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Spectrum of Antimicrobial Susceptibility of Escherichia coli Isolated from Urine Samples of a Tertiary Care Hospital of Bangladesh

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Abstract— *Escherichia coli* is a major etiological agent of Urinary tract infection (UTI) worldwide. People of all ages and gender are prone to UTI, but age over 20 years and women are more susceptible. The purposes of this study were to identify the *E. coli* isolates from suspected UTI samples and determine the prevalence and antimicrobial susceptibility pattern of the isolates. A total of 200 midstream urine samples were collected from suspected UTI patients between January 2016 and May 2016 from a tertiary care hospital. Presence of *E. coli* isolates in 138 samples was confirmed after microbiological and biochemical tests. Antimicrobial susceptibility testing of *E. coli* isolates revealed high level of resistance to Nalidixic Acid, Cefotaxime, and Ceftazidime. High level of sensitivity was found to Amikacin, Tobramycin, Netilmicin which can be used as effective antimicrobials, but before that clinician should determine antimicrobial susceptibility of UTI pathogen of suspected patients. To avoid antibiotic resistance, use of antibiotics should be monitored and drug regulations must be strictly followed.

Keywords— *Escherichia coli*, Urinary Tract Infection, antimicrobial susceptibility, and antibiotic resistance.

1. Introduction

Escherichia coli are Gram-Negative, facultative anaerobe and nonsporulating bacteria. They belong to Enterobacteriaceae family and very common in human gut. Majority of *E. coli* strains are not injurious but some of them can cause illness. *E. coli* is often responsible for urinary tract infection (UTI) and other illness like diarrhea, abdominal pain, pyogenic infection, sepsis, fever and occasionally vomiting. [1] UTI has already become a great challenge for the clinicians because of frequent incidence, recurrence, complications and antimicrobial resistance [2]. *E. coli* is considered as leading pathogen to cause UTI worldwide and even rated for more than 80% UTI cases in several countries [2]. Women are much more vulnerable to UTI than man as uropathogenic *E. coli* easily enter the bladder of women through their short urethra which is near to anus [3]. Complicated UTI happens in patient with functional or physical anomalies of the genitourinary tract [2]. Diagnosis of UTI is usually done based on symptom, urine analysis and urine culture [4].

To treat *E. coli* generated UTI, antibiotic resistance is still a great barrier worldwide especially in developing countries where overuse and misuse of antibiotics are very common [5]. Moreover, drug regulations, guidelines and monitoring of use of antimicrobials are poor in developing countries. In last 25 years, clinical isolates of *E. coli* have exposed high prevalence of resistance to first line antibiotic [6]. This threat has become worse in case of emergence of multidrug resistant (MDR) *E. coli* isolates from clinical samples. MDR *E. coli* pathogenic

isolates often show resistance to three or even more antibiotics and therefore, responsible for great public health crisis with high morbidity and mortality [7].

The objectives of this study were to screen the clinical specimen of suspected UTI patients for the presence of *E. coli*, identify the prevalence of isolates among different age groups and determine the antibiogram of *E. coli* isolates.

2. Materials and Method

2.1. Samples. This study was conducted from June 2015 to December 2015 in the Department of Microbiology of Primeasia University, Banani, Dhaka. The study samples were obtained from clinical samples of UTI suspected patients admitted in a tertiary care hospital in Bangladesh. A total of 200 midstream urine samples were collected aseptically in sterile wide mouth glass containers from male and female patients of different age group and transported in ice-box to the laboratory immediately for urine analysis.

2.2. Isolation of E. coli. Quantitative bacteriuria was determined using 0.01mL calibrated wire loop to plate onto Blood agar, which allowed the growth of most of the bacteria including *E. coli*. The same steps were also done with MacConkey Agar to isolate gram-negative bacteria and Eiosine-Methylene Blue Agar to visualize characteristic features of *E. coli* colonies. All plates were incubated aerobically at 37^0C for 24 hours for growth. After incubation the number of colonies was calculated for the diagnosis of UTI. The culture showing at least 10^5 cfu/ml considered as pathogenic *E. coli* positive sample while less than 10^5 cfu/ml considered as non-significant growth of *E. coli*. Gram staining was performed from isolated colonies and then pure culture of isolated colonies was prepared to perform biochemical and physical examination to confirm the presence of *E. coli* isolates.

