



CULTURE AND ANTIBIOTIC SENSITIVITY OF *ESCHERICHIA COLI* ISOLATED FROM PATIENTS WITH URINARY TRACT INFECTIONS

Mohammad Asaduzzaman^{*1}, Farha Matin Juliana², Tasnuva Ferdous¹, Disha Halder¹, Salma Begum¹, Mousumi Khatun¹, Md. Jhaangir Alam¹, Nazmul Hossain¹, Biswajit Das¹ and Sakib Hossen¹

¹Department of Biochemistry, Primeasia University, Dhaka, Bangladesh.

²Department of Biochemistry and Molecular Biology, Jahangirnagar University, Dhaka, Bangladesh.

***Corresponding Author: Mohammad Asaduzzaman**

Department of Biochemistry, Primeasia University, Dhaka, Bangladesh.

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ABSTRACT

Background: *Escherichia coli* is the commonest cause of community and nosocomial urinary tract infections (UTI). Antibiotic treatment is usually empirical, relying on susceptibility data from local surveillance studies. We therefore set out to determine the levels of resistance to antimicrobial agents amongst all urinary isolates obtained over a 12-month period. **Methods:** Antimicrobial susceptibility to nalidixic acid, levofloxacin, cefixime, cotrimoxazole, ceftriaxone, ciprofloxacin, gentamicin, colistin, nitrofurantoin, amikacin, meropenem was determined for 1037 *Escherichia coli* urinary isolates obtained from community and hospitalized patients in Dhaka. **Results:** High resistance rates were observed against most of the antibiotics examined here. Nalidixic Acid stood to be the most resistant type of antibiotic. 87.58% resistant for males and 76.71% for females. Cefixime 69.37% for males and 52.64% for females. Cotrimoxazole 59.01% for males and 44.64% for females. Ciprofloxacin 24.84% for males and 17.34% for females. Amikacin 12.61% for males and 13.62% for females. Resistance to Amikacin and Meropenem was found to be lower. **Conclusion:** With the exception of nalidixic acid, resistance to agents commonly used as empirical oral treatments for UTI was extremely high. Levels of resistance to levofloxacin and cefixime render them unsuitable for empirical use. Continued surveillance and investigation of other oral agents for treatment of UTI in the community is required.

KEYWORDS: UTI, Multidrug Resistance, Uropathogen, Antibiotics, *Escherichia coli*.

INTRODUCTION

Antibiotic resistance is an increasing threat to life and to morbidity and mortality. Urinary Tract Infection (UTI) is a very common infection all over the world, but it is more prevalent in developing South Asian countries like Bangladesh. UTI can be nosocomially ubiquitous in the clinical environment, so that the prevalence rate of uropathogens is being alarmingly accelerated. To prevent these pathogens, different types of antibiotics and their super generations are used indiscriminately in different doses in misused and overused forms. So, uropathogens are becoming resistant to efficacious drugs by adopting different mechanisms of mutations and genetic transformations. UTI is one of the most common diseases encountered in medical practices these days and encompasses a broad range of clinical fields that are associated with the common finding of positive urine cultures. Besides, every year, about 150 million people are affected by UTIs. Worldwide, at a cost of about US \$6 billion, even UTIs have demonstrated significant morbidity and mortality. Urinary tract infections are more common in females than in males, because of the

short length of the urethra and its proximity to the anus. Pregnancy and sexual activity also make women more susceptible to UTI. The etiology of UTIs and the antibiotic susceptibility of urinary pathogens, both in the community and hospitals, have changed over the past years, and recently, antibiotic resistance has become a major global problem.^[1]

Escherichia coli is the predominant cause of both community and nosocomial urinary tract infections (UTI). In the UK, trimethoprim or nitrofurantoin are usually recommended for empirical treatment of episodes of uncomplicated cystitis in the community,^[2] whilst parenteral cephalosporins and aminoglycosides are reserved for complicated infections or pyelonephritis. In North America, a cutoff point of 20% has been suggested as the level of resistance at which an agent should no longer be used empirically.^[3] A UK study of the antimicrobial susceptibility of bacterial pathogens causing UTI in 1999-2000 showed high levels of resistance to trimethoprim, amoxicillin and oral cephalosporins,^[4] whilst a study of three collections of

Escherichia coli strains obtained from patients in East London in 1991, 1999 and 2004 showed rates of trimethoprim resistance of over 30%.^[5] The emergence of strains producing extended spectrum- β lactamases (ESBL's) and others exhibiting quinolone resistance now threatens the empirical use of both cephalosporins and ciprofloxacin,^[6] seriously limiting treatment regimens. In order to determine current levels of resistance to antibiotics commonly used locally for empirical treatment, we reviewed susceptibility to ampicillin, amoxicillin/clavulanate, trimethoprim, nitrofurantoin, cefalexin, gentamicin, ciprofloxacin, and cefpodoxime amongst all *Escherichia coli* urinary isolates obtained in our laboratory over a 1 year period. A multidrug resistance pattern of the isolated organism was observed in this present study.

