Isolation, identification and antimicrobial susceptibility pattern analysis of *Escherichia coli* isolated from clinical samples of Bangladesh

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ABSTRACT :

Urinary tract infection (UTI) is one of the most common infections of the world caused by mainly *Escherichia coli*. The purpose of this study was to identify *E. coli* as causative agent of UTI in patient of different age groups and to investigate their responses against commonly used antibiotics. Altogether, 480 urine samples were analyzed by culture method. The samples were equally streaked on Blood agar, MacConkey, and Eosin Methylene Blue Agar and then incubated at 37°C for 24 hours. After 24 hours of incubation, *E. coli* was identified on the basis of morphological characteristics of colony on culture media. For further confirmation of the presence of *E. coli*, Gram staining and conventional biochemical tests were also performed. Disk diffusion method was used for susceptibility testing against seventeen different antibiotics on Muller Hinton agar. Among the 480 urine samples, 81 samples were positive for *E. coli*. It was found that the females were more prone to UTI than males. The result of antibiotic sensitivity test on *E. coli* isolates demonstrated that they were highly sensitive to Amikacin, Gentamycin, Netilmycin, Imipenem, Meropenem, Pipracillin-Tazobactam, Tobramycin, Nitrofurantoin , Azithromycin, Levofloxacin, and Ciprofloxacin. *E. coli* was found intermediate sensitive to third-generation Cephalosporins such as Cefixime, Cefotaxime, Ceftazidime, Ceftriaxone and least sensitive to Cotrimoxazole and Nalidixic acid. Thus, all antibiotics used in present study except Cotrimoxazole and Nalidixic acid, could be the choice for empirical treatment of UTI.

Keywords: Urinary tract infection, *Escherichia coli*, antibiotics, antimicrobial resistance.

INTRODUCTION:

E. coli is an important normal flora of human and other mammals. It is widely distributed in the environment and also causes a variety of infection including urinary tract infection. Urinary tract infection (UTI) is the most common infection of human population. UTI is defined as the persistence of actively growing microorganisms within the urinary tract. Microorganisms causing UTI almost come from the skin at or near the opening of the urethra. In case of UTI, most susceptible groups are neonates, girls, young women and men. In case of adult person, it occurs more commonly in women than men because the female urethra is much shorter and closer to anus, therefore up to 40% women develop UTI at least once during their lives and a significant numbers of these women suffer recurrent UTI [1]. On the other hand, the secretion of male prostate contains the bactericide substances and Zn which play a vital role in countering with *E. coli* and prevent this kind of infection in men. Although antibiotics are widely available, UTI still remains one of the most common clinical complications. Because now a day's antibiotic resistance has become an important phenomenon due to widespread use of antibiotic by patient without testing antibiogram. Antibiotic resistance results serious public health issue in the management of UTI particularly in developing country like Bangladesh. To ensure appropriate treatment, it is obligatory to have knowledge about the organisms causing UTI and their susceptibility to antibiotics [2]. Therefore, the purpose of this study was to isolate, and identify *E. coli* from clinical samples and to determine their susceptibility to commonly available antibiotics which may help the physician to choose appropriate treatment for the prevention of UTI.

MATERIALS & METHODS:

For this study, a total of 480 specimens were collected from a private hospital between June, 2015 to December, 2015. Using the standard operation procedures, clean-catch midstream morning urine specimens were collected using sterile wide mouth glass containers. Until laboratory analysis, the samples were then kept cooled in an ice-box. The time between sample collection and sample analysis did not exceed one hour. Using calibrated wire loops, 0.01 ml urine sample was plated onto Blood agar, MacConkey agar and Eiosin-Methylene Blue agar. The plates were then incubated aerobically at 37°C for 24 hours. The number of colonies was counted for the diagnosis of UTI. The samples showing number of colonies greater than 10^5 cfu /ml after 24 hours were considered as pathogenic count for *E. coli*. If the colony forming unit (cfu) remained less than 10^5 cfu / ml, it was considered as non–significant growth in case of *E. coli* or negative sample. From discrete colonies, Gram

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staining and further sub culturing was done to obtain pure cultures. A battery of biochemical tests was performed to identify *E. coli.*