2.3. Biochemical identification. Several biochemical tests involved Indole test, Methyl red test, Voges-Proskauer test, Citrate utilization test, Urease test, Catalase test, Oxidase test, H_2S and Gas production test and Motility test were performed with the pure culture to identify *E. coli.*

2.4. Antimicrobial susceptibility testing. According to the Clinical and Laboratory Standards Institute (CLSI) guidelines Kirby Bauer's disk diffusion method was applied to determine the antibiogram of the isolates [8]. Commercially available discs (Oxoid Ltd, England) were used to measure antimicrobial susceptibility of the isolates. The concentration of drugs applied for the disc diffusion testing were Cefotaxime (30 µg), Co-trimoxazole (23.75 µg sulfamethoxazole/ 1.25 µg trimethoprim), Levofloxacin (5 µg), Ceftazidime (30 µg), Ceftriaxone (30 µg), Ciprofloxacin (30 µg), Gentamycin (10 µg), Amikacin (30 µg), Piperacillin-Tazobactam (10 µg) , Tobramycin (10 µg), Netilmicin (30 µg), Nitrofurantoin (30 µg) and Nalidixic Acid (30 µg) (Table 1). Inoculum was prepared to 2 ml Muller Hinton broth and incubated at 37^0C for 4 hours to develop fresh culture. The fresh culture then adjusted to McFarland 0.5 standard. A sterile cotton swab was immersed into the suspension and streaked on the surface of Muller Hinton agar plate. Then the plate was subjected to dry in room temperature for few minutes. Antibiotic discs were placed onto the surface of agar aseptically with sterile forceps and then plates were incubated at 37^0C for 24 hours. After incubation the diameters of zone of complete inhibition were measured by millimeter calipers.

2.5. Quality Control. Stranded reference strain *E. coli* (ATCC 25922) was used as quality control for culture and sensitivity testing. The zone of inhibition of isolates were classified as sensitive and resistant following the criteria of CLSI [9].

3. Result

A total of 200 urine specimens of UTI suspected patients were tested for the isolation of *E. coli* and followed by antimicrobial susceptibility was performed. Among 200 samples, 69% (138) showed positive growth of *E. coli* with colony count over 10^5 CFU/ml. The presence of *E. coli* isolates further confirmed by microbiological and biochemical tests.

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Among these 138 positive samples, 83 (60.14%) were female and 55 (39.86%) were male patients. Though different age groups of positive male and female showed different percentage, age category between 21-40 years showed the highest percentage (31.16%) of susceptibility among 138 positive samples. The next susceptible age group were 41 to 60 years with 34 positive samples (24.64%), over than 60 years with 31 positive samples (22.46%), and 0 to 20 years with 30 positive samples (21.74%) (Figure 1).

The positives isolates were subjected to Kirby Bauer's disk diffusion method to determine the susceptibility to common antibiotics. *E. coli* isolates exhibited highest level of resistance to Nalidixic Acid (93.48%), Cefotaxime (86.23%), Ceftazidime (86.23%), Co-trimoxazole (62.32%), Ceftriaxone (60.14%), Ciprofloxacin (56.52%), Levofloxacin (52.89%) and were highly sensitive to Amikacin (90.58%), Tobramycin (79.72%), Netilmicin (79.72%), Gentamycin (67.39%) and Nitrofurantoin (67.39%) (Table 1).