Methods

Study Design

This cross-sectional study was designed to assess the antibiotic resistant pattern of *Escherichia coli* cultured from UTI patients.

Place of Data Collection

IbnSina Diagnostic & Consultation Center, Badda, Dhaka.

IbnSina Diagnostic & Consultation Center, Doyaganj, Dhaka.

Place of Data Analysis

Department of Biochemistry, Primeasia University

Data Collection Time

February 2016 to January 2017.

Data Analysis Time

January-April 2021.

Sample Collection and Bacteriological Assessment

Early morning midstream urine samples will be collected. Sterile wide container (China) with screw cap tops as described by Kolawale *et al.*^[7] will be used for sample collection. On the label were the name, age, sex and time of collection. All the patients will be properly instructed on how to collect the urine samples aseptically and taken to the laboratory immediately for culture. In the diagnostic laboratory, each well mixed urine sample (1 μ l) will be inoculated on MacConkey agar (Oxoid) and Blood agar (Oxoid) media plate under class-II laminar airflow (NUVO SanajiMalzemelzeni, ImalatVcTicaret A.S, Turkey). The inoculum on the plate was streaked out for discrete colonies with a sterile wire loop sterilized by auto loop sterilizer (Germany) following standard procedures.^[8,9,10] The culture plates will be incubated at 35°-37°C by an incubator (Germany) for 24-48 hours and observed for the growth of bacteria through formation of colonies. All the bacteria will be isolated and identified using morphological, microscopy (Japan)

and biochemical tests like TSI (HiMedia), MIU (HiMedia) and Simmons Citrate (HiMedia) agar following standard procedures.^[8,11]

Antibiotic Susceptibility Assessment

The disc diffusion technique was used for antibacterial susceptibility testing of the isolates.^[12,13,14] using commercial antibiotics containing discs. We will use the commercial antibiotic disc Bacterial susceptibility was determined by the Kirby-Bauer disc diffusion method using antibiotic containing discs from Oxoid Ltd, UK. Eleven types of antibiotic discs were used in this study. Those were Amoxyciline (AML), Doxycycline (DO), Nalidixic acid (NAL), Ciprofloxacin (CIP), Cephalexin (CL), Cefradine (CEF), Ceftriaxone (CRO), Cefixime (CFM), Gentamycin (GEN), Netilmycin (NET), Nitrofurantoin (NIT), gentamycin (5 μ g, HiMedia). Interpretation of results will be analysed using zone sizes. Zones of inhibition \geq 21mm will be considered sensitive, 16-20mm intermediate and <15 mm resistant. Isolates will be classified as either sensitive or resistant based on the definition of the Clinical and Laboratory Standard Institute.^[13]

Statistical Analysis

Data will be assessed using the free software GNU PSPP stable release 1.4.1/ September 5, 2020. It has a graphical user interface and conventional command-line interface. It is written in C and uses GNU Scientific Library for its mathematical routines. The name has "no official acronymic expansion".

RESULTS

Of the total 12943 (male-3638 & female-9305) urine samples collected from UTI suspected patients, 1236 (9.55%) samples were positive and 11707 (90.45%) samples were negative in selected areas (Badda, Gulshan, Baridhara, Rampura, Doyagonj, Gandaria, Jatrabari, Sayedabad, Dhaka, Bangladesh) (Figure-1).

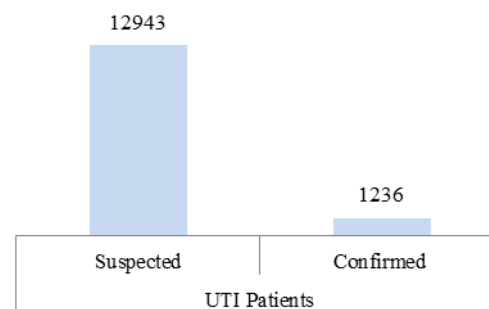


Figure-1: Suspected UTI patient versus UTI patients.

Among 1236 UTI patients 275 patients were male and 961 patients were female (Figure-2).

