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Pattern of Antimicrobial Resistance to *Escherichia Coli* Among the Urinary Tract Infection Patients in Bangladesh

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Abstract: Urinary Tract Infection (UTI) is very common in our day-to-day clinical practice. Among all the organisms *Escherichia coli* (*E coli)* is the most common but antimicrobial resistance becomes an alarming issue for UTI management now a days. Aim of this study is to assess the pattern of antimicrobial resistance to *E coli* among the UTI patients in Jashore, Bangladesh. This observational study was conducted from February, 2017 to January, 2018 in the district of Jashore, Bangladesh. We recruited 696 patients of both sex with UTI only infected by *E coli*. We had excluded the patients with UTI caused by other than *E coli*, female during menstruation, pregnancy, history of taking antibiotics within last 21 days, catheterization within 1 month and pelvic organ & genito urinary tract surgery within 6 months. Evaluation of antimicrobial resistance was done according to the standard bacteriological methods. Mean age of our study cases was 41.46±17.21 years with the range from 15 to 91 years. More than 85% participants were female with a sex ratio was 8:1.5. Reproductive age group ranged from 21 to 50 years was affected most commonly which constituted approximately two-third of our study. Our study revealed that the maximum antimicrobial resistance to *E coli* was Cotrimoxazole (95.0%), followed by Ceftazidime (75.7%), Gentamicin (70.3%), Amikacin (69.0%), Imipenam (58.9 %,), Cefixime (58.0%), Ciprofloxacin (57.3%), Azithromycin (56.0%), Cefuroxime (46.6%), Cefotaxime (37.4%), Ceftriaxone (35.2%), Meropenem (32.2%), Nitrofurantion (4.7%). With the high magnitude of antimicrobial resistance to *E coli* among the UTI patients even with extended generation of Cephalosporins, Carbapenams, Ciprofloxacin, Cotrimoxazole, Azithromycin and Aminoglycosides, our recommendation as first line empirical treatment option in UTI should be Nitrofurantoin due to low resistance pattern.

Keywords: UTI, *E coli*, Antibiotic Resistance, Antibiotic Susceptibility

1. Introduction

Urinary tract infection (UTI) is one of the most common infectious diseases in the community practice with approximately 150 million people affected in the world each year accounting for nearly 25% of all infections. [1-2] Among all organisms *E coli* causes 80-90% in uncomplicated UTIs. [3] A retrospective analysis using The Surveillance Network®, USA in 2012 reported the most common pathogen isolated from female who visited U.S. outpatients in 2012 was *E coli* (64.9%). [4] Urinary tract infection causes morbidities including

pyelonephritis and cystitis which are resulted by presence of microorganisms in the urinary tract. [5] Though UTIs are related to minimum morbidity, the annual financial burden of the US alone costs at around 2 billion dollar. [6] However UTI is a common scenario in our daily clinical practice, but the increasing antimicrobial resistance is associated with treatment failure and overburden of healthcare cost around the globe. [7] Evidence shows that antimicrobial resistance pattern to urinary *E coli* is growing gradually not only in the developing countries but also in the developed countries. In India from 2008 to 2013, the trend of antimicrobial resistance for E *coli* to third generation Cephalosporin, Fluoroquinolone and Carbapenams were increased from 70% to 83%, 78% to 85% and 10% to 13% respectively. [8] From 2000 to 2014, in German, Sweden, Spain and UK antimicrobial resistance against *E coli* were increasing to ciprofloxacin from 2.2% to 20.2%, 0% to 7.3%, 14.7% to 30.8% and 0.5% to 15.3% respectively and to Trimethoprim from 22.5% to 36.8%, 8.8% to 16.9%, 25.1% to 37.3% and 14.9% to 46.0% respectively. In UK Nitrofurantoin resistance to urinary *E. coli* were increased from 0% to 6% in the same duration. [9] In USA from 2005 to 2009, increased *E coli* resistance to Ampicillin from 39% to 43%, Cefazolin from 4% to 7%, Trimethoprime-Sulfamethoxazole 17% to 25%, Fluoroquinolone from 7% to 16%, Gentamicin from 3% to 7%, and extended-spectrum Cephalosporins from 1% to 3%. [10] A time series analysis over five years in an Australian Tertiary Hospital reported significantly raised antimicrobial resistance in *E coli* against different antibiotics. [11] In the Study for Monitoring Antimicrobial Resistance Trends (SMART) in Canada and United States (US) confirmed increasing resistance in *E*. coli. after evaluation of resistance trend in 3498 *E coli* induced UTI between 2010 to 2014 and found Extended-Spectrum Beta-lactamase (ESBL) phenotype increasing (7.8–18.3%, $P < 0.0001$) in US and susceptibility to Cephalosporins and Fluoroquinolones was significantly lower. But in Canada, no significant increasing trend (10.4-13.0%. $P = 0.079$) of ESBL was found and lower than US rates. [12] Reports of US also confirmed increasing resistance in *E*. coli. by retrospective study using The Surveillance Network®, USA in 2012 with a comparison with 2003 reports showing increasing resistance against Ciprofloxacin (3.6% to 11.8%) and Trimethoprim-Sulfamethoxazole (17.2% to 22.2%) with a lower resistance against Nitrofurantoin (from 0.7% to 0.9%). [4]

