ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue V May 2025

Bacterial Uropathogens Causing Urinary Tract Infection with Antibiotic Susceptibility Pattern of *E. Coli*: A Cross Sectional Study

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DOI: https://doi.org/10.51584/IJRIAS.2025.100500096

Received: 09 May 2025; Revised: 20 May 2025; Accepted: 22 May 2025; Published: 18 June 2025

ABSTRACT

Background: Urinary tract infection (UTI) is a major public health burden and affect the quality of life with significant morbidity of affected individuals. The aim of this study was to determine bacterial uropathogens along with antimicrobial susceptibility pattern of *E. coli* among patients with symptoms of UTIs in a tertiary care hospital in Dhaka, Bangladesh.

Methods: This cross-sectional observational study was conducted from May, 2023 to April, 2024 at Bangladesh Medical College Hospital (BMCH), Dhaka, Bangladesh and laboratory work was carried out in Microbiology department of Bangladesh Medical College (BMC), Dhaka, Bangladesh. A total of 170 urine samples were collected from both outpatient and inpatient department patients with clinical evidence of one or more symptoms of UTI, such as dysuria, increased frequency, hesitation, urgency and abdominal pain. Each patient's clean-catch midstream urine was collected in a sterile screw-capped universal container. Patients' written consents were also collected before specimen collection. Urine samples were sent to microbiology laboratory for further study. A specimen was considered positive for UTI if an organism was cultured at a concentration of $\geq 10^5$CFU/ ml or when an organism was cultured at a concentration of 10^4CFU/ml and >5 pus cells per high power field were observed on microscopic examination of urine. Identification of bacterial pathogens was made on the basis of Gram reactions, morphology, motility test, biochemical and cultural characteristics. Antimicrobial susceptibility testing of *E. coli* was performed by disc diffusion method following the National Committee for Clinical laboratory Standards (NCCLS) guidelines.

Results: A total 170 urine samples were collected from symptomatic UTI patients, among which 120 (70.59%) patients were female and 50 (29.41%) patients were male. In OPD, male and female patients were 37 (74%) and 86 (71.67%) and in IPD, male and female patients were 13 (26%) and 34 (28.33%) respectively. Out of 50 male patients, common age group of symptomatic UTI was 70-79 years whereas in 120 female patients, the majority of the UTIs were reported within the age group of 19-29 years, 50-59 years and 60-69 years ($p < 0.001$). Among five risk factors observed for UTI, the most common risk factor in female was DM 37 patients, followed by HTN 28 patients, H/O UTI in last one year 21 patients, previous H/O taking antibiotics in last six months 15 patients & CKD 3 patients. In male, commonest risk factor was HTN 20 patients, followed by DM 14 patients, previous H/O taking antibiotics in last six months 7 patients, CKD 5 patients & H/O UTI in last one year 4 patients. Culture positive samples were detected 62(36.47%) from 170 urine samples. Out of 62 culture positive isolates, *E. coli* 34(20%) was found to be the most common pathogen followed by *Klebsiella* spp. 13(7.65%), *Pseudomonas* spp. 7(4.12%), *Enterobacter* spp. 4(2.35%) and *Enterococci* spp. 4(2.35%). *E. coli* was highly susceptible to mecillinam 28(82.35%), nitrofurantoin

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue V May 2025

30(88.23%) and meropenem 28(82.35%). Susceptibility to netilmicin 26(76.47%), ceftazidime 24(70.58%), amikacin 24 (70.58%) were also detected.

Conclusion: We observed that *E. coli* was highly susceptible to mecillinam, nitrofurantoin and meropenem. As mecillinam and nitrofurantoin are oral antimicrobial agents, clinician can choose these drugs for the treatment of UTI. We attempted to investigate the susceptibility patterns of *E. coli*; however, the results were not representative of the entire Bangladeshi situation. Therefore, a mass study is required to find out the real scenarios of antibiotic susceptibility pattern of uropathogens.

Keywords: Bacterial uropathogens, UTI, *E. coli*, antibiotic susceptibility.

