

Antimicrobial Sensitivity Pattern of *Escherichia coli* Causing Urinary Tract Infection in Bangladeshi Patients

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Abstract Objectives: Urinary tract infection (UTI) is a common bacterial infection in the Bangladesh community. There has been an increasing resistance by Escherichia coli to the commonly available antibiotics. The aim of the present study was to determine the prevalence of UTI, the common causative bacteria & antimicrobial susceptibility patterns of E. coli responsible for urinary tract infections (UTIs) to currently used antimicrobial agents. Methods and Results: In this study, three hundred urine specimens from clinically suspected UTI patients were collected from both outpatient and inpatient department during the period of February 2015 to January 2016 from a tertiary level hospital in the central part of country. The inclusion criteria included patients presenting with symptoms suggestive of UTI at the study site and who gave informed written consent to participate in the study. The exclusion criteria included patients on antibiotics within the last 2 weeks, and those with recent history of instrumentation. The urine samples received were processed using standard methods. Antimicrobial sensitivity patterns were performed on all E. coli isolates obtained from urine samples by disc diffusion method. Among 300 urine samples, (59%) yielded significant bacteriuria; 123 samples (41%) showed no growth. Out of 177 urine samples which showed significant bacterial growth, 72 (40.7%) samples comprised of males and 105 (59.3%) of females. Females within the age group of 20-29 years(26.67%) and elderly males of >60 years(34.7%) showed higher prevalence of UTI. 75.7% of isolates were found to be Escherichia coli, 7.9% Klebsiella pneumoniae, 5.6% Proteus mirabilis, Pseudomonas aeruginosa 5.1%, 1.7% Enterococci faecalis, 2.8% Staphylococci saprophyticus and 1.1% were Staphylococcus aureus. E. coli as the predominant cause of UTI, showed the highest percentage of resistance to cotrimoxazole, nalidixic acid and amoxicillin. The isolates were most sensitive to Imipenam, Meropenam, Nitrofurantoin and Amikacin. Klebsiella pneumoniae was the second most prevalent pathogen. Conclusion: E. coli was the most frequent isolate. Imipenam, Meropenam, Nitrofurantoin and Amikacin were shown to be very effective against E. coli organisms.

Keywords: Urinary Tract Infections, Causative agents, Escherichia coli, Antimicrobial resistance

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1. Introduction

Urinary tract infections (UTIs) are one of the most common infectious diseases, and nearly 10% of people will experience a UTI during their lifetime. [1,2] The prevalence is increased by several factors. Poor socioeconomic status is reported to be a major risk factor with indigent patients having a fivefold increased risk. [3] Other risk factors include increased age, high parity, poor perineal hygiene, history of recurrent UTI, diabetes mellitus, neurogenic bladder retention, anatomic or functional urinary tract abnormality, and increased frequency of sexual activity. [4,5] UTI affect patients in all age groups and both sexes. [6] 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. [7] The infections may be symptomatic or asymptomatic, and either type of infection can result in serious sequelae if left untreated. [8]

Although several different microorganisms can cause UTIs, including fungi and viruses, bacteria are the major causative organisms and are responsible for more than 95% of UTI cases. [9] Organisms that cause UTI are those from the normal vaginal, perineal, and fecal flora. [10,11] The vast majority of uncomplicated UTIs are caused by the Gram negative bacillus Escherichia coli, with other pathogens including Enterococci, Staphylococcus saprophyticus, Klebsiella spp. and Proteus mirabilis. [12] Escherichia coli is the most prevalent causative organism of UTI and is solely responsible for more than 80% of these infections. [12,13] An accurate and prompt diagnosis of UTI is important in shortening the disease course and for preventing the ascent of the infection to the upper urinary tract and renal failure. [13]

There have been reported cases of resistance to antibiotics by these UTI causing organisms. [14,15] The

extensive and inappropriate use of antimicrobial agents has invariably resulted in the development of antibiotic resistance which, in recent years, has become a major problem worldwide. [16] Following frequent use of broad spectrum antibiotics, the prevalence of these resistant bacteria is mainly due to widespread use of antibiotics in people and animal feeds. [14,17] These resistance properties are easily transferred between bacteria of different genera through plasmids and other means. To ensure appropriate treatment, knowledge of the organisms that cause UTI and their antibiotic susceptibility is mandatory. [18]

Therapy is based on information determined from the antimicrobial resistance pattern of the urinary pathogens. However, because of the evolving and continuing antibiotic resistance phenomenon, regular monitoring of resistance patterns is necessary to improve guidelines for empirical antibiotic therapy. [13,19,20]