As antimicrobial resistance is increasing all over the world even in the developed countries, so we have designed this study with the aim to see the effectiveness of different antimicrobials used against urinary tract infection with *E coli* in terms of antimicrobial resistance in Jashore, Bangladesh.

2. Methods

2.1. Study Design and Settings

This observational study was conducted from February 2017 to January 2018 in the district of Jashore, Bangladesh. We recruited 694 *E coli* positive UTI patients 15 years and above of both sex. Female patients during menstruation, pregnancy period, patients gave history of taking antibiotics within last 21 days, catheterization within 1 month, pelvic organ and genitourinary tract surgery within 6 month were excluded from the study. The purpose of the study was explained to each of the participants and verbal consent was taken from the participants regarding use of their urine culture report in our study. Institutional consent was approved from ~~the~~ superintendent of Jashore Medical College & Hospital.

2.2. Patients Profile

Selected patients were both male and female of 15 years and above whom presenting to the physicians with the complaints of burning or painful micturition, increased frequency of micturition or lower abdominal pain.

2.3. Urine Sampling

Patients who had given informed consent to participate in the study been instructed verbally the collection of a clean catch mid-stream urine sample. Each participant was requested to give the early morning first voiding urine sample. After collecting the urine sample in a sterile container were processed for urine microscopy and culture in the same day in a renowned private pathological laboratory in Jashore, Bangladesh.

2.4. Definition

Microscopic examination revealed pus cell >5/HPF in the centrifuged deposit of urine was considered as our study participants. The Microscope model CX23-LED. OLYMPUS (Japan) was used to identify pus cell in urine sample in our study.

2.5. Bacterial Isolation and Identification

The urine sample which revealed pus cell >5/HPF in microscope were inoculated aseptically on Chromogenic agar (Hicrome UTI agar), Blood agar and MacConkey agar media and incubated 24 hours at 37˚C aerobically. Completing the incubation period, we counted the number of bacteria per ml of urine. After passing overnight incubation period we had counted only the *E coli* colony in the urine culture. The other bacterial colonies were excluded from our study.

2.6. Antimicrobial Susceptibility Testing

After identification of E *coli* in the urine sample we further demonstrated for antimicrobial susceptibility testing by Mueller-Hinton (MH) agar using Kirby-Bauer disc diffusion method against a panel of 13 antibiotics; Imipenum (10mcg), Ceftriaxone (30mcg), Ciprofloxacin (5mcg), Cotromoxazole (25mcg), Ceftazidime (30mcg), Cefotaxime (30mcg), Gentamycin (10mcg), Nitrofurantoin (300mcg), Azithromycin (15mcg), Cefixime (5mcg), Cefuroxime (30mcg), Meropenem (10mcg), Amikacin (30mcg). *E coli* ATCC 25922 were used as control strains for interpretations of antibiotics susceptibility testing. Antibiotics susceptibility

was denoted as sensitivity and resistance by using the diameter of zone of inhibition as per the Clinical Laboratory Standard Institute (CLSI) guidelines. [13]

2.7. Statistical Analysis

All the data were analyzed by using Statistical Package for Social Sciences (SPSS) version 23. Categorical data was grouped as % and numbers and mean with standard deviation (SD) measured from continuous data.

3. Results

A total 696 eligible patients of both sex aged above 15 years whose urine culture grown *E coli* were included in the our study. Out of total patients 102(14.7%) were male and 594(85.3%) patients were female gender. So, the majority patients in our study were female with a male and female sex ratio of 1.5:8 [Figure-1].

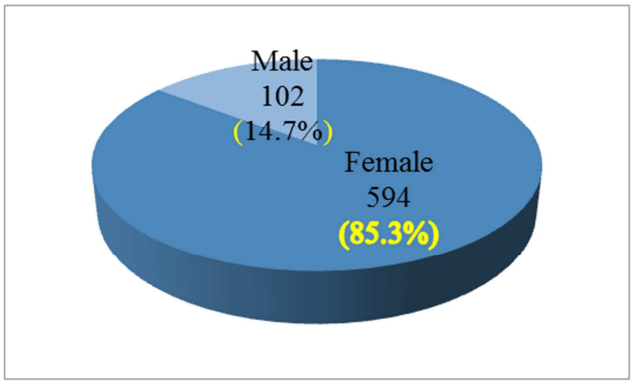

Figure 1. *Sex distribution of the study population.*

The mean age of our study population was 41.46±17.21 with the age range from 15 to 91 years. The highest numbers of patients were from 2nd to 5th decades which constituted 62% of the total study population [Table 1].