INTRODUCTION

Urinary tract infection (UTI) is a major public health burden and affect the quality of life with significant morbidity of affected individuals. Any infection of the urinary tract involving the urethra, urinary bladder, ureters, or kidneys is termed as UTI. Most common causative agents are bacteria, followed by fungi such as Candida [1]. UTIs are the third most prevalent type of infection in human worldwide, behind respiratory tract infections and alimentary tract infections [2]. Almost 35% of all health care associated infections are UTIs [1]. Globally, almost 150 million people receive a UTI diagnosis each year, costing the economy more than $6 billion in US dollars [2]. Gram-positive bacteria account for 10%–15% of infections, while Gram-negative bacteria account for 80%–85% [3]. Of all bacteria, *E. coli* is responsible for 75–95% of UTI cases [4].

Broad-spectrum antibiotics are frequently administered for empirical treatment since antibiotics have proven to be particularly successful in the management of UTIs [5]. The empirical selection of antibiotics for UTI treatment is aided by susceptibility data from nearby microbiological facilities; however, these data are limited to complex UTIs since uncomplicated UTI specimens are infrequently reported to laboratories [6]. Antimicrobial therapy has reduced the fatality rate from UTIs, but overuse of these treatments can increase the chance of developing multidrug resistance (MDR) or other complications [2].

Antibiotic misuse and the availability of these drugs over-the-counter have led to the emergence of antibiotic resistance against common infections on a global scale [7]. Antibiotic susceptibility (AS) screening for organisms causing UTIs must be done continuously due to the serious public health concern of the rising occurrence of drug resistance among uropathogens [8].

The antibiotic susceptibility pattern of microorganisms differs between hospitals and between different geographic areas [9]. So, the aim of this study was to determine bacterial uropathogens along with antimicrobial susceptibility pattern of *E. coli* among patients with symptoms of UTIs in a tertiary care hospital in Dhaka, Bangladesh.

MATERIALS AND METHODS

This cross-sectional observational study was conducted from May, 2023 to April, 2024 at Bangladesh Medical College Hospital (BMCH), Dhaka, Bangladesh and laboratory work was carried out in Microbiology department of Bangladesh Medical College (BMC), Dhaka, Bangladesh. A total of 170 urine samples were collected from both outpatient department (OPD) and inpatient department (IPD) patients with clinical evidence of one or more symptoms of UTI, such as dysuria, increased frequency, hesitation, urgency and abdominal pain. Each patient's clean-catch midstream urine was collected in a sterile screw-capped universal container. All patients were instructed on collecting samples aseptically to avoid contamination. Patients' written consent was also collected before specimen collection and the study was approved by concerned authority. Urine samples were sent to microbiology laboratory for further study.

Inclusion criteria: i) Patients aged ≥19 years; ii) clinical evidence of one or more symptoms of UTI, such as dysuria, frequency, hesitation, urgency and abdominal pain, was recorded in patients included in the analysis; and iii) bacterial count value must be $\geq 10^5$ CFU/mL in the urine at mid flow to be deemed culture positive.

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue V May 2025

Exclusion criteria: i) Patients with a history of recent antibiotic therapy (within the last 72 hours), ii) patients with polymicrobial infections involving more than two bacterial species and pregnant females with asymptomatic bacteriuria, iii) patients with urinary catheter were excluded from our study.

Sample collection, processing, identification and biochemical characterization of pathogens:

Clean catch midstream urine sample was collected into a sterile container aseptically. Each container was closed carefully, labeled properly as name, age, gender, ward and serial number for tracing and was transferred to laboratory for further investigation. Samples were processed according to a previously described methodology [10]. A sterile platinum wired calibrated loop was used which delivered 0.001 ml of urine. A loopful urine sample was plated on Cystine-Lactose-Electrolyte Deficient (CLED) agar media (Hi Media Laboratories, India). The inoculated plates were incubated at 37^0C for 24 hours and extended to 48 hours in culture negative cases. The plates were then examined macroscopically for bacterial growth. A specimen was considered positive for UTI if an organism was cultured at a concentration of $\geq 10^5$CFU/ ml or when an organism was cultured at a concentration of 10^4CFU/ml and >5 pus cells per high power field were observed on microscopic examination of the urine [11] – [13]. Identification of bacterial pathogens was made on the basis of Gram reactions, morphology, motility test, biochemical and cultural characteristics [14].

