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Urine Analysis and Determination of Drug Sensitivity Pattern of Isolated *Escherichia coli* from Urinary Tract Infected Patients

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Authors' contributions

This work was carried out in collaboration between all authors. Authors KF, TA and SN designed the study. Authors SP, AC and MJH managed the experimental process and analyses of the raw data. Author TA wrote the protocol and the first draft of the manuscript. Authors TA and SB managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Urinary Tract Infections are one of the most common infections in medical practice. UTI is the common causes of complications, mortality, morbidity and economic loss especially in the developing countries like Bangladesh. Prediction of the agents causing UTI and knowledge of their antimicrobial susceptibility patterns is important for the empirical therapy of UTI.

Aims: The aim of the present study was to isolate *Escherichia coli* from urine samples of patients suspected with UTI and determination of antimicrobial susceptibility patterns of the isolated *E. coli* to commonly used antibiotics.

Place and Duration of Study: Urine samples were collected between February, 2015 to April, 2015 from a tertiary care hospital located in Dhaka city, the capital of Bangladesh. The study was

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conducted in Centre for Excellence Laboratory (CEL), Microbiology Department of Primeasia University, Dhaka-1213, Bangladesh.

Methodology: A total of 288 urine samples were collected during the study period. Urine sample was streaked on Cystine-Lactose-Electrolyte Deficient (CLED) agar, MacConkey agar, and blood agar media for isolation of *E. coli*. Gram staining and various biochemical tests were performed for confirmation of *E. coli.* Antimicrobial sensitivity test was determined by Kirby Bauer's disc diffusion method on Mueller Hinton agar plate using commonly used antibiotics. For statistical analysis, Statistical Package for Social Sciences (SPSS) software, version 20 was used in our study.

Results: Sixty-six (22.92%) samples showed positive culture for *Escherichia coli*. *E. coli* was more commonly isolated from female (51.51%) than male (48.48%) patients. Results from antimicrobial sensitivity revealed that, *E. coli* showed high level of sensitivity to Piperacillin-Tazobactam (100%), Tobramycin (100%), Netilmicin (100%), Meropenem (98.48%), Gentamicin (96.97%), Imipenem (95.45%), Levofloxacin (90.91%), Amikacin (87.87%), and Nitrofurantion (81.82%). This bacterium showed moderate level of sensitivity to Ciprofloxacin (60.60%) and Azithromycin (57.58%) and least level of sensitivity to Nalidixic acid (33.33%), and Co-trimoxazole (22.73%).

Conclusion: Therefore, it can be concluded that Piperacillin-Tazobactam, Tobramycin and Netilmicin could be most effective drugs in the study area for the empirical treatment of UTI.

Keywords: Urinary tract infections; Escherichia coli; antibiotic resistance.

1. INTRODUCTION

Urinary tract infections (UTIs) are the most common extra intestinal infections [1]. A urinary tract infection (UTI) is an infection that affects any part of the urinary tract. UTIs are characterized by symptoms such as dysuria, frequency, urgency, irritation of urinary tract, discomfortable pressure, bloody urine with strong smell, fever and flank pain [2]. UTIs affect people of all age from the neonate to the geriatric age group and both sex [3]. Different factors like age, gender, immuno-suppression, kidney tumors, pregnancy, stone, catheterization, neurological diseases, congenital or acquired anomalies of bladder, enlarge prostrate, diabetes mellitus etc. may affect the prevalence of UTIs [4]. During entire lifetime, nearly 10% of people will experience UTIs and in every year, about 150 million people are diagnosed with UTI across the world [5,6,7].

Microorganisms that cause UTIs almost come from the skin at or near the opening of the urethra. Occasionally, Fungi and viruses cause UTIs, but bacteria are most commonly reported as causative agents of UTIs. Among the Gram-negative bacteria, *Escherichia coli* is the most common etiological agent of UTI and approximately 90% of first UTI in young women are caused by *E. coli* [8]. The other Gram-negative pathogens usually associated with UTIs are *Klebsiella* sp., *Proteus mirabilis* and *Pseudomonas aeruginosa*. On the other hand, Enterococci, group B streptococci and coagulase negative *Staphylococci* are the most frequently encountered Gram-positive bacteria in UTI [9]. These microorganisms use different virulence factors and pathogenic mechanisms to colonize and infect the urinary tract.

