ampicillin,linezolidand co-trimoxazole were found to be largely ineffective. Similar findings were also observed by Jhora et al and Shahnaz et al[5,28].

Most isolates of *Klebsiella spp.*are susceptible to fluoroquinolones, aminoglycosides, and carbapenems[8], therefore *Klebsiella pneumoniae* demonstrating maximum sensitivity towards amikacin and meropenem, andgood sensitivityto gentamicin was expected. Nitrofurantoin showing fair sensitivityand quinolonesshowing less sensitivity contradicts with the findings of Haque et al and Bouza et al[8,15]. *Klebsiella spp.* are intrinsically resistant to penicillins and can acquire resistance to third- and fourth-generation cephalosporins owing to the production of plasmid-mediated extended-spectrum beta-lactamases (ESBLs), so penicillin-G being totally useless and ampicillin, amoxycillin, cefuroxime, cefixime and ceftriaxone being found to be highly resistant supports the findings of other researchers[8,15].

S. saprophyticus has shown high sensitivity to carbapenem and gentamicin, and moderate sensitivity to ciprofloxacin which was similar to the findings of Jhora et al[2].The organism being absolutely resistant to cefixime and ceftazidime, and highly resistant to ceftriaxone and co-trimoxazole was supportive of the findings of Haque et al[15]but contrary to the findings of Jhora et al[2].

Amikacin found to be completely ineffective against *S. aureus*in a previous study[14]has shown 100% susceptibility in the present one and in another study from Bangladesh[29] while high sensitivity towards gentamicin and remarkable resistance to cefixime (83.33%) were consistent with the findings of other researchers[14,29].

Pseudomonas spp. being fully susceptible to amikacin and carbapenemhas been contradictory to but showing total resistance to ceftriaxone, co-trimoxazole and nalidixic acid, and 75% resistance to nitrofurantoin has been supportive of the findings of Sanjee et al [14]. Carbapenem (meropenem) having been considered as the most effective anti-pseudomonal drug in our study was also observed by Begum et al in a study in Bangladesh in 2013[30].

Almost all the uropathogens have shown remarkable amount of susceptibilityto gentamicin from aminoglycoside group and meropenem from carbapenem group. In addition, gentamicin and nitrofurantoin were also found to be strongly effective and quinolones were observed to be fairly sensitive. All the drugs from 2nd, 3rd and 4th generation cephalosporins (cefuroxime, cefixime,ceftriaxone, cefepime), co-trimoxazole and co-amoxiclav were found to be either ineffective or less effective against most of the uropathogens, which is a red alert for empirical treatment of uncomplicated UTIs.

This study has clearly demonstrated that the uropathogens are becoming resistant to the most commonly prescribed antibiotics for the treatment of uncomplicated UTIs. Extended spectrum â-lactamase (ESBL) of Gram negative uropathogens help them to gain resistance against 3rd and 4th generation cephalosporins[8,15] whereas resistance to carbapenem antibiotic group is often due to loss of outer membrane proteins and up-regulation of active efflux pumps or production of metallo-â-lactamase (MBL)[31]. Major factors known to influence the evolution and transfer of multi drug resistance among microorganisms are incomplete doses, ease of access, over prescription, prescription of higher generation antimicrobials, prescribing antibiotics without laboratory results and indiscriminate use of antimicrobials in agriculture and livestock sectors[32]. As drug resistance is mainly an acquired property which can also be lost in any time, the resistance profile of some drugs shows rises and downfalls with course of time towards a particular pathogen[33].

No fundamentally new classes of antibiotic drugs have been developed since the 1950s, and the means of diagnosing bacterial infections remains largely

unchanged since the 19th century which accounts for the death of 700,000 people each year from infection by drug-resistant pathogens and parasites. If left unchecked, by 2050, drug-resistant bacteria could kill 10 million people each year - more than currently death from cancer, knocking 2.0-3.5% off of the global GDP[34]. To prevent the emergence of MDR pathogens globally continuous surveillance and monitoring is a must.

The present study, conducted on a small number of samples in a small District Hospital, Bangladesh, hardly represents the total scenario. As the resistance pattern of the uropathogens is ever changing and continuous, we recommend for a broad-based longitudinal study that can reflect the broader perspective which may serve as a basis for the development of the national antibiotic guideline and timely revision of the existing guideline in response to the emerging MDR pathogens. We do emphasize molecular level researches on the mechanism of drug resistance coupled with computational biology for identification of potent drug target so that novel therapeutic agents are designed against MDR pathogens.

Conclusion:

The present study found *E. coli* to be the predominant uropathogen, followed by Klebsiella spp. and S. saprophyticus. High resistance was documented towards second-, third- and fourth-generation cephalosporins. Amikacin and meropenem were found to be the most effective drugs with over 95% overall sensitivity but resistance to oral antibacterials was increasing. The rapid emergence of antimicrobial resistance is a serious warning sign that we must be cautious with overprescribing and indiscriminate use of antibiotics. Appropriate antimicrobial drugs should be prescribed in accordance with culture and sensitivity reports, and empirical therapy must be considered on the recent antibiogram profile of a particular geographic area.

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