Antimicrobial susceptibility testing was performed using Kirby Bauer's disk diffusion method [3] on Mueller-Hinton agar according to Clinical and Laboratory Standards Institute (CLSI) guidelines. The antibiotic discs and their concentrations consisted of Azithromycin (15µg), Cefotaxime (30 µg), Ceftazidime (30 µg), Ceftriaxone (30 µg), Ciprofloxacin (30 µg), Co-trimoxazole (1.25/23.75 µg), Gentamycin (10 µg), Cefixime (5 µg), Imipenem (10µg), Meropenem (10 µg), Levofloxacin (5 µg), Amikacin (30 µg), Netilmicin (30 µg), Tobramycin (10 µg), Piperacillin-Tazobactam (10 µg), Nitrofurantoin (30 µg) and Nalidixic acid (30 µg).

By the standard method of inoculation, an inoculating needle was touched to a single well-isolated colony and inoculated to 2 ml of Muller Hinton broth. The broth culture was then allowed to incubate at 37°C for 4 hours to obtain young culture. The turbidity of the actively growing broth cultures was then adjusted to a McFarland 0.5 standard (3×10^8 cfu/ml). To inoculate on Muller Hinton agar medium, a sterile non toxic cotton swab was dipped into the standardized suspension. The excess broth was purged by pressing and rotating the swab firmly against the inside of the tube above the fluid level. The swab was then streaked evenly in three directions over the entire surface of the agar plates to obtain a uniform inoculum. A final sweep was made of the agar rim with the cotton swab. This plate was then allowed to dry for 3 to 5 minutes, before the discs were applied.

Antibiotic impregnated discs were then applied to the surface of the inoculated plates with a sterile forcep. All discs were gently pressed down into the agar with sterile forcep to ensure complete contact with agar surfaces. Within 15 minutes, after the discs were applied, the plates were inverted and placed into an incubator at 37°C for 24 hours. The zone of inhibition was recorded in millimeter (mm). *E. coli* (ATCC 25922), was used as quality control strain. Isolates were considered as sensitive or resistant on the basis of zone of inhibition following the criteria of Clinical and Laboratory Standards Institute (CLSI) guidelines.

RESULTS & DISCUSSION:

A total of 480 urine samples were collected and analyzed. Among the cultures screened, 81 samples showed positive growth of *E. coli*. The positive growth of *E. coli* was confirmed by cultural, microscopic, and various biochemical tests which results are presented in Table 1.

Of these 81 positive cases, 17 (21%) isolates were from male and rest 64 (79%) isolates were from female (Figure 1). This result indicated that the female patients had higher prevalence of UTI than in males. This result is consistent with other studies performed in Turkey and Iran [4, 5]. A numbers of factors are associated with high prevalence of infection in females such as shorter and wider urethra in females than in males, lack of antimicrobial properties of prostatic fluid as in males, hormonal changes which affect the mucosal adherence of bacteria and trauma of urethra during sexual intercourse.

Features	*E. coli*
Colony on Blood agar	Non-hemolytic, large, gray, moist colony
Colony on MacConkey agar	Red colonies, circular, low convex, smooth, translucent, Lactose fermenters colony
Colony on Eosin-Methylene Blue agar	Colony with green metallic sheen
Gram staining	Gram negative, rod shaped, pink color
Voges-Proskauer	Negative
Methyl red	Positive
Indole	Positive
Motility	Positive
H_2S production	Negative
Gas production	Positive
Oxidase	Negative
Citrate utilization	Negative
Catalase	Positive
Urease	Negative