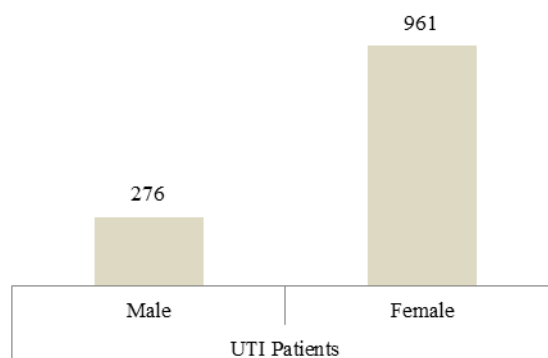


Figure-2: Gender distribution among UTI patients.

Table-1: Distribution table of Urinary Tract Infection (UTI) patients by age groups and gender (n=1236).

Age (Years)	<10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	81-90	>90
Male	53	05	19	16	26	38	73	38	05	02
Female	103	83	181	113	149	130	124	56	14	08
Total	156	88	200	129	175	168	197	94	19	10

In our study, table-1 showed the distribution table of urinary tract infections affected patients by their age groups and gender. The highest number of study subjects goes to the 21-30 year age group (200 patients=181 female + 19 male) and followed by the 61-70 year age group (197 patients=124 female + 73 male), the 41-50 year age group (175 patients=149 female + 26 male), the

51-60 year age group (168 patients= 130 females + 38 males) and the 10 year age group (156 patients=103 females + 53 males) respectively. The most prevalent frequency of female and male patients affected by uropathogens was found in the 21-30 year and 61-70 year age groups respectively.

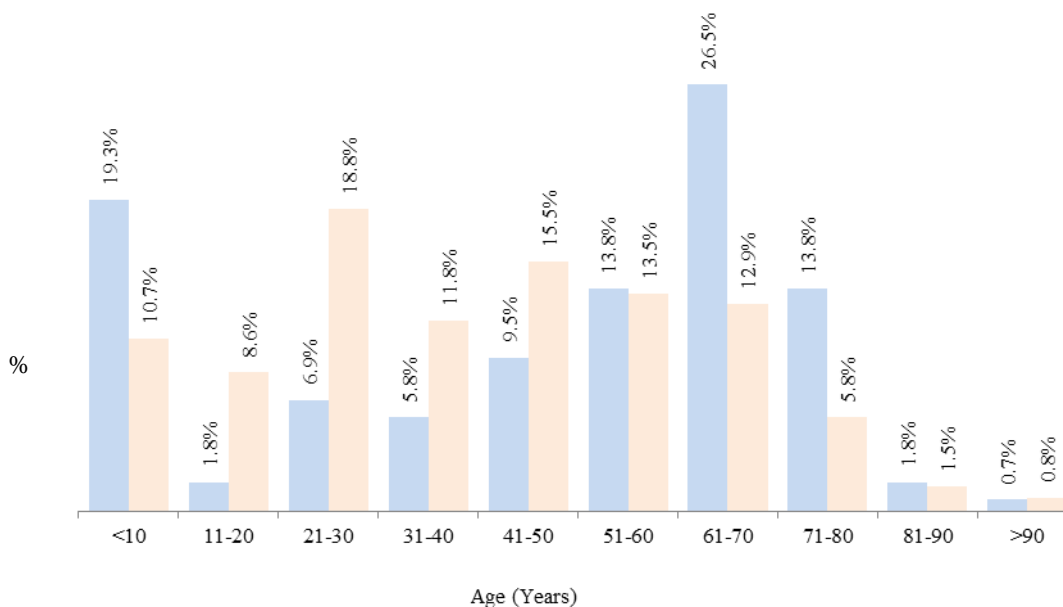


Figure-3: UTI percentage among different age groups of male (N=275) and female (N=961).

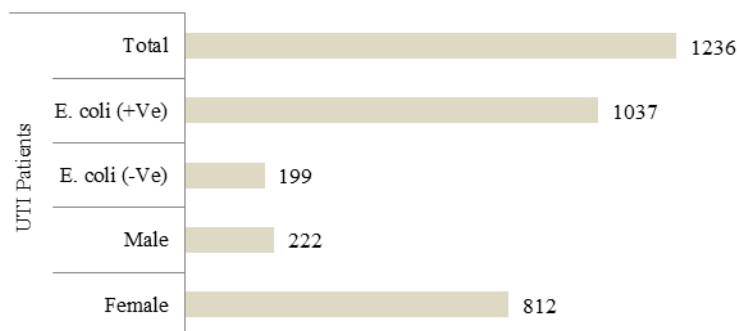
The percentage of male patients was more prone than female patients (19.3% > 10.7%) under the 10 year age group. In between 11-20, 21-30, 31-40, and 41-50 years of age group, female UTI infection (8.6%, 18.8%, 11.8%, and 15.5% respectively) is higher than male (1.8%, 6.9%, 5.8%, and 9.5% respectively). In between 51-60, 61-70, 71-80 and 81-90 years of age, male