Antimicrobial susceptibility testing:

Antimicrobial susceptibility testing of *E. coli* was performed by disc diffusion method following the National Committee for Clinical laboratory Standards (NCCLS) guidelines [15]. All discs were obtained from Oxoid Ltd. Antibiotics used for uropathogens were amoxyclav (30µg), ceftriaxone (30µg), amikacin (30µg), ciprofloxacin (5µg), co-trimoxazole (25µg), gentamicin (10µg), micellinum (25µg), nalidixic acid (30µg), nitrofurantoin (300µg), cefuroxime (30µg), piperacillin/ tazobactam (110µg), meropenem (10µg), levofloxacin (5µg), netilmicin (30µg), cefepime (30µg), cefixime (5µg), ceftazidime (30µg), aztreonam (30µg).

Statistical analysis:

Statistical Package for Social Science (SPSS) version 20 was used for data analysis. Chi-squared Test (χ^2) was done to analyze the data.

RESULTS

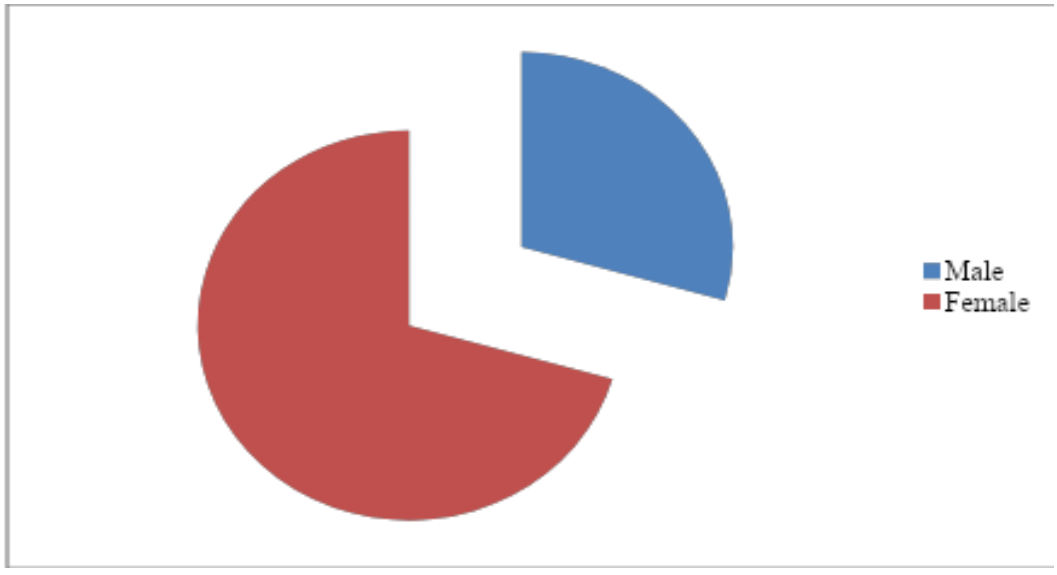
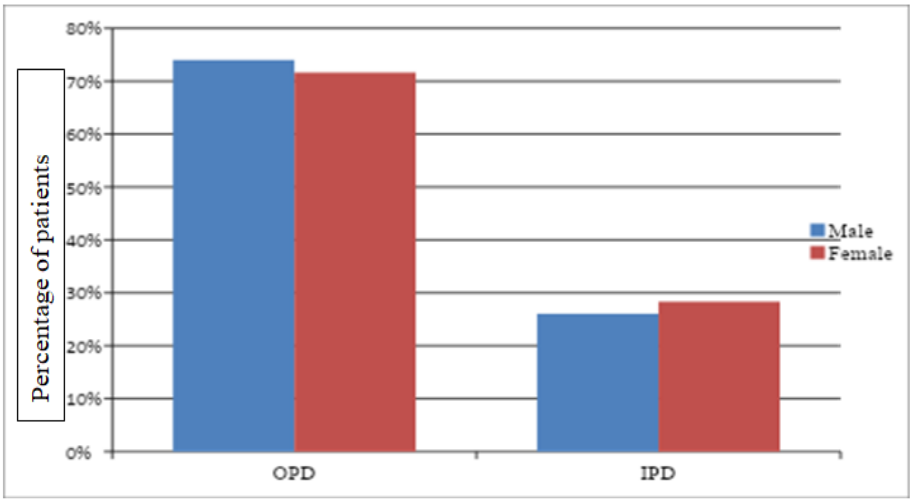