Table 1: Cultural, microscopic and biochemical properties of *E. coli*

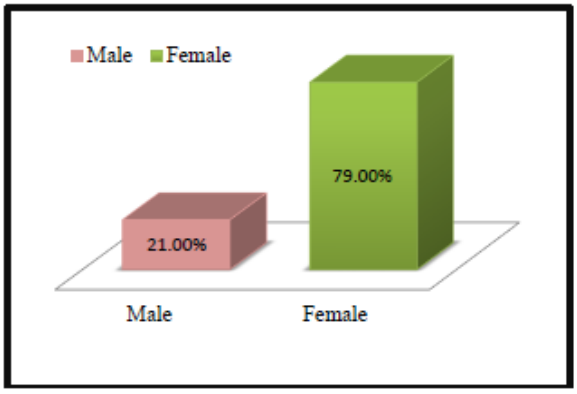

Figure 1: Prevalence of *E. coli* according to sex

The most susceptible age group of patients to UTI was 31-45 years (41.98%) followed by 16-30 years (27.16%), 46-60 years (19.75%), > 60 years (7.4%) and 0-15 years (3.7%) (Figure 2). This study suggests that UTI is more commonly occur in the age group between 16- 60 years. This reason of occurrence may be due to frequent sexual intercourse, use of contraceptive spermicidal agents, diaphragms and menopause for women and enlargement of the prostate gland for men. This type of finding has earlier been reported in India and Italy [6, 7, 8].

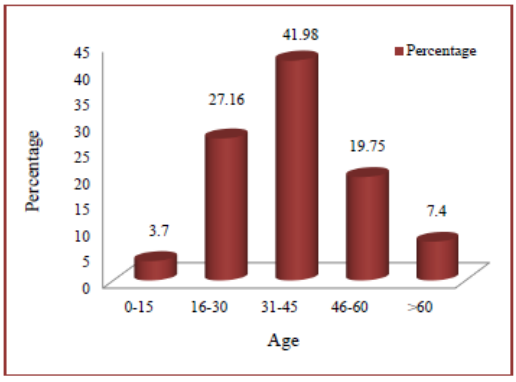

Figure 2: Prevalence of *E. coli* according to different age group

In the present study, the selected isolates were examined for their susceptibility to common antibiotics by disc diffusion method [3]. It was found that, 100% of the isolates were sensitive to Gentamycin, Netilmicin, Amikacin, Imipenem, Meropenem, Piperacillin-Tazobactam followed by 96% sensitive to Tobramycin (Figure 3). Previous studies conducted in India and Kenya also showed high sensitivity to Gentamycin [9, 10, 11]. 95.65% sensitivity to Amikacin, Imipenem and 91.30% sensitivity to Meropenem were found in another study conducted in India [12]. These findings were further supported by another study where the sensitivity rate of *E. coli* to Amikacin was found 93-100% [13]. For Piperacillin-Tazobactam, 90.6% sensitivity and for Tobramycin, 100% sensitivity was recorded in Pakistan [13]. Therefore, from this study it was found that Carbapenems (Imipenem, Meropenem), Aminoglycoside (Gentamycin, Netilmicin, Amikacin, Tobramycin) and Piperacillin-Tazobactam were the most sensitive drugs against the isolated *E. coli* strains.

