

## Original Article

# Bacterial Isolates and Antibiotic Sensitivity Pattern from Urinary Tract Infection in a Private Diagnostic Laboratory in Dhaka, Bangladesh

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Urinary tract infection (UTI) is a common infection encountered by clinicians and despite the widespread availability of antimicrobial agents, UTI has become difficult to treat because of appearance pathogens with increasing resistance to antimicrobial agents. The objectives of this study were to determine the pathogens causing UTI and to determine the antibiotic sensitivity status among these isolates in a diagnostic laboratory in Dhaka city. A laboratory based cross sectional survey was conducted in a diagnostic Centre in Dhaka Bangladesh from July 2016 to December 2016. A total of 553 urine samples were collected from each patients and processed in microbiology laboratory to isolate pathogens and antibiotic susceptibility test using standard procedure. Among 553 urine samples, 158 (28.57%) samples was found to be culture positive of which 39 (24.70%) were isolated from male patients and 119 (75.30%) from female patients. *Escherichia coli* (43.67%) were found to be the predominant pathogen followed by *Staphylococcus* spp. (16.45%), *Enterococcus* spp. (13.39%), *Klebsiella* spp. (13.29%), *Candida* spp. (5.70%), *Acinetobacter* spp. (4.43%), *Pseudomonas* spp. (3.80%) and *Proteus* spp. (1.27%). The carbapenem group antibiotics (imipenem and meropenem) was found to be resistant in 0 to 5.1% of the Gram negative isolates. On the Other hand, most of the Gram positive isolates showed sensitivity to linezolid and vancomycin. This study showed that *E. coli* isolates were the predominant pathogens and showed resistance to commonly prescribed drugs resulting in a very few options for drug to treat UTIs.

**Keywords:** Urinary tract infection (UTI), Uropathogens, Antimicrobial susceptibility, Antimicrobial agents.

## Introduction

Urinary tract infection (UTI) is the common bacterial infectious disease in community practice with high rate of morbidity and financial cost all over the world. About 150 million people are suffering from UTI each year Worldwide, costing 6 billion US dollars<sup>1</sup>. Nearly about 10% of people will experience UTI during lifetime<sup>2</sup>. UTIs refer to the presence of microbial pathogens within the urinary tract and it is usually classified by the site of infection of urinary bladder (cystitis), kidney (pyelonephritis) or urine (bacteriuria). Almost 95% of cases of UTI are caused by bacteria that typically multiply at the opening of the urethra and travel up to the bladder.

A limited predictable spectrum of organism is responsible for urinary tract infection. Most of the UTI are caused by Gram negative bacteria like *E. coli*, *Klebsiella* spp., *Pseudomonas aeruginosa*, *Proteus* spp., *Acinetobacter* spp. *Serratia* and *Morganella morganii*. UTI can be caused by Gram positive bacteria too. Presence of *Enterococcus* spp., *Staphylococcus* especially coagulase negative *Staphylococci* and *Streptococcus*

*agalactiae* were also reported<sup>3</sup>. UTI is much more common in women than in men due to anatomical and physiological reason; by virtue of its position, urogenital tract is more vulnerable to bacterial infection caused by internal and external flora

Studies from India, Navaneeth *et al.*, Bangladesh, Iqbal *et al.*, and Nepal, Srinivassa *et al.* have reported an increased resistance of the urinary pathogens to commonly used antibiotics<sup>4-6</sup>. The aim of this study was to determine microbial etiologic agents responsible for urinary tract infection and to evaluate their *in vitro* susceptibility pattern to commonly used antimicrobial agents in a private practice set up of Dhaka city. This study is important for clinicians in order to facilitate the effective treatment and management of patients with symptoms of urinary tract infection.

## Materials and Methods

**Study design:** A prospective cross-sectional study was conducted in the dept. of Microbiology, Popular diagnostic centre ltd. Dhaka, Bangladesh. A total of 553 samples were collected during this study period from July 2016-Dec 2016.

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### Inclusion/Exclusion criteria

Study population consisted of all the patients who visited the concerned laboratory for urine culture examination.

### Sample collection and processing

Study population were instructed on how to collect clean-catch mid-stream urine. Freshly voided midstream urine (10-20 ml) from each patient was collected in a wide mouth sterile container supplied by the laboratory and bring to the laboratory as early as possible, usually within 1 hour after collection<sup>7</sup>.

### Microscopy

The urine samples were mixed thoroughly, centrifuged and examined microscopically for wet mount preparation.