Table 1. *Age distribution of the study population.*

Age in years	Number	Percentage (%)
< 20 years	74	10.6
21-30 years	178	25.6
31-40 years	109	15.7
41-50 years	144	20.7
51-60 years	79	11.4
61-70 years	77	11.1
> 70 years	35	5.0
Total	696	100.0
Mean ±SD	41.46(±17.21)	Range 15-91 years

We had tested thirteen different commonly used antibiotics in urinary tract infection for evaluating the antibiotics susceptibility testing in our study. Among them Cotrimoxazole showed the highest number of antibiotics resistance to E coli which constituted 95% of the total patients. Nitrofurantoin constituted 4.7%, the lowest rate of antibiotics resistance to E coli. The rest antibiotics in our study showed resistance to *E coli* as follows, Ceftazidime (75.7%), Gentamicin (70.3%), Amikacin (69.0%), Imipenam (58.9 %,), Cefixime (58.0%), Ciprofloxacin (57.3%), Azithromycin (56.0%), Cefuroxime (46.6%), Cefotaxime (37.4%), Ceftriaxone (35.2%), Meropenem (32.2%) [Table 2].

Table 2. *Antibiotics resistant and sensitive pattern of the patients.*

Name Antibiotics	Resistant	Sensitive
Imipenum	410(58.9 %%)	286(41.1%)
Ceftriaxone	245(35.2%)	451(64.8%)
Ciproflaxacin	399(57.3%)	297(42.7%)
Cotrimoxazole	661(95.0%)	35(5.0%)
Ceftazidime	527(75.7%)	169(24.3%)
Cefotaxime	260(37.4%)	436(62.6%)
Gentamicin	489(70.3%)	207(29.7%)
Nitrofurantion	33(4.7%)	663(95.3%)
Azithromycin	390(56.0%)	306(44.0%)
Cefixime	404(58.0%)	292(42.0%)
Cefuroxime	324(46.6%)	372(53.4%)
Meropenem	224(32.2%)	472(67.8%)
Amikacin	480(69.0%)	216(31.0%)

4. Discussion

Urinary tract infections are second most common infections after respiratory tract infections among the human beings which may affects urethra, bladder or kidneys. [14] *E coli*, a Gram negative bacillus which is responsible for more than 80% cases of urinary tract infections worldwide. [15] It is well postulated that UTI is more common among the woman due to the closer alignment of urethra to the anus and shorter urethra which we have found in our present study at a ratio of 8:1.5. [16-19]

Common risk factors causing UTIs are sexually active pre-menopausal women, use of spermicides for contraceptive, frequent change of sexual partners, age of the first UTI, maternal history of UTI, wearing tight undergarments, deferred voiding habit, pregnancy, DM and immunosuppression due to any cause. [20-22] So, reproductive age plays a vital role as a risk factor for developing UTI in both sex. In the meanwhile our study reveals that maximum age of UTI affected from 2nd to 5th decades which constitutes 62% of the total cohorts.

Antibiotics resistance to the bacterial infections can be a great barrier for the effective treatment options even in case of UTI. The antibiotics resistance is closely related ~~with~~ to greater mortality or morbidity and burden of total healthcare costs. Abuse of antibiotics, lack of patients education, unauthorized sale of antibiotics, limited access of health care systems, inadequate surveillance or regulatory systems, and non-human use of antimicrobial such as in animal production are main causative factors for resulting antibiotics resistance in the developing country. [23] Here we have used common form of antibiotics testing in our study to detect their resistance pattern to *E coli* in UTI patients.

a. Cotrimoxazole: A few years back Cotrimoxazole was a commonly practicing antibiotic in many infections but its use becomes limited due to cutaneous side effects now a days. The present study shows that the highest antibiotics resistance to *E coli* is Cotrimoxazole and which constitutes 95% of the total study population.