The pathogens causing UTIs are almost always predictable [21], with *Escherichia coli* being the primary etiologic agent among both outpatients and inpatients accounting for 75 to 90% of urinary tract infection isolates. [22] Therefore, constant monitoring of drug resistance is required because only limited data describing multidrug resistance among UTI isolates is available. [21]

2. Methods

This prospective study was carried out in Centre for kidney disease & Urology Hospital, Dhaka during the period of February 2015 to January 2016. The study was conducted after due ethical approval which was subjected to the hospital administrations. After providing written informed consent, every patient was asked about symptoms suggestive of UTI (e.g., urgency, dysuria, urinary frequency, loin pain, and nausea). Midstream urine specimens from three hundred clinically suspected UTI patients that fulfilled the above criteria were selected. The participants were also selected on the basis of inclusion and exclusion criteria. The patients comprised both sexes and all age groups. Samples were collected using a sterile container that was refrigerated (4°C), and processed within 1 hour of collection. These samples were subjected to routine microscopy, culture, and sensitivity according to standard practice. A loopful urine sample was inoculated on MacConkey agar and blood agar plates and incubated aerobically at 37°C overnight and for 48 h in negative cases and read to assess growth of significant bacteriuria. A specimen was considered positive for UTI if an organism was cultured at a concentration of at least 105 single bacteria colonies per mL of urine and>5 pus cells per high-power field were observed on microscopic examination. [23] Then isolates were Gram-stained. Bacterial identification was based on standard culture and biochemical characteristics of isolates. E. coli was identified as medium, pink-to-red colonies and confirmed by positive indole test, whereas K. pneumonia were large, pink-to-mauve colonies, which were confirmed by negative oxidase and indole tests. P. mirabilis was assessed as small pale-to-colorless colonies testing negative to indole and oxidase and positive to urease. Enterococcus faecalis identified by the presence of small,

turquoise colonies with coccoid morphology, which tested negative for catalase and positive for bile esculin. *Pseudomonas aeruginosa* can be isolated as clear colonies which will test positive for oxidase. Confirmatory tests include production of the blue-green pigment pyocyanin. *Staphylococcus aureus* was identified with the corresponding laboratory tests: catalase, coagulase, and mannitol test. Identified and pure isolates were maintained in nutrient agar slants and incubated at 37°C for 24 hrs. *S. saprophyticus* was identified as catalase positive, coagulase-negative and novobiocin-resistant species.

The disc diffusion method was used to determine the antimicrobial susceptibility of isolates. Standard inoculums were inoculated on Muller-Hinton agar and incubated at 37°C for 24 h. After 24 h, antimicrobial susceptibility and resistance was determined by isolate growth zone diameter.

3. Results

Of the 300 tested samples, total 177(59%) urine samples showed significant bacterial growth The majority 105(59.3%) of the isolates were from female while the remaining were from male. The highest prevalence of UTI in females was found in the age group of 20-29 years (26.67%); however in males the highest susceptible age group to UTI was ≥ 60 years (34.7%). (Table 1).

Table 1. Age and sex distribution of patients with positive UTI

Age (years)	UTI group(Male) (n=72)		UTI group (Female) (n=105)	
	n	%	n	%
0-9	6	8.3	2	1.9
10-19	3	4.2	9	8.57
20-29	6	8.3	28	26.67
30-39	9	12.5	27	25.71
40-49	9	12.5	12	11.43
50-59	14	19.5	15	14.29
<u>></u> 60	25	34.7	12	11.43

Table 2. Distribution of organisms isolated from patients with urinary tract infection

Bacterial pathogens	Frequency (%)	
Escherichia coli	134(75.7%)	
Klebsiella pneumoniae	14 (7.9%)	
Pseudomonas aeruginosa	9(5.1%)	
Proteus mirabilis	10(5.6%)	
Enterococci faecalis	3 (1.7%)	
Staphylococcus saprophyticus	5 (2.8%)	
Staphylococcus aureus	2 (1.1%)	

75.7% of isolates were Escherichia coli, 7.9% Klebsiella pneumoniae, 5.6% Proteus mirabilis, Pseudomonas aeruginosa 5.1%, 1.7% Enterococci faecalis, 2.8% staphylococci saprophyticus and 1.1% Staphylococcus aureus.