### Culture

A calibrated sterile Nicrome wire loop for the semi-quantitative method was used for the plating. It has a 4 mm diameter loop to deliver 0.01 ml of liquid. A loopful of the well mixed urine sample was inoculated on Hi-Chrome (Himedia, India), MacConkey and Blood agar media (Mast diagnostic, UK) and then incubated at 37<sup>0</sup> C aerobically for 24 hrs and for 48 hrs in negative cases. They were then screened for bacterial growth. A significant bacterial count was considered as any count equal to or in excess of 100,000 CFU/ml. A less than 100 CFU/ml was interpreted as negative. Bacterial identifications were carried out based on standard culture and biochemical characteristics of isolates.

### Antibiotic susceptibility testing

In the present study, antimicrobial susceptibility testing was done on Mueller-Hinton agar media (Mast diagnostic, UK) using disk diffusion (Kirby Bauer's) method according to the Clinical Laboratory Standard Institute (CLSI) guidelines using the following antimicrobial agents: Ampicillin 10µg, Amikacin 30µg, Aztreonam 30µg, Cefepime 30µg, Ceftriaxone 30µg, Ceftazidime 30µg Cefixime 5µg, Cefoxitin 30µg, Cloxacillin 5µg Cotrimoxazole 25µg, Ciprofloxacin 5µg, Imipenem 10µg, Gentamicin 10µg, Meropenem 10µg, Netilmicin 30µg, Nitrofurantion 300µg, Levofloxacin 5µg Vancomycin 30µg, Linezolid 30µg, Netilmicin 30µg, Penicillin 10µg. Reference strains of *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 were used for quality control for antimicrobial susceptibility tests<sup>8</sup>.

### Statistical analysis

The statistical analysis of data was performed using Microsoft Excel-2011 version. Discrete values were expressed as percentage. Descriptive statistics were used to summarize patient characteristics and the prevalence of antimicrobial sensitivity.

## Results

Out of 553 urine samples, only 158 (28.57%) was found to be culture positive, having a bacterial load of  $e^{10^5}$  cfu/ml. Of 158 culture positive samples, 39 (24.70%) were from male patients and 119 (75.30%) from female patients and other 395 (71.43%) sample were negative or normal flora.

**Table 1.** Growth of Urine culture among the study population (n=553)

Culture	Frequency (%)	Male (%)	Female (%)
Positive	158 (28.57)	39(24.68)	119(75.32)
Negative	395 (71.43)	155(39.00)	241(61.00)
Total	553(100)	194(35.00)	360(65.00)

Most of the patients were in age group of 21 to 40 years, of which 38(17.92%) cases were positive. In this age group, 34(98.12%) were female and 4(1.88%) were male. In the age group of less than 20 years, number of female patients was also higher than male patient which is 16(61.54%) and 10(38.46%) cases respectively. Overall, female cases were more common than male, shown in Table 2.

**Table 2.** Age and sex distribution of culture positive patient(n=158)

Age	No	Growth (%)	Female (%)	Male (%)
< 20	105	26(24.76)	16(61.54)	10(38.46)
21-40	212	38(17.92)	34(98.12)	4(1.88)
41-60	152	60(39.47)	48(80.00)	12(20.00)
61-80	77	29(37.66)	19(65.52)	10(34.48)
> 80	7	5(71.42)	2(40.00)	3(60.00)

The most common isolates in this study was found to be Gram negative bacilli which accounts for 66.45% of the total isolates. Of the Gram negative bacilli, the predominant isolate was the *E. coli* (43.96%) followed by other bacilli like *Klebsiella spp.* (13.29%), *Acinetobacter spp.* (4.43%), *Pseudomonas spp.* (3.80%) and *Proteus spp.* (1.27%) among the major isolates. The most prominent Gram positive bacteria identified was *Staphylococcus spp.*, accounts for 16.45% of total isolates, followed by *Enterococcus spp.* (13.39%). *Candida spp.* was responsible for (5.70%) of cases. Table 3 shows the detailed frequency of all the isolates.

**Table 3.** Frequency of positive isolates with based on sex.

Name of the isolates	Frequency (%)	Male (%)	Female (%)	
Gram negative(N=105)	<i>E. coli</i>	69 (43.67%)	16 (10.12%)	53 (33.55%)
	<i>Klebsiella spp.</i>	21 (13.29%)	3 (1.90%)	18 (11.39%)
	<i>Acinetobacter spp.</i>	7 (4.43%)	2 (1.27%)	5 (3.16%)
	<i>Pseudomonas spp.</i>	6 (3.80%)	3 (1.90%)	3 (1.90%)
	<i>Proteus spp.</i>	2 (1.27%)	1 (0.63%)	1 (0.63%)
Gram positive(N=44)	<i>Staphylococcus spp.</i>	26 (16.45%)	8 (5.06%)	18 (11.39%)
	<i>Enterococcus spp.</i>	18 (13.39%)	3 (1.90%)	15 (9.50%)
Fungus (N=9)	<i>Candida spp.</i>	9 (5.70%)	3 (1.90%)	6 (3.80%)
Total	158(100%)	39 (24.68%)	119 (75.32%)	