Increasing bacterial resistance to antibiotics is a world-wide problem. The prevalence of antimicrobial resistance in patients with UTI is increasing as in almost all cases of UTI, empirical treatment initiates before the laboratory results of urine culture are available. Antimicrobial resistance can vary according to geographical and regional location [10,11]. For this reason, knowledge of the etiological agents of UTIs and their antimicrobial resistance patterns in specific geographical locations may aid clinicians to choose the appropriate antibiotics for empirical treatments. Hence, aim of the current study was to identify *E. coli* from suspected UTI infected males and females of different age groups, analysis of their sensitivity and resistance patterns against locally available antibiotics commonly prescribed by the clinicians in order to find suitable antimicrobial agents which may help the clinicians to choose the right empirical treatment for the study area.

2. MATERIALS AND METHODS

2.1 Sample Collection

This study was carried out in the Microbiology laboratory of the Department of Microbiology,

Primeasia University, Banani, Dhaka-1213. 288 urine samples were collected from patients attending the internal patient department (IPD) and outpatient department (OPD) of a tertiary care hospital located in Dhaka city. Patients of all age groups including age 15 to ≥48 having clinical evidence of urinary tract infection, determined by treating physicians, were included in this study. The duration of the study was three months from February, 2015 to April, 2015.

Clean catch midstream urine was collected from each patient into a 20 ml calibrated sterile screw-capped universal container which was distributed to the patients. After collection of the samples, they were transported to the Microbiology laboratory of Primeasia University as soon as possible in an insulated foam box with ice to maintain a temperature ranging from 4℃ to 6℃.

2.2 Chemical Analysis

COMBINA 11S dipstick was used for chemical analysis of the urine specimens within one hour of specimen collection. Specimens were thoroughly mixed several times by inversion and before analysis no centrifugation was done.

2.3 Microscopic Analysis

About 12 ml of each specimen was centrifuged at 1500 rpm for 5 minutes. The supernatant was discarded leaving only around 1 ml of pellet. The concentrated pellet was mixed well and 20 µl of the suspension was placed on a glass slide, the suspension was covered with cover slip and observed under microscope for the presence of pus cell, RBC, albumin, nitrate, glucose, ketone bodies in urine of UTI patients.

2.4 Microbiological Analysis

A loopful uncentrifuged urine sample was placed on Cystine-Lactose-Electrolyte Deficient (CLED) agar, MacConkey agar, and blood agar media. Inoculated plates were incubated at 37℃ for 24 h and for 48 h for negative cases. A sample was considered positive for pathogenic count of *E. coli* if the number of colonies was greater than 10^5 cfu/ml [12]. From discrete colonies, Gram staining and further sub culturing was done to obtain pure culture. *E. coli* was identified on the basis of standard culture and biochemical characteristics of isolates.

2.5 Antibiotic Susceptibility Tests

Antimicrobial susceptibility testing of the isolated *E. coli* was done by the standard Kirby Bauer's disc diffusion method on Mueller Hinton agar plate. Antimicrobial agents tested were Piperacillin-Tazobactam (10 µg), Tobramycin (10 µg), Netilmicin (30 µg), Meropenem (10 µg), Gentamicin (10 µg), Imipenem (10 µg), Levofloxacin (10 µg), Amikacin (30 µg), Nitrofurantion (30 µg), Ciprofloxacin (30 µg), Azithromycin (15 µg), Nalidixic acid (30 µg), and Co-trimoxazole (1.25/23.75 µg). By sterile pipette 0.1 ml of test culture was poured on plate and the culture used was spread homogenously on the medium using sterile glass spreader. Antibiotic discs were applied aseptically to the surface of the inoculated plates after 3 to 5 minutes with the help of sterile forceps. The plates were then inverted and incubated at 37℃ for 24 h. After incubation, the plates were examined and the diameters of the zone of complete inhibition were measured in mm by following the recommendations of the criteria of the Clinical and Laboratory Standards Institute (CLSI) [13]. *Escherichia coli* (ATCC 25922) was used as quality control strains in our study.

2.6 Statistical Analysis

Chi square test was conducted to find out the significant difference between male and female of different age groups in relation to the prevalence of *E. coli*. *P* value of <0.05 at 95% level of confidence interval was considered statistically significant. The statistical analysis was performed by the Statistical Package for Social Sciences (SPSS) software, version 20.