Table 3. Anti-microbial sensitivity pattern of Escherichia coli

Antibiotics	E.coli(134)			
Antibiotics	Sensitive	Resistant		
Imipenam	121(90.3%)	13(9.7%)		
Meropenam	120(89.6%)	14(10.5%)		
Nitrofurantoin	119(88,8%)	15(11.2%)		
Amikacin	117(87.3%)	17(12.7%)		
Levofloxacin	96(71.7%)	38(28.4%)		
Ceftazidime	86(64.2%)	48(35.8%)		
Netilmicin	84(62.7%)	50(37.3%)		
Mecillinam	79(58.9%)	55(41.1%		
Ceftriaxone	72(53.7%)	62(46.3%)		
Gentamycin	70(52.2%)	64(47.8%)		
Cefuroxime	68(50.8%)	66(49.3%)		
Ciprofloxacin	43(32.1%)	91(67.9%)		
Amoxycillin	19(14.2%)	115(85.8%)		
Nalidixic acid	17(12.7%)	117(87.3%)		
Cotrimoxazole	3(2.2%)	131(97.8%)		

E. coli isolates showed resistance to co-trimoxazole, nalidixic acid and amoxicillin in larger percentage respectively. E. coli isolates were highly sensitive to Imipenam, Meropenam, Nitrofurantoin and Amikacin

4. Discussion

Our study showed a high prevalence of UTI in females (59.3%) than in males (40.7%) which correlates with other findings which revealed that the frequency of UTI is greater in females as compared to males. [24,25] 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. [26,27]

Females of the age group 20–29 years were found more susceptible (26.67 %) to UTI. These findings correlate with other reports which showed that females are more prone to UTIs than males during adolescence and adulthood. [28,29] The factors of this increasing incidence of UTI in young age females are associated with high sexual activity, recent use of a diaphragm with spermicide, and a history of recurrent UTIs. [30]

In our study it was found that the elderly males (≥60 years) had a higher incidence of UTI (34.7%) when compared with the elderly females. This finding is similar to a study conducted at a tertiary care hospital in Jaipur, Rajasthan, India. [31] The main cause behind this increasing incidence of UTI with advancing age in males is due to prostate enlargement and neurogenic bladder. [25] This factor is also reported by other authors whose studies showed that the prostate disease in males is responsible for the increase in incidence of UTI and decrease in female: male ratio in patients above 50 years. [32]

Of the 300 tested sample, 177 samples showed growth of pathogens among which the most prevalent were *E. coli* 134(75.7%) followed by *Klebsiella pneumoniae* 14(7.9%). Other isolated bacteria from UTI cases were *P.aeruginosa* (5.1%), *Proteus mirabilis* (5.6%), *S.aureus* (1.1%),

Enterococci faecalis(1.7%) and S.saprophyticus (2.8%). Our study, as with previous studies, shows that E. coli is the predominant etiology of UTI. [33,34,35] The reason of the highest rate of E.coli is that they are normal fecal flora and uropathogenic strains of E.coli have an adherence factor called P fimbriae, or pili, which mediate the attachment of E.coli to uroepithelial cells. [36]

Our results also correlates with others in which *Klebsiella pneumoniae* was reported as the second most frequently isolated organism in UTI. [37,38] The results of our study show that among the heterogeneous causative organisms of UTI, Enterobacteriaceae are the predominant pathogens, followed by Gram-positive cocci. These findings are consistent with reports published from other countries. [39,40]

The highest percentages of resistance of Escherichia coli causing urinary tract infections were found for cotrimoxazole (97.8%), nalidixic acid (87.3%), amoxicillin (85.8%), whereas the highest percentages of sensitivity were seen for imipenem (90.3%), meropenem (89.6%), nitrofurantoin (88,8%) and amikacin (87.3%). These results correlates with a study done in Comilla Medical College, Bangladesh. [41] Khotaii et al. reported resistance rates of 87.5% to ampicillin, 67.5% to trimethoprim sulfamethoxazole. [42] This significantly higher bacterial resistance to antibiotics in our region may be due to a higher rate of antibiotic usage, even in the absence of a prescription. Reducing the number of prescriptions of a particular antibiotic can lead to a decrease in resistance rates. [43,44] Another study conducted in India showed that meropenem was highly sensitive against Gram negative bacilli. [45] In other study, meropenem and imipenem were found to be 98% and 100% sensitive, respectively, against highly resistant gram negative bacilli. [46] A study done in King Fahd Hospital, Saudi Arabia showed that meropenem was 95.8% sensitive followed by amikacin (93.7%) and imipenem (91.71%) against extended spectrum β lactamase producing E. coli. [47]

5. Conclusion

It is concluded that most of the urinary tract infections in human are caused by *E. coli*. Regular monitoring of antimicrobial susceptibility for E.coli is recommended to improve treatment.

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