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INCIDENCE OF ANTIBIOTIC RESISTANT *ESCHERICHIA COLI* IN UTI SUSPECTED PATIENTS-A SINGLE CENTERED STUDY

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The evaluation of antimicrobial sensitivity patterns has a significant role in prescribing antimicrobial drugs to treat patients suffering from urinary tract infections (UTIs). This study assessed the etiology and antimicrobial susceptibility of the common uropathogens as those were detected in Dhaka, Bangladesh. This study was conducted following the culture and identification of pathogenic microorganisms from patients of all age groups and sexes. A total of 110 urine samples were collected from suspected UTI patients for culture and sensitivity as advised by the physicians. Female patients (76.4%) were dominant over male patients (23.6%) and patients were 0 to 90 years of age. From these samples, 25 *Escherichia coli* were isolated from culture-positive cases to determine antibiotic sensitivity patterns. Most of these isolates were resistant to Azithromycin (100%), Nalidixic Acid (80%), Cotrimoxazole (54%), Ciprofloxacin (44%) and Cefixime (44%). The present study emphasized the necessity of standard microbiological screening techniques to evaluate the etiological characteristics required to effectively treat UTI patients.

Keywords: *Escherichia coli*, Urinary Tract Infection (UTI), biochemical test, antibiotic sensitivity, multi-drug resistance, Dhaka

INTRODUCTION

Urinary tract infections (UTIs) are the second most common type of infection in humans which cost millions of dollars for treatment in most countries including Bangladesh (1). UTI refers to an infection of the kidneys, ureters, bladder or urethra by invasion of pathogenic microorganisms resulting in an inflammatory response of the urothelium. Urinary tract infection can occur in both males and females in different age groups, but a variety study shows that women are more prone to develop a UTI in comparison to men (2). One of the main factors behind this is the physiology and anatomy of a woman's body. The distance between the urethra and the anus is much shorter in females than in males which easily allows the possibility of microbial flora to access the urinary bladder. The rapid proliferation of these microorganisms inside the urethra causes an inflammatory response in the host's body.

The family members of Enterobacteriaceae are often found to be associated with UTIs in humans. *Escherichia coli* is the main uropathogenic microorganism that causes the maximum cases of UTIs in human in different age groups both males and females (3, 4). After *E. coli* the most common UTI pathogens are *Klebsiella* spp., *Proteus* spp., *Pseudomonas* spp., *Enterobacter* spp., *Citrobacter* spp., *Morganella* spp. etc. (5). The most common gram-positive microorganisms include *Enterococcus* spp., *Staphylococcus saprophyticus* and different groups of streptococci (6). *Candida* spp., is also known to cause UTI and UTI-related infections in the human body (7).

There are several types of antimicrobial drugs that are commonly used to treat UTI patients by physicians but the increasing trend of antibiotic resistance in various pathogens compromises the effectiveness of these drugs in both developed and developing countries. including Bangladesh. The sensitivity pattern of these pathogenic microorganisms reveals great variability due to the geographical, historical and lifestyle of individuals (8). Several social and other factors are responsible for this type of behavior of microorganisms which include the use of a wide spectrum of antibiotics in common health-related problems, misuse of antimicrobial drugs and also the incomplete and inappropriate consumption of doses of antibiotics by patients (9-11). The easy access to these drugs in local pharmacies, the unawareness of people and the lack of implementation of laws related to antibiotic selling and use are the important causes of the increase in antibiotic resistance. The objective of this study was to isolate and identify *Escherichia coli,* the causative microorganisms responsible for urinary tract infections and determine their antibiotic susceptibility pattern.

MATERIALS AMD METHODS

Urine collection and culture: Urine samples were collected and analyzed from 110 patients of all age groups suffering from UTIs during the time period between June 2022 to August 2022 at Medinova Pvt. Limited, Dhaka,

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Bangladesh. All the urine samples were collected from both hospitalized and outdoor patients. Midstream urine samples were collected from all the patients in a sterile container. Urine samples were streaked aseptically into 5% Blood agar and MacConkey agar plates by using a sterile inoculating loop (1 microliter). All the plates were incubated at 37ºC for 24 hours. Plates were observed for bacterial growth after incubation at 37ºC overnight (12-14). All culture-positive plates were kept for further processing and identification of microorganisms however all the culture-negative plates were kept for an additional 24 hours to confirm the negative results.