**Table 4.** Distribution of Antibiotic sensitivity among the bacterial isolates

AntibioticSusceptibility	Gram negative isolates					Gram positive isolates	
	<i>E. coli</i> n=69(%)	<i>Klebsiella</i> n=21(%)	<i>Acinetobacter</i> n=7(%)	<i>Pseudomonas</i> n=6(%)	<i>Proteus</i> n=2(%)	<i>Staphylococcus</i> n=26(%)	<i>Enterococcus</i> n=18 (%)
Ampicillin	3(4.34)	3(14.28)	0(0.00)	0(0.00)	0(0.00)	2(7.69)	16(88.88)
Cephadrine	6(8.69)	5(23.80)	0(0.00)	0(0.00)	1(50.00)	-	-
Ceftriaxone	21(30.43)	14(66.66)	2(28.57)	2(33.33)	1(50.00)	-	-
Cefixime	20(28.98)	12(57.14)	1(14.28)	0(0.00)	1(50.00)	-	-
Ceftazidime	43(62.31)	15(71.42)	3(42.85)	3(50.00)	2(100.0)	-	-
Cefepime	47(68.11)	16(76.19)	4(57.14)	4(66.66)	2(100.0)	-	-
Imipenem,	65(94.20)	20(95.23)	6(85.71)	5(83.33)	2(100.0)	25(96.15)	16(88.88)
Meropenem	65(94.20)	20(95.23)	6(85.71)	5(83.33)	2(100.0)	25(96.15)	16(88.88)
Cotrimoxazole	26(37.63)	10(47.61)	1(14.28)	0(0.00)	1(50.00)	12(46.15)	-
Gentamycin	53(76.81)	16(76.19)	5(71.42)	4(66.66)	2(100.0)	22(84.61)	-
Netilmicin	58(84.05)	17(80.95)	5(71.42)	5(83.33)	2(100.0)	23(88.46)	-
Amikacin	62(89.85)	19(90.47)	6(85.71)	5(83.33)	2(100.0)	24(92.30)	-
Ciprofloxacin	27(39.13)	12(57.14)	2(28.57)	2(33.33)	1(50.00)	11(42.30)	12(66.66)
Levofloxacin	30(43.47)	12(57.14)	2(28.57)	2(33.33)	1(50.00)	12(46.15)	12(66.66)
Doxycycline	13(18.84)	8(38.09)	0(0.00)	0(0.00)	0(0.00)	9(34.61)	13(72.22)
Tertracycline	13(18.84)	8(38.09)	0(0.00)	0(0.00)	0(0.00)	9(34.61)	13(72.22)
Nitrofurantoin	61(88.40)	13(61.90)	6(85.71)	4(66.66)	2(100.0)	22(84.61)	16(88.88)
Aztreonam	36(52.17)	13(61.90)	4(57.14)	3(50.00)	2(100.0)	-	-
Cefoxitin	53(76.81)	17(76.19)	5(71.42)	4(66.66)	2(100.0)	18(69.23)	-
Cloxacillin	-	-	-	-	-	18(69.23)	-
Tigecycline	-	-	-	-	-	26(100.0)	18(100.0)
Lenizolid	-	-	-	-	-	26(100.0)	18(100.0)
Vancomycin	-	-	-	-	-	24(92.30)	16(88.88)

## Discussion

This study provides valuable data to isolate and to identify the pathogen which cause urinary tract infection and monitor the status of antimicrobial sensitivity among uropathogens to improve efficient empirical treatment. In addition to that the susceptibility pattern of these bacteria is very important to avoid development of drug resistant<sup>9</sup>. In our study isolation and identification of uropathogens were performed and 158(28.57%) urine sample showed significant growth of bacteria so, remaining majority 395

(71.43%) of the cases showed either insignificant bacteriuria or no growth with urine from the suspected cases of UTI.

This study resembles to the study conducted by Yusuf *et al.*, 2015<sup>10</sup> and Barber *et al.*, 2013<sup>11</sup> and they showed 28% significant bacterial growth.

The reason of low growth rate may be due to irrational use of antibiotic which is available in the local market in this country and these are given without prior culture and antibiotic sensitivity pattern. In addition to that, incomplete doses are another factor.