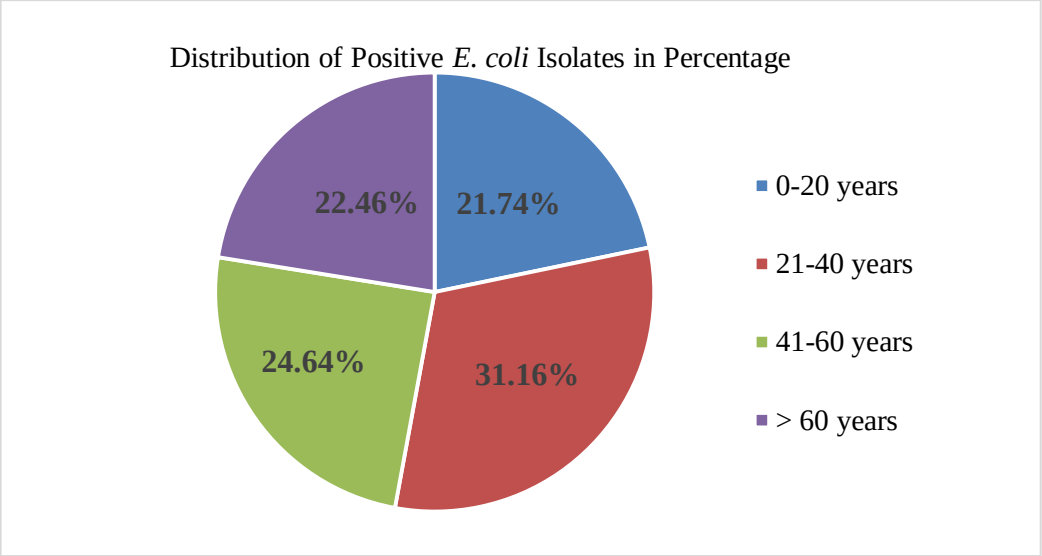

Figure 1: Distribution of *E. coli* isolates of urinary samples among different age groups

Table 1: Susceptibility of clinical isolates of *E. coli* to thirteen different antibiotics

SI. No	Antibiotic	Concentration	Resistant		Sensitive	
			Number	Percentage	Number	Percentage
1.	Cefotaxime (CTX)	30 µg	119	86.23	19	13.77
2.	Co-trimoxazole (COT)	23.75µg/1.25µg	86	62.32	52	37.68
3.	Levofloxacin (LE)	5 µg	73	52.89	65	47.11
4.	Ceftazidime (CAZ)	30 µg	119	86.23	19	13.77
5.	Ceftriaxone (CTR)	30 µg	83	60.14	55	39.86
6.	Ciprofloxacin (CIP)	30 µg	78	56.52	60	43.48
7.	Gentamycin (GEN)	10 µg	45	32.61	93	67.39
8.	Amikacin (AK)	30 µg	13	9.42	125	90.58
9.	Piperacillin-Tazobactam (PIT)	10 µg	49	35.50	89	64.50
10.	Tobramycin (TOB)	10 µg	28	20.28	110	79.72

11.	Netilmicin (NET)	30 µg	28	20.28	110	79.72
12.	Nitrofurantoin (F)	30 µg	45	32.61	93	67.39
13.	Nalidixic Acid (NA)	30 µg	129	93.48	9	6.52

4. Discussion

E. coli are widely distributed microorganisms in the environment and responsible for causing various types of infection in hospital and the community [5]. *E. coli* has been recorded as major etiological agent responsible for 70 to 80% UTI [10,11]. In this present study, 69% positive *E. coli* isolates were found among 200 study samples. Among 138 positive samples 60.14% were belonged to female patients while 39.86% belonged to male patients. Similar elevated prevalence rate of UTI incidence in female were reported in different studies conducted in Pakistan (69.80%), Saudi Arab (60.35%), Iran (86.24%) and Turkey (82.30%) [12,13,14,15]. Several factors including small urethra, deficiency of germicidal substances in prostatic fluid, hormonal imbalance is considered for the higher incidence of infection in female [3]. An epidemiological study conducted by Foxman, stated that almost 50% women would have UTI during their lifespan and alarmingly one in every three women would need antimicrobial medication to treat UTI before or during their early adulthood [16].

In this study, age category between 21 to 40 years was identified as most susceptible group (31.16%) to *E. coli* generated UTI. This outcome is somewhat consistent with the similar study conducted in Bangladesh by Tanzina *et al* where the age category between 31 to 45 was most vulnerable group [3]. Rest of the age categories revealed almost same susceptibility to *E. coli* which can be ordered as age category 41 to 60 years (24.64%), category greater than 60 years (22.46%) and category 0 to 20 years (21.74%) (Figure 1). Result of age category between 0 to 20 is alarmingly high in comparison with the study of Tanzina *et al* but consistent with the prediction of Foxman [16]. In all age groups, except over 60 years, female found highly susceptible to UTI. The infection rate was very high in females in 21 to 40 age group (72.09%), followed by 41 to 60 age (61.76%), 0 to 20 age (60%) while age group greater than 60 showed higher infection rate to male (58.06%). This study indicating that people of all ages and genders are significantly susceptible to *E. coli* generated UTI.