Bacterial colony analysis and identification: Blood (5%) agar (Himedia, India Ltd.) plates were used for the identification of the growth of Gram-positive microorganisms and MacConkey agar (Himedia, India Ltd.) media were used for the identification of Gram-negative bacteria. Usually, all the UTI-causing microorganisms grow both on blood and MacConkey agar media except for the *Streptococcus* spp. which only grows on blood agar plates. All the gram-negative bacteria were identified with biochemical tests and gram stains (12, 13-15).

Routine microscopic examination: After performing the culture all urine samples were centrifuged and examined microscopically for the presence of white blood cells (pus cells), red blood cells, *Candida* spp., parasites, casts and crystals. The presence of pus cells in the minimum range of 5-6 cells per focus indicates that the patient might have developed a UTI (12-13).

Antibiotic sensitivity/susceptibility test (AST): All the culture-positive microorganisms were tested for antibiotic sensitivity. A single touch of an isolated colony was picked from the bacterial colony and prepared a bacterial suspension with normal saline at 0.5 McFarland standard. By using a sterile one-time cotton swab stick the suspension was lawn into the Muller-Hinton agar media 21 different types of antibiotics were used for the observation of the antibiotic sensitivity pattern of the isolated *E. coli*, the disc diffusion method was used in the Muller-Hinton and Muller-Hinton Blood agar media for the sensitivity test for gram-negative and fastidious bacteria, respectively. With the help of a disk dispenser, the antibiotic disks were dispensed into the agar plates (16, 17). After performing the following procedure all the plates were incubated at 37ºC for 18 to 24 hours. After the incubation period, all the plates were observed and the zone of inhibition was in millimeters with a meter scale. The zone of inhibition for individual microbial agents was indicated as susceptible, intermediate and resistant. The sensitivity and resistance values were determined by comparing the zone diameters with those suggested in the CLSI (Clinical and Laboratory Standard Institute) guideline 2021.

RESULTS & DISCUSSION

Enrolled patients:

A total of 110 urine samples for the growth and sensitivity of *E. coli* were included in this study. The female patients were dominant in the suspected cases of UTI. About 84 (76.4%) female patients and 26 (23.6%) male patients were advised of a urine culture by the physician in June 2022. The maximum number of patients was between the age group of 22 to60 years. As for the children only 8 cases of UTI were observed. Female patients (76.4%) were predominant as shown by the orange bars over the male patients (23.6%) mostly in the group of 21-60 years as shown by the blue bars.

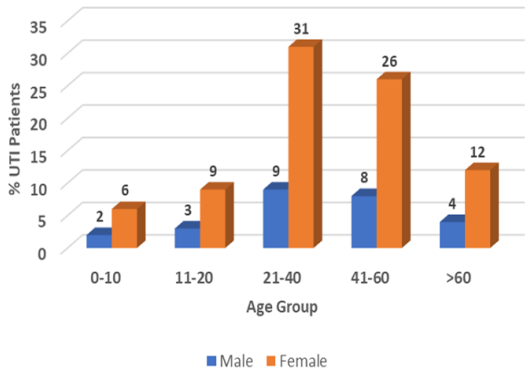

Figure 1. Age groups and gender distribution of the suspected patients suffering from UTI.

Isolation of causative microorganisms:

The urinary tract is usually sterile but accidental bacterial invasion can occur causing upper and lower urinary tract infections which can be both symptomatic or asymptomatic (8, 16, 18, 19). Gram-negative bacteria *Escherichia coli* and *Klebsiella* spp. are found to be the most common causes of UTI (18, 19). In the present study, 25 causative microorganisms were identified among the 110 cases. For the male patients, the culture positivity rate was 11.5% and for female patients, the rate was 26.2%. All of the UTIs were caused by *Escherichia coli* (100.0%).

Table 1. Number of uropathogens isolated from 25 urine culture-positive cases (n=25).

No. of uropathogens	No. of isolates
E. coli	25

Antibiotic susceptibility pattern of the isolates:

Microbial resistance and sensitivity towards the commonly consumed antibiotics were experimented to understand the current pattern of drug-resistant bacteria. This procedure helps physicians prescribe the patients use suitable antibiotics with appropriate dosage. But the rapidly increasing drug resistance in pathogenic bacteria has made the treatment of patients more challenging. Scientists are now seeking alternatives and developing new sensitive drugs to alleviate from such conditions. Bacteria can become resistant by following a variety of mechanisms that help them to avoid the deleterious effects of antibiotics. Mutation is a common way of developing drug resistance. Drug-resistant genes can be transmitted to other susceptible bacteria by plasmids, bacteriophages, transposons, etc. (20, 21).