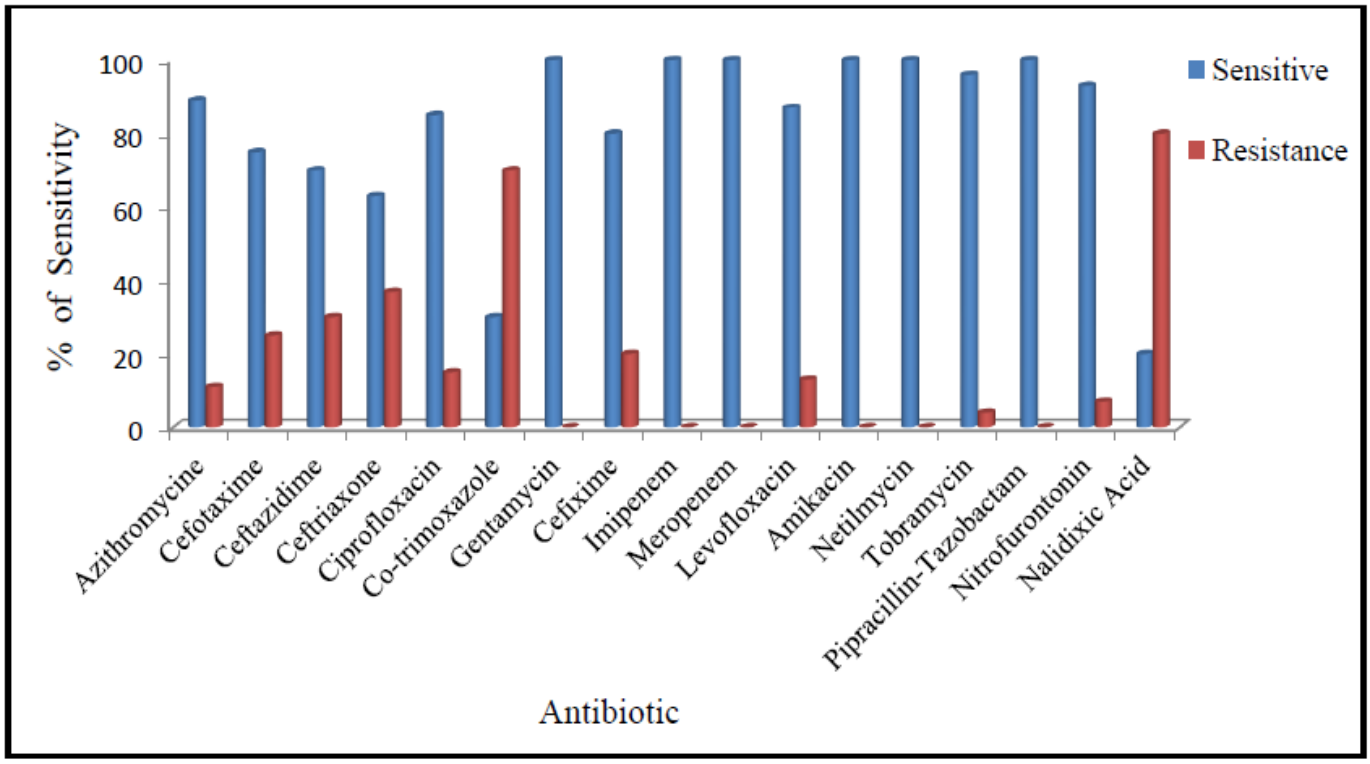

Figure 3: Antibiotic sensitivity pattern of *E. coli* against seventeen different antibiotics

From this study, sensitivity to Nitrofurantoin was found 93%, which is close to another study where sensitivity to Nitrofurantoin was found 93.48% [14]. In another study where Grude and his colleague found 97% sensitivity to Nitrofurantoin. [15]. High-level sensitivity of *E. coli* to Nitrofurantoin may reflect limited indication, narrow spectrum of activity, narrow tissue distribution, and limited contact of this antibiotic with bacteria present outside of the urinary tract [16].

In case of Macrolide such as Azithromycin, 89% of the isolates were found sensitive. In a study conducted in India, it was found that more than 60% isolates showed sensitivity to Azithromycin which is lower than our present study [17].

Antimicrobial sensitivity to third-generation Cephalosporins such as Cefixime, Cefotaxime, Ceftazidime, and Ceftriaxone *were found 80%, 75%, 70% and 63% respectively. This result is similar to another study conducted in Iran where more than 60% isolates were sensitive to third-generation* Cephalosporins [1].

Quinolones group antibiotic especially second and third generation Quinolones for example Ciprofloxacin and Levofloxacin were found effective against 85% and 87% isolates but first generation Quinolone such as Nalidixic acid was found effective only to 20% isolates. Increased resistance of *E. coli* against first generation Quinolones may be due to the overuse of these drugs for the treatment of UTI [18] or generalized use of fluroquinolones in animals feed which in turn lead to the transmission of resistance mechanism to strains from animals to humans [19].

From this study, it can be inferred that Co-trimoxazole and Nalidixic acid were virtually useless against *E. coli*, because they were sensitive against 30% and 20% of the isolated organisms respectively. Similar result was also found in a study which showed that the Co-trimoxazole and Nalidixic acid were sensitive to 18% and 22% of *E. coli* isolates [14]. It has been reported in studies from Nepal and other countries that resistance of *E. coli* to Co-trimoxazole is increasing day by day [20, 21, 22, 23, 24].

CONCLUSION:

Our result suggests that the incidence of UTI was higher in females than males. The bacterial sensitivity profile reveals that Carbapenems, Aminoglycoside, Piperacillin-Tazobactam, Nitrofurantoin, Ciprofloxacin, Levofloxacin, Azithromycin, and third-generation Cephalosporins are highly effective and Co-trimoxazole and Nalidixic acid were least effective against the isolated *E. coli*. It is recommended that for appropriate treatment and prevention of bacterial resis-

tance, the clinicians should prescribe antibiotic after having the culture sensitivity results.

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