Antibiotic resistance of bacteria has become a global health concern. This resistance rate is very high in developing countries [17]. Few studies in Bangladesh showed very elevated resistance rate among uropathogens as well as *E. coli* isolates [3,18]. The present study also revealed increasing resistance result of *E. coli* isolates. In this study commercially available discs were used to determine antimicrobial susceptibility of *E. coli* isolates. The highest resistance was found to Nalidixic Acid (93.48%). This finding of high resistance to Nalidixic Acid correlates with the previous study in Bangladesh conducted by Setu *et al* where resistant rate was 63.31% [18]. Moreover, high level of resistance of UTI isolates to Nalidixic Acid have also been recorded in India (77.7%) and Saudi Arabia (50.6%) [13,19]. A study in Pakistan also showed increasing resistance (over 60%) of UTI strain against Nalidixic Acid [20]. On the other hand, similar study in Iraq found lower resistance rate (25.9%) of *E. coli* strain against Nalidixic Acid [21]. Resistance to Nalidixic Acid thought to be occurred due to overabundant usage of Nalidixic Acid as first-generation quinolone for treating UTI or acquiring Nal resistant *E. coli* through zoonosis [3,22]. In our present study next level of resistance were found to Cefotaxime (86.23%), Ceftazidime (86.23%), Co-trimoxazole (62.32%), Ceftriaxone (60.14%), Ciprofloxacin (56.52%), and Levofloxacin (52.89%). These findings significantly showed high level of resistance of *E. coli* isolates to third-generation cephalosporin which is inconsistent with another study in Bangladesh where resistance to all cephalosporin were less than 40 % [3]. Another study of the home country showed 56.23% of resistance of UTI strains against Ceftriaxone which supports our current findings [18]. Present results were also parallel with the report of India where isolates showed high level of resistance (66.58%) to third generation cephalosporin [23]. Several studies reported increasing rate of resistance of urinary *E. coli* to Co-trimoxazole worldwide [24,25]. In Iraq, among all cephalosporins, Cefazoline and Cefixime were found highly resistant [21]. Poor drug regulation and monitoring, distribution of antibiotic without prescription from local shops, improper choice of antibiotics during treatment, uncontrolled use of antibiotics, acquisition of resistant bacteria through selection process or by R plasmids are main reasons for antibiotic resistance [18].

From this study, it was found that more than 90% of the isolates were sensitive to Amikacin, followed by

Tobramycin (79.72%), Netilmicin (79.72%), Gentamycin (67.39%) and Nitrofurantoin (67.39%) (Table 1). These findings were resembling with the result of two different studies in Bangladesh suggesting that these antibiotics can be used as antimicrobial therapy to treat UTI patients in Bangladesh [3,18]. High level of sensitivity of UTI isolates to Amikacin were also found in Saudi Arabia (98.90%), southwest India (96.5%), Pakistan (87.3%) and Iraq (71.6%) [1,11,13,21]. From these studies, Gentamycin and Tobramycin were also reported as highly sensitive in India, Saudi Arabia and Iraq. Current result further strengthened by the study in Turkey where high level of sensitivity of urinary *E. coli* were found against Nitrofurantoin (98.9%) and Gentamycin (91%) [25]. From these findings, Amikacin, Tobramycin, Netilmicin, Gentamycin and Nitrofurantoin can be considered as effective antimicrobials to treat UTI but before that, antimicrobial susceptibility of UTI pathogen must be investigated.

5. Conclusion

In conclusion, it is clear that great numbers of UTI were caused by pathogenic *E. coli* and prevalence of UTI in women is higher than men. From the antimicrobial susceptibility profile, Amikacin, Tobramycin and Netilmicin were found effective for empirical therapy of UTI. It is also recommended that physician should investigate antibiotic susceptibility of UTI pathogen to prescribe a patient. To prevent antibiotic resistance, use of antibiotics should be monitored and controlled. Future studies are recommended to identify other isolates of UTI and use all antibiotics to determine elaborate pattern of susceptibility.

6. Conflict of Interests: The authors declare that there is no conflict of interests regarding the publication of this paper.

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