One study supports our results where they have found Cotrimoxazole is one of the most resistant antibiotics to *E coli*. [24] Increasing resistance trends in US and Canada and lower susceptibility also support our finding. [4, 12, 25]

b. Nitrofurantoin: It is an oral form, cheap, less side effects and most effective antibiotics which are commonly used in UTIs patients in our country. We have found that Nitrofurantoin is only 4.7% resistance to *E coli* in the present study and accounted the lowest resistance form antibiotics to it. So, Nitrofurantoin is still highly effective to E coli positive UTIs patients in ~~Jessore~~ Jashore, Bangladesh. Some studies favor and some goes against the result of ~~85~~ our present study. [18, 26, 27] But less increasing resistance (from 0.7% to 0.9%) and higher susceptibility (>80%) in US support our report. [4, 25]

c. Ciprofloxacin: The quinolone derivative, Ciprofloxacin is used empirically not only in UTIs but also in various forms infections due to its safety profile and cheap in price. Its oral form is widely used in uncomplicated UTI patients. Ciprofloxacin develops highly resistance to various infectious agents recently due to its inappropriate and misuse. This study has revealed 57.3% resistance to *E coli* positive UTIs patients. This resistance rate of Ciprofloxacin to *E coli* is higher than the other studies conducted in Bangladesh. Even Ciprofloxacin resistance rate (79.66%) to *E coli* is higher in one study in Saudi Arabia. [28-30] A multicenter study of 13 countries in the Asia-Pacific region from 2010–2013 reported lower susceptibility to Ciprofloxacin support our study. [31]

d. Azithromycin: Azithromycin is one of the macrolides which is highly popular for its dose convenient in use. Macrolides are used more often with some sexually transmitted disease (STD) caused urinary problems usually in combination with other antibiotics to eliminate severe UTIs. Unfortunately it shows 56% resistance to *E coli* positive urine in these study populations. One study from West Bengal, India reported Azithromycin resistance to *E coli* positive UTIs patients near about 30%. [32]

e. Aminoglycosides: The most common Aminoglycosides are using in UTIs patients in our country are Gentamycin and Amikacin. Aminoglycosides are used usually in combination with other antibiotics to combat severe UTIs. Gentamycin and Amikacin are cheap, used parentally and less side effects except nephrotoxicity but this study reveals high resistance rate, 70.3% and 69.0% respectively to *E coli*. A very recent study, the enzymatic resistance against Aminoglycosides to *E coli* in northeastern Poland found that Gentamycin resistance rate was 59% and 11.4% in Amikacin. [33]

f. Cephalosporins: Second and third-generation Cephalosporins are commonly used empirical agents for both uncomplicated and complicated UTIs patients in any context. They are relatively expensive but safe even in case of pregnancy. Previously it was thought that they are highly susceptibility to UTIs patients but the present study shows highly resistance to *E coli* eg: Cefuroxime (46.6%), Cefixime (58.8%), Ceftiaxone (35.2%), Cefotaxime (37.4%), and ceftazidime (75.7%). A recent study reported that Cefuroxime (72.41%) and Ceftriaxone (66.58%) were higher resistance rate to urinary *E coli* than that of the present study. [34] But one study conducted in the Ayatollah Rouhani Teaching Hospital of Babol Medical Sciences University in North of Iran reported the resistance rate of Ceftriaxone, Cefotaxime and Cefixime were 40.4%, 45.6% and 43.9% respectively which are near similar to our study. [24] But the *E coli* exhibited higher resistance rate to Ceftazidime (81.36%) and Cefotaxime (76.27%) in a study of Saudi Arabia. [30]

g. Carbepenam: Imipenem and Meropenam of the Carbepenams group are the commonly agents using for the management of severe form of infections caused by extended –spectrum ß- lactamase (ESBL) positive *E coli* in our country. Carbepenems are treated as a reserved drug to eliminate multi-drug resistance gram negative bacilli but unfortunately Meropenem and Imipenem are 32.2% and 58.9% resistance to *E coli* positive UTI patients respectively in our study. Imipenem resistance rate to *E coli* is 32.5% in a study of Pakistan. [35] Another study in Bangladesh has shown Meropnem resistance rate to E coli is 10.5%. [36]

Antibiotics resistance depends on the genetic mutation and locality. So, geographical variation may be a determinant of difference magnitude of antimicrobial resistance pattern.

5. Limitation of the Study

Though the study includes significant number of cases but there are some potential limitations also. This includes-study conducted in a limited area, associated comorbidities were not evaluated, associated clinical presentations were not evaluated and distinctions of bacteriuria from UTI were not done.

6. Conclusion

High magnitude of antimicrobial resistance against *Escherichia coli* was observed among the UTI patients in Bangladesh even with extended generation of Cephalosporins, Carbapenems, Ciprofloxacin, Cotrimoxazole, Azithromycin and Aminoglycosides. Moreover, out of all other antibiotics Nitrofurantoin shown the least resistance against *Escherichia coli*. So, we recommended Nitrofurantoin as first line empirical treatment option in UTI patients.

Authors' Disclosures of Potential Conflicts of Interest

The author(s) indicated no potential conflicts of interest.

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