3. RESULTS

Over a 3-month period, 288 urine samples from inpatients and outpatients departments of a tertiary care hospital were analyzed. Total 66 (22.92%) urine samples showed the significant growth of *E. coli*. Among the positive sample, 45 (68.18%) patients were from urban and rest 21 (31.82%) from rural. The percentage of illiterate and literate was 39.39% and 60.61% respectively. In according to socioeconomic condition, 20 (30.30%) were recorded as poor, 40 (60.61%) were medium and 6 (9.09%) were rich. In our study, married patients were higher (77.27%) than single (22.73%) patients (Table 1).

Table 1. Demographic factors of positive sample of the study population

Variables	Number (%)
Location	
Urban	45 (68.18%)
Rural	21 (31.82%)
Educational level	
Illiterate	26 (39.39%)
Literate	40 (60.61%)
Socioeconomic condition	
Poor	20 (30.30%)
Medium	40 (60.61%)
Rich	6 (9.09%)
Marital status	
Single	15 (22.73%)
Married	51 (77.27%)

34 (51.51%) samples from females and 32 (48.48%) from males showed positive growth for *E. coli.* These results indicated that the prevalence of UTI caused by *E. coli* was higher in female patients than in male. From Chi square (χ2) test, it was also found that the prevalence of the *E. coli* was significantly different (P < 0.00001) between female and male of different age groups at 95% confidence interval level (χ2 =24.53; degree of freedom = 3) (Table 2).

The highest susceptible age group of female patients to UTI was 15-25 (41.18%) followed by 26-36 years (32.35%), 37-47 years (14.71%), and ≥48 years (11.76%); however in males the highest susceptible age group was ≥48 years (62.50%) followed by 37-47 years (21.88%), 26-36 years (9.38%), and 15-25 (6.25%) (Table 2).

The frequency of common urinary abnormal findings according to male and female patients in urine such as pus cell, RBC, albumin, glucose, nitrate and ketone bodies are presented in Fig. 1. The common urinary abnormal findings for female patients are RBC (76.40%) followed by pus cell (73.21%), nitrate (66%), ketone bodies (60%), glucose (55.55%), and albumin (53.76%). On the other hand, for male patients the common urinary abnormal findings are albumin (46.24%) followed by glucose (44.44%), nitrate (44%), ketone bodies (40%), pus cell (26.79%), and RBC (23.60%) (Fig. 1).

Table 2. Distribution of *E. coli* in relation to sex and age of patients

Age group	Male (%)	Female (%)	Chi square (χ2) value	*P* value
15-25	2 (6.25)	14 (41.18)		
26-36	3 (9.38)	11 (32.35)		
37-47	7 (21.88)	5 (14.71)	24.53	<.00001
≥48	20 (62.50)	4 (11.76)		
Total	32	34		

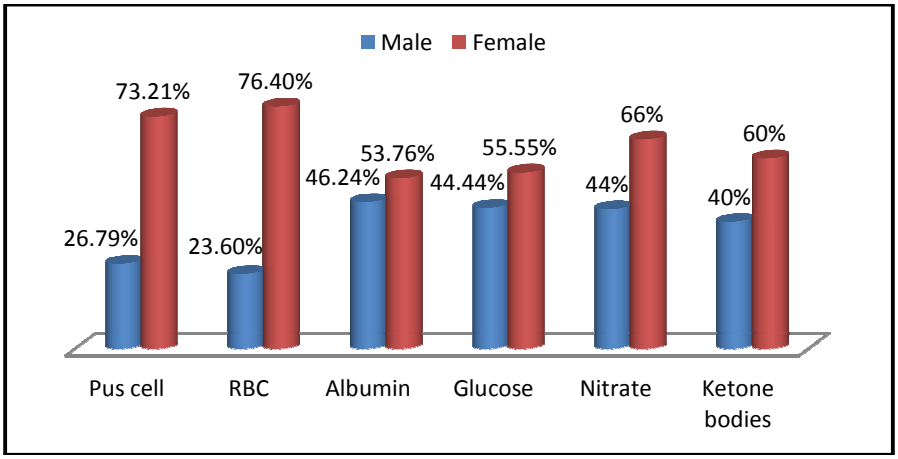

Fig. 1. Frequency of sex based distribution of pus cell, RBC, albumin, glucose, nitrate, and ketone bodies in UTI patients

Antimicrobial susceptibility test revealed that *E. coli* isolates were highly sensitive to Piperacillin-Tazobactam, Tobramycin, Netilmicin, Meropenem, and Gentamicin followed by Imipenem, Levofloxacin, Amikacin, and Nitrofurantoin and least level of sensitive to Nalidixic acid and Co-trimoxazole (Table 3).

