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Article

Prevalence and antibiotic susceptibility pattern of pathogens from urinary tract infections (UTI) in a private diagnostic laboratory in Bangladesh

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Abstract: Urinary tract infection (UTI) is one of the commonest infections 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 aim and 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 Popular Diagnostic Centre Ltd. Dhanmondi, Dhaka-1205, Bangladesh from July 2016 to December 2016. A total of 553 freshly voided midstream urine samples (10-20 ml) were collected in a wide mouth sterile container from patients and processed in microbiology laboratory to isolate pathogens and antibiotic susceptibility test using standard procedure. Among 553 urine sample, the culture positivity in urine samples was found to be 158 (28.57%) 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%), *Psudomonas* spp. (3.80%) and *Proteus* spp. (1.27%). Carbapenem group (Imipenem, Meropenem) were the most effective antibiotic with resistance between 0 and 5.1% of the gram negative isolates and Linezolid and Vancomycin was most effective in gram positive isolates. Nitrofurantoin was most effective both gram negative and gram positive isolates. This study finding showed That *Escherichia. coli* isolates were the predominant pathogens and showed increasing pattern to the commonly prescribed drugs in private practice that in turn leaves the clinicians with very few alternative options in drug for the treatment of UTIs.

Keywords: urinary tract infection (UTI); uropathogens; antimicrobial susceptibility; antimicrobial agents

1. 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. Worldwide, about 150 million people are suffering from UTI/year, costing 6 billion US dollar **(**Gonzalez *et al.*, 1999). Nearly about 10% of people will experience in UTI during lifetime (Hoberman *et al.*, 1997). 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 *Esherichia coli, Klebsiella* spp., *Pseudomonas aeruginosa, Proteus* spp., *Acinetobacter* spp., *Serratia* and *Morganella margani*.

UTI also caused by Gram positive bacteria like *Enterococcus* spp., *Staphylococcus* especially coagulase negative *Staphylococci* and *Streptococcus agalactiae* (Shaaban *et al*., 2012**)**. 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.,* 2002, Bangladesh, Iqbal *et al.,* 1997 and Nepal, Srinivassa *et al.,* 199 have reported an increased resistance of the urinary pathogens to commonly used antibiotics. The aim of this study was to determine microbial etiologic agents responsible for urinary tract infection and to evaluate them 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.

2. Materials and Methods

2.1. Study design

A prospective cross-sectional study was conducted in the dept. of Microbiology, Popular Diagnostic Centre Ltd. Dhaka, Bangladesh. Total 553 samples were collected during this study period from July 2016 - Dec 2016.

2.2. Inclusion/Exclusion criteria

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

2.3. Sample collection and processing

Study population were instructed on how to collect clean-catch mid-stream urine into sterile container after carefully cleaning the genitalia, especially around the opening the urethra. Samples were collected wide mouthed, leak proof container supplied by the laboratory and brought to the laboratory as early as possible, usually within 1 hour after collection.

2.4. Microscopy

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

2.5. Culture

A calibrated sterile Nicrome wire loop for the semi-quantitative method was used for the plating. It has a 4 mm diameter to deliver 0.01 ml. 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 37^0 C aerobically for 24 hrs and for 48 hrs in negative cases.
They were then examined for bacterial growth. A significant bacterial count was taken as any count equal to or in excess of 100,000 CFU/ml. A less than 100 CFU/ml was interpreted as negative.

2.5.1. Cultural observation

Color, size, and colony morphology are observed from the incubated plates.

2.5.2. Microscopic examination of pathogens

Slides were prepared from each different colonies observed on the plates and gram staining was performed. The results such as the gram positive or gram negative, shape of the bacteria are observed from the examinations.

2.6. Biochemical examination

The selected colonies based on the cultural, microscopic and microbiological examinations, were subjected to biochemical examination (starch hydrolysis, lipid hydrolysis, casein hydrolysis, triple sugar iron agar test, oxidase test, catalase test, nitrate reduction test, indole production test, methyl red test, voges-proskauer test, citrate utilization test, urease test) for confirmation of the pathogens (Cheesbrough, 2006).

2.7. 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 10 µg, Amikacin 30 µg, Aztreonam 30 µg, Cefepime 30 µg, Ceftriaxone 30 µg, Ceftazidime 30 µg, Cefixime 5 µg, Cefoxitin 30 µg,

Cloxacillin 5 µg, Cotrimoxazole 25 µg, Ciprofloxacin 5 µg, Imipenem 10 µg, Gentamicin 10 µg, Meropenem 10 µg, Netilmicin 30 µg, Nitrofutantion 300 µg, Levofloxacin 5 µg Vancomycin 30 µg, Linezolid 30 µg, Netilmicin 30 µg, Penicillin 10 µg. Reference strains of *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 were used for quality control for antimicrobial susceptibility tests (CLSI, 2014).