In the present study, 21 commonly available antibiotics were tested to observe the susceptibility and resistance patterns. Antibiotic sensitivity patterns were observed in cases of Imipenem (100%), Amikacin (100%), Colistin (100%), Tigecycline (100%), Meropenem (96%), Gentamycin (96%), Doripenem (96%), Piperacillin (88%) and Nitrofurantion (80%). Azithromycin (100%), Nalidixic Acid (80%), Cotrimoxazole (54%), Ciprofloxacin (44%) and Cefixime (44%). A few antibiotics showed intermediate level sensitivity such as Amoxiclav (36%), Ciprofloxacin (28%). From Figure 2, we can state that most of the isolates were highly resistant to most of the tested antibiotics.

The total culture-positive rate was 22.7% among the total 110 patient samples. Most of these patients have plenty of pus cells in routine microscopic examination. However, the low positivity rate indicates that there might be a strong chance of the patients having a prior antibiotic history. The presence of antibiotics is one of the most challenging cases in detecting pathogenic microorganisms. Sometimes patients go to a medicine shop directly instead of visiting a doctor. In most of

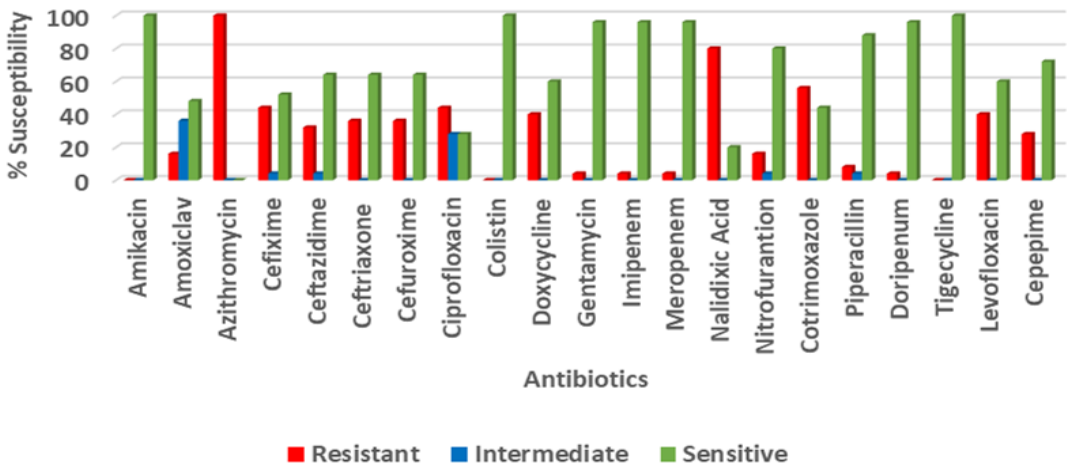

Figure 2. Antibiotic susceptibility pattern of *E. coli* isolates.

these cases, patients are prescribed antibiotics by the local medicine shop or pharmacy. This is a very common trend in Bangladesh. After the administration of antibiotics into the patient's body, there is a strong possibility of missing the culture ability of microorganisms on the culture plates. The presence of these antibiotics could have been detected by doing the antibiotic assay tests which was also one of the limitations of the study.

It is very important to prescribe antibiotics after an antibiotic sensitivity test and it should be advised in the appropriate dosage. The patients must be instructed correctly to take the medications just as prescribed and should also be informed properly about the deleterious effects of misuse of the antibiotics leading to drug resistance which not only causes harm to the individual but also to the people in their surroundings (21-23).

CONCLUSION

Though urinary tract infection is a common phenomenon, the treatment procedure is becoming more complicated due to multi-drug-resistant pathogenic bacteria. Only a few antibiotics are still in susceptible conditions. But resistance to these antibiotics is an obvious condition for the future. Development of new drugs is a must and patients should be advised properly to take the antibiotic medication to control the condition of rapidly occurring resistance traits.

CONFLICTS OF INTERESTS

The authors have declared that no competing interests exist.

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