infection (13.8%, 26.5%, 13.8% and 1.8% respectively) is higher than female infection (13.5%, 12.9%, 5.8% and 1.5% respectively). Above 90 years of age, female infection (0.8%) is higher than male (0.7%) but here the number of patients was very small (Figure-3).

Table-2: Distribution of *E. coli* mediated UTI among UTI patients.

Organism	Percentage (n=1037)		
	Male	Female	Total
<i>E. coli</i>	222(18.0%)	815(65.9%)	1037(83.9%)

In a total of 1236 UTI patients, *Escherichia coli* was present in 1037 (male 222 or 18.0% and female 815 or 83.9%) (Table-2).

**Figure-4: *E. coli* distribution.**

Among 1236 UTI patients *E. coli* found in 1037 patients, and did not found in 199 UTI patients. Males accounted

for 222 of the *Escherichia coli* confirmed patients, while females accounted for 815 (Figure-4).

Table-3: Antibiotic Susceptibility pattern of *Escherichia coli* among male UTI patients (n=222) and female UTI patients (n=815).

Antibiotic	Patients	Sensitive		Resistant	
		Number	Percentage	Number	Percentage
Amikacine	Male	194	87.39%	28	12.61%
	Female	704	86.38%	111	13.62%
Meropenem	Male	213	96.02%	9	3.98%
	Female	794	97.48%	21	2.52%
Colistin	Male	167	75.16%	55	24.84%
	Female	635	77.95%	180	22.05%
Nalidixic Acid	Male	28	12.42%	194	87.58%
	Female	189	23.23%	626	76.77%
Cefixime	Male	68	30.63%	154	69.37%
	Female	331	40.61%	484	59.39%
Levofloxacin	Male	48	21.41%	174	78.59%
	Female	312	38.29%	503	61.71%
Ceftriaxone	Male	89	40.09%	133	59.91%
	Female	451	55.34%	364	44.66%
Nitrofurantoin	Male	160	71.90%	62	28.10%
	Female	674	82.66%	141	17.34%
Ciprofloxacin	Male	125	56.52%	97	43.48%
	Female	524	64.25%	291	35.75%
Gentamicin	Male	139	62.61%	83	37.39%
	Female	550	67.48%	265	32.52%
Cotrimoxazole	Male	91	40.99%	131	59.01%
	Female	386	47.36%	429	52.64%