Prior antibiotic therapy before sending urine sample for culture and sensitivity and other clinical conditions like non-gonococcal urethritis could be the factors responsible for insignificant bacteriuria or no growth<sup>11</sup>.

In this study showed the prevalence was high in females than males for each isolates 75.31% and 24.68% respectively, so we can say strictly female suffers more than male from UTI. Other studies also showed that urinary tract infection was more common in females. Akhter *et al.*, 2012<sup>12</sup> and Yusuf *et al.*, 2015<sup>10</sup> was found 71.6% and 66.6% respectively. It is well established that female are more commonly infected with UTI than male due to anatomical position of urethra, influence of hormone and pregnancy<sup>13</sup>. The international studies have shown that UTIs in women are very common; therefore, one in five adult women experience UTI in her life and it is extremely common, clinically apparent, worldwide patient problem.

The most frequently isolated bacteria in this study was *E. coli* (43.67%) followed by *Staphylococcus spp.* (16.45%), *Enterococcus spp.* (13.39%), *Klebsiella spp.* (13.29%), *Acinetobacter spp.* (4.43%), *Candida pp.* (5.70%), *Pseudomonas spp.* (3.80%) and *Proteus spp.* (1%.27). These results correlated with the study by Rana *et al.*<sup>14</sup> who found the predominance of *E. coli* (46.66%) followed by *Staphylococcus spp.* (15.8%), and also with Shaaban *et al.*<sup>15</sup>, who also found that *E. coli* is most prevalent isolates in urine accounting up to 43%, followed by *klebsiella pneumoniae* (14.1%), *Pseudomonas aeruginosa* and *Proteus mirabilis* (9.4%).

Predominance of *E. coli* among the different uropathogens, was also found by Oluremi *et al.*<sup>16</sup> and by Chakupurakal *et al.*<sup>17</sup>. The dominance of *E. coli* is followed by *Staphylococcus spp.* (16.45%) in this study resembles to the study done by Jha and Bapat,<sup>18</sup> which was the second most organisms causing UTI.

Antibiotic susceptibility test reveals that higher percentage of susceptibility for Imipenem and Meropenem, which accounts for 96% of the cases, followed by Nitrofurantoin (88%), Amikacin (88%), Netilmicin (88%), shown in table-4. This study resembles to the study conducted by Farjana *et al.*<sup>19</sup> where higher percentage of susceptibility was seen for Amikacin (88%). Second is the Nitrofurantoin which considered as an appropriate agent for first line treatment of community acquired UTIs.

Ampicillin was found to show the higher resistance rate (98%) which resembles to the study conducted by Nerukar *et al.*<sup>20</sup> which showed that isolates of most of the species exhibited a high rate of resistance to ampicillin. Resistance to antibiotics develops due to its frequent misuse<sup>21</sup>.

According to the above results, among antibiotics used for susceptibility test for Gram negative bacteria, Amikacin, Netilmicin and Nitrofurantoin was found to be the most effective antibiotics followed by Gentamicin, Cefoxitin, Cefepime and Ceftazidime for the isolates respectively were sensitive.

In present study, *E. coli* and *Klebsiella* showed maximum sensitivity to nitrofurantoin. Almost all Gram negative isolates are least sensitive to Cephalosporin and fluoroquinolones groups. *Pseudomonas spp.* was found to resistant against all commonly used antibiotic and sensitive to Imepenem, Meropenem. The higher rate of resistance might be a result of the irrational use of antibiotics<sup>21</sup>.

*Staphylococcus spp.* and *Enterococcus spp.* was found to be sensitive to Linezolid (100%), Vancomycin (94%). This is comparable with study of Jayshri *et al.*<sup>21</sup>. The antimicrobial susceptibility pattern confirms that most of the urinary isolates in our environment are resistant to the commonly used antibiotic including the cephalosporin and fluoroquinolones. In particular, the high resistance of the gram negative isolates to the fluoroquinolones is worrisome as these are reserve drugs for treating resistance infections<sup>22</sup>.

### Conclusion

A drug resistance among bacterial pathogen is an evolving process, regular surveillance and monitoring is necessary to provide physicians knowledge on the updated and most effective empirical treatment of UTIs. Periodic reassessment of in vitro susceptibility pattern of urinary pathogens to serve as a guide for antibiotic therapy since these organism exhibit resistances to first-line drugs used for UTI. In order to prevent decrease resistance to antibiotics, the use of antibiotics should be kept under supervision, should be given in appropriate doses for an appropriate period of time.

In the present study, community-acquired UTI and nosocomial UTI were not been distinguished. This was the main limitation of the study.

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