2.8. Statistical analysis

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

3. Results

Out of 553 urine samples, the culture positive ($\geq 10^5$ cfu/ml) was found 158 (28.57%) of which 39 (24.70%) were isolated from male patients and 119 (75.30%) from female patients and other 395 (71.43%) sample were negative or normal flora (Table 1).

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, which were 38 (17.92%) cases positive; among them 34 (98.12%) were female and 4 (1.88%) cases were male. In the age group of less than 20 years' female is also predominant than male which is 16 (61.54%) cases and 10 (38.46%) cases respectively. Overall female was more common than male, shown in Table 2.

Table 2. Age and sex distribution of 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 have been the gram negative bacilli which accounts for 66.45% of the total positive isolates. In 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 (Table 3). In gram positive organism identified was 27.85% which is followed by *Staphylococcus* spp. (16.45%) and *Enterococcus* spp. (13.39%). *Candida* spp. was responsible for (5.70%) of cases (Table 3).

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

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

Antibiotic Susceptibility	Gram negative isolates					Gram positive isolates	
	E. coli	***Klebsiella***	***Acinetobacter***	***Pseudomonas***	***Proteus***	***Staphyloccus***	***Enterococcus***
	n=69(%)	**n=21 (%)**	**n=7 (%)**	**n=6 (%)**	**n=2(%)**	**n=26 (%)**	**n=18 (%)**
Ampicillin	3(4.34)	3(14.28)	0(0.00)	0(0.00)	0(0.00)	2(7.69)	16(88.88)
Cephradine	6(8.69)	5(23.80)	0(0.00)	0(0.00)	1(50.00)	-	-
Cefriaxone	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)

4. Discussion

This study provides valuable data to isolate and 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 (Behzadi *et al.,* 2010). In our study isolation & 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 and Barber *et al.,* 2013 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. (Barber *et al.,* 2013).

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 females. Akhter *et al.,* 2012 and Yusuf *et al.,* 2015 was found 71.6 % and 66.6% respectively.

It is well established that female is more commonly infected with UTI than male due to anatomical position of urethra, influence of hormone and pregnancy **(**Jones *et al.,* 1999) 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 species was *E. coli* 43.67% followed by *Staphylococcus* spp. 16.45%, *Enterococcus* spp. 13.39 %, *Klebsiella* spp. 13.29%, *Acinetobacter* spp. 4.43%), *Candida* spp. 5.70%, *Psudomonas* spp. 3.80% and *Proteus* spp. 1%. These results agree with Alshwaikh *et al.,* 2014 who found that *E. coli* (46.66%) followed by *Staphylococcus* spp. (15.8%), and also agree with Shaaban *et al.,* 2012 who found that *E. coli* 43% followed by *Klebsiella pneumonia* 14.1%, *Pseudomonas aeruginosa* and *Proteus mirabilis* 9.4%.

Among the different uropathogens, the most predominant organism was found to be *E. coli* 43.67 %, which is confirmed to the study done by Oluremi *et al*., 2011 and also in study conducted by Chakupurakal *et al.,* 2010

where predominant organism was *E. coli* and in number of reports worldwide where the particular organisms is identified as most common uropathogens. The dominance of *E. coli* is followed by *Staphylococcus spp.* 16.45% in this study resembles to the study done by Jha *et al.,* 2005 which was the second most organisms causing UTI. In sectional study by University of Florida USA of a group of patient, 81 patients met the inclusion criteria of this study of these 81 patients 89% had UTI *E. coli* (Mcloughlin *et al.,* 2003).

Antibiotic susceptibility test reveals that higher percentage of susceptibility for Imipenem & Meropenem 96%, followed by Nitrofurantoin (88%), Amikacin (88%), Netilmicin (88%), shown in Table 4. This study resembles to the study conducted by Farjana *et al.,* 2009 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.,* 2012 which show that isolates of most of the species exhibited a high rate of resistance to ampicillin. Resistance to antibiotics develops due to its frequent misuse. (Nerukar *et al.,* 2012)

According to the above result, 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* have maximum sensitive to nitrofurantoin. Almost all gram negative organisms are very less sensitive to cephlosporin and flouroquinolones groups, *Pseudomonas species* was found resistant against all commonly used and maximum sensitive was imepenem, meropenem. The results of present study show higher rate of resistance in a private diagnostic laboratory, which may be a result of the irrational use of antibiotics (Dund *et al.,* 2015).

Staphylococcus & *Enterococcus spp*. was sensitive to Linezolid (100%), Vancomycin (94%). This is comparable with works of Dund *et al.,* 2015. 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 (Aderounmu *et al.,* 2006)

5. Conclusions

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 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.

Conflict of interest

None to declare.

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