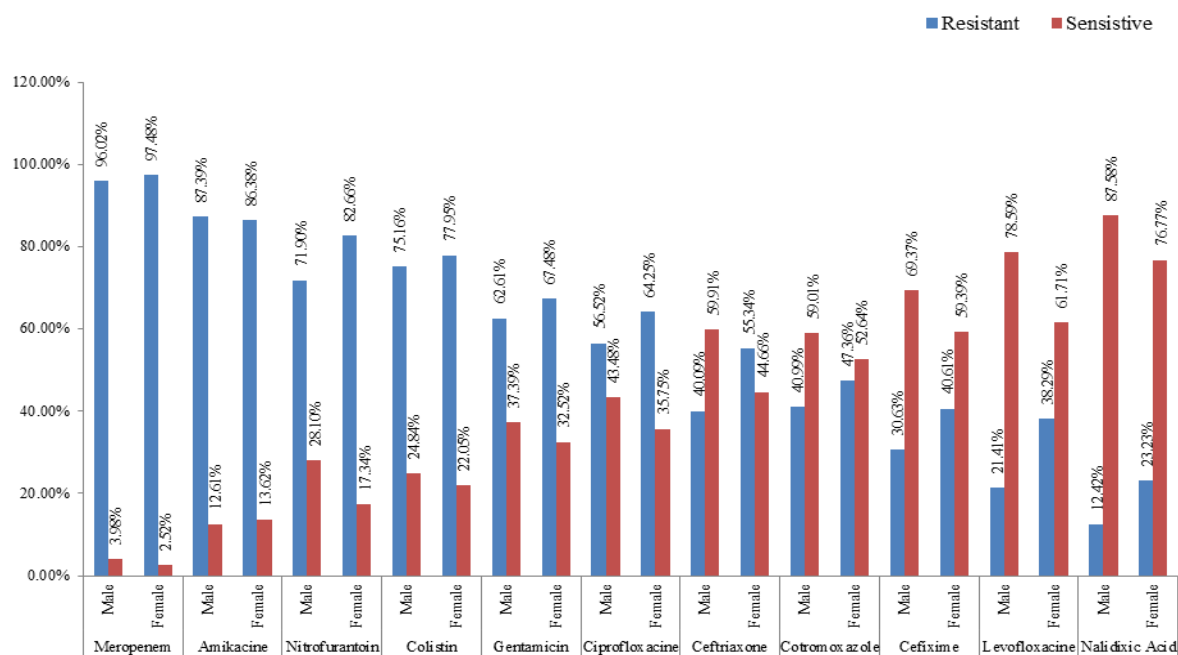


Figure-5: Antibiotic Susceptibility pattern of *E. coli* among male UTI patients (n=222) and female UTI patient.

Table-3 and Figure-5 described that Meropenem antibiotic was found resistant to 3.98% of male and 2.52% of female UTI patients. Same as for amikacine 12.61% for male and 13.62% for female, nitrofurantoin 28.10% for male and 17.34% for female, colistin 24.84% for male and 22.05% for female, ceftriaxone 59.9% for male and 44.64% for female, cotrimoxazole 59.01% for male and 52.64% for female, levofloxacin 78.59% for male and 61.71% for female, levofloxacin 78.59% for male and 76.71% for male and 76.71% for female, 69.

DISCUSSION

Knowledge of local susceptibility patterns is important for the selection of appropriate sensitive of UTI in the primary healthcare setting.^[15] A recent study,^[16] found that the frequency of urinalysis requests has declined over time, which probably corresponds with a decline in urine culture and susceptibility testing. This decline will have the unintended effect of decreasing the amount of information available for assessment of local resistance trends.^[16]

Urinalysis is a relatively simple, office-based test that can be used to evaluate patients with urinary complaints. It is important that clinicians are aware of the regional antibiotic resistance rates before initiating experimental antimicrobial therapy for UTI treatment, as it is well-described that urinary infection with a resistant pathogen is more likely to lead to bacteriological/clinical failures.^[17] Antibiotics are available as non-prescription drugs in medicine shops and irrational use is not uncommon. Adherence to treatment protocol and compliance with treatment course of antimicrobials need to be emphasized at different levels.

The present study was carried out to identify *Escherichia coli* from urinary tract infected patients who came to a health check center in Dhaka city. For the confirmation of the presence of this disease causing agent, various cultural and biochemical techniques were performed. Among 1236 UTI patients, *Escherichia coli* infection was found in 1037 (male 222 and female 815). The percentage of male patients was more than female patients (19.3% > 10.7%) under 10 year age group. In between 11-20, 21-30, 31-40, and 41-50 years of age group, female UTI infection (8.6%, 18.8%, 11.8%, and 15.5% respectively) is higher than male (1.8%, 6.9%, 5.8%, and 9.5% respectively). In between 51-60, 61-70, 71-80 and 81-90 years of age, male infection (13.8%, 26.5%, 13.8% and 1.8% respectively) is higher than female infection (13.5%, 12.9%, 5.8% and 1.5% respectively). Above 90 years of age, female infection (0.8%) is higher than male (0.7%) but here the number of patients was very small.

High resistance rates were observed against most of the antibiotics examined here. Lowest to highest resistant type followed by meropenem 3.98% for male and 2.52% for female, amikacine 12.61% for male and 13.62% for female, nitrofurantoin 28.10% for male and 17.34% for female, colistin 24.84% for male and 22.05% for female, ciprofloxacin 59.9% for male and 52.64% for female, cefixime 69.37% for male and female, levofloxacin 78.59% for male and 66.71% for male and 76.71% for female. The multidrug resistance pattern of the isolated organism was also observed in this present study.

Measures for prevention and containment of antimicrobial resistance are necessary in Bangladesh. It should be taken as a national priority and the establishment of a national alliance or regulation

governing the use of antimicrobials should be considered.

CONCLUSION

In conclusion, data from local laboratories exaggerates the nalidixic acid, levofloxacin, cefixime, and cotrimoxazole resistance problems among *Escherichia coli* urine isolates from general practice. For optimal interpretation of cumulative susceptibility data in the primary healthcare setting, it is necessary to take into account the type of UTI (uncomplicated vs. complicated), as well as the sex and age of each patient.

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