Table 3. Antimicrobial sensitivity pattern of *E. coli* against commonly used antibiotics

Antibiotic's name	*E. coli* (n=66)	
	Sensitive	Resistant
Piperacillin-Tazobactam	100% (66/66)	0% (0/66)
Tobramycin	100% (66/66)	0% (0/66)
Netilmicin	100% (66/66)	0% (0/66)
Meropenem	98.48% (65/66)	1.52% (1/66)
Gentamicin	96.97% (64/66)	3.03% (2/66)
Imipenem	95.45% (63/66)	4.55% (3/66)
Levofloxacin	90.91% (60/66)	9.09% (6/66)
Amikacin	87.87% (58/66)	12.12% (8/66)
Nitrofurantion	81.82% (54/66)	18.18% (12/66)
Ciprofloxacin	60.60% (40/66)	39.39% (26/66)
Azithromycin	57.58% (38/66)	42.42% (28/66)
Nalidixic acid	33.33% (22/66)	66.67 (44/66)
Co-trimoxazole	22.73% (15/66)	77.27% (51/66)

4. DISCUSSION

In present study, 288 urine samples were collected of which 66 (22.92%) samples showed positive growth for *E. coli*. Result of our study revealed that female (51.51%) was more commonly infected with *E. coli* than male (48.48%) patients. This finding is similar with other studies conducted in various parts of the world [12,14,15,16]. A numbers of factors are responsible for high prevalence of UTI in females such as shorter and wider urethra, trauma of urethra during sexual intercourse, hormonal changes, and lack of prostatic fluid containing antimicrobial properties [17,18].

Female patients in the age ranges of 15-36 (41.18%) years were found highly susceptible to UTI than other age groups. Our finding is consistent with other studies conducted in India and Italy [19,20,21]. The reasons behind this high prevalence of UTI in this age ranges of female are sexual intercourse, use of contraceptive spermicidal agents, diaphragms, menopause and a history of recurrent UTIs. However, the highest susceptible age group for male was in the age ranges of ≥48 years (62.50%). Increasing incidence of UTI with advancing age in males is associated with prostate enlargement and neurogenic bladder [22]. It has been reported that the prostate disease is responsible for the increase in the occurrence of UTI in males and decrease in ratio between female and male patients above 50 years [23].

Escherichia coli was found 100% sensitive to Piperacillin-Tazobactam, Tobramycin and Netilmicin. A study conducted in 2016 by Akter T et al. [16] in Bangladesh where *E. coli* was found 100% sensitive to Piperacillin-Tazobactam and Netilmicin which support our study. Besides, 100% sensitivity to Tobramycin was also recorded in another study conducted by Sabir S et al. in Pakistan [24].

Furthermore, our study showed that *E. coli* was 98.48% sensitive to Meropenem, 96.97% to Gentamicin, 95.45% to Imipenem and 90.91% to Levofloxacin. Similar types of sensitivity were also recorded by other studies conducted in Bangladesh, Pakistan and Italy [16,21,24]. Sensitivity of *E. coli* to Amikacin and Nitrofurantion was found 87.87% and 81.82% respectively. Sabir S et al. [24] reported 99.6% sensitivity of *E. coli* to Amikacin and 95.1% to Nitrofurantion which were higher than our findings.

Escherichia coli showed moderate level of sensitivity to Ciprofloxacin (60.60%) and Azithromycin (57.58%). A study performed in India by Shalini JM et al. [25] where 69.56% sensitivity to Ciprofloxacin was recorded which is close to our result. Sensitivity to Azithromycin

(89%) was recorded in another study conducted in Bangladesh by Akter T et al. [16].

Escherichia coli showed least level of sensitivity to Nalidixic acid (33.33%) and Co-trimoxazole (22.73%). These findings are similar with another study where Manikandan S et al. [26] found sensitivity to Nalidixic (19.4%) acid and Co-trimoxazole (38.6%).

5. CONCLUSION

From our study, it can be concluded that females are more susceptible to UTI than male. Regular surveillance of antimicrobial susceptibility pattern for *E. coli* is recommended to improve empirical treatment for UTI and Piperacillin-Tazobactam, Tobramycin and Netilmicin could be the first choice of drug in the study area for the empirical treatment of UTI caused by *E. coli*.

CONSENT

All authors declare that written informed consent was obtained from the patients for publication of this paper and accompanying images.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethical committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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