Figure 1: Gender distribution of symptomatic UTI cases (n=170)

The gender distribution of adults is shown in figure 1. A total 170 urine samples were collected from symptomatic UTI patients, among which 120 (70.59%) patients were female and 50 (29.41%) patients were male.

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue V May 2025

Table I: Distribution of symptomatic UTI cases from both IPD and OPD



In OPD, male and female patients were 37 (74%) and 86 (71.67%) and in IPD, male and female patients were 13 (26%) and 34 (28.33%) respectively are shown in Table I.

Table II: Age and sex distribution of urine samples collected from symptomatic UTI patients (n=170)

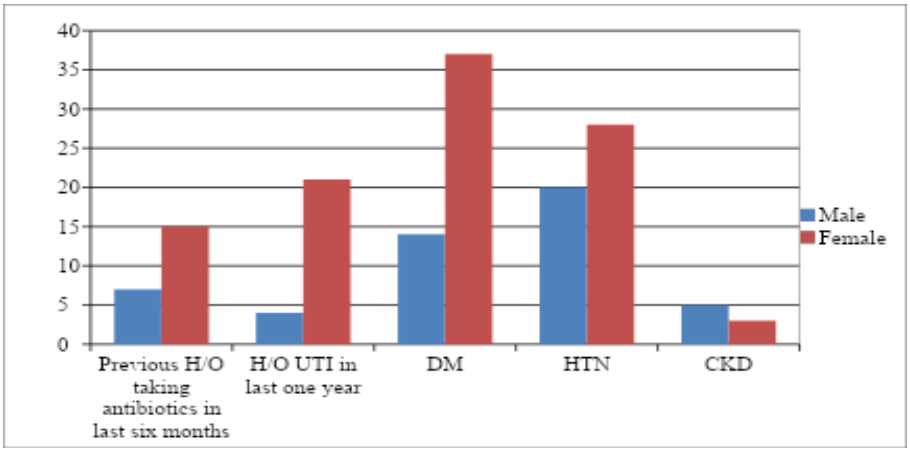
Age group	Male	Female	p-value
19-29 yrs	1	25	
30-39 yrs	2	18	
40-49 yrs	4	11	
50-59 yrs	5	25	
60-69 yrs	10	24	<0.001
70-79 yrs	24	10	
80-89 yrs	2	6	
90-99 yrs	2	1	
Total	**50 (29.41%)**	**120 (70.59%)**	

Chi-squared Test (χ^2) was done to analyze the data

p-value <0.001 which is statistically significant

The age and sex distribution of urine samples collected from symptomatic UTI patients shows that out of 50 male patients, common age group of symptomatic UTI was 70-79 years whereas in 120 female patients, the majority of the UTIs were reported within the age group of 19-29 years, 50-59 years and 60-69 years (p <0.001) (Table II).

Table III: Risk factors in symptomatic group of UTI cases



ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue V May 2025

Table III shows risk factors of UTI cases in both male and female patients. Out of five risk factors observed for UTI, the most common risk factor in female was DM 37 patients, followed by HTN 28 patients, H/O UTI in last one year 21 patients, previous H/O taking antibiotics in last six months 15 patients & CKD 3 patients. In male, commonest risk factor was HTN 20 patients, followed by DM 14 patients, previous H/O taking antibiotics in last six months 7 patients, CKD 5 patients & H/O UTI in last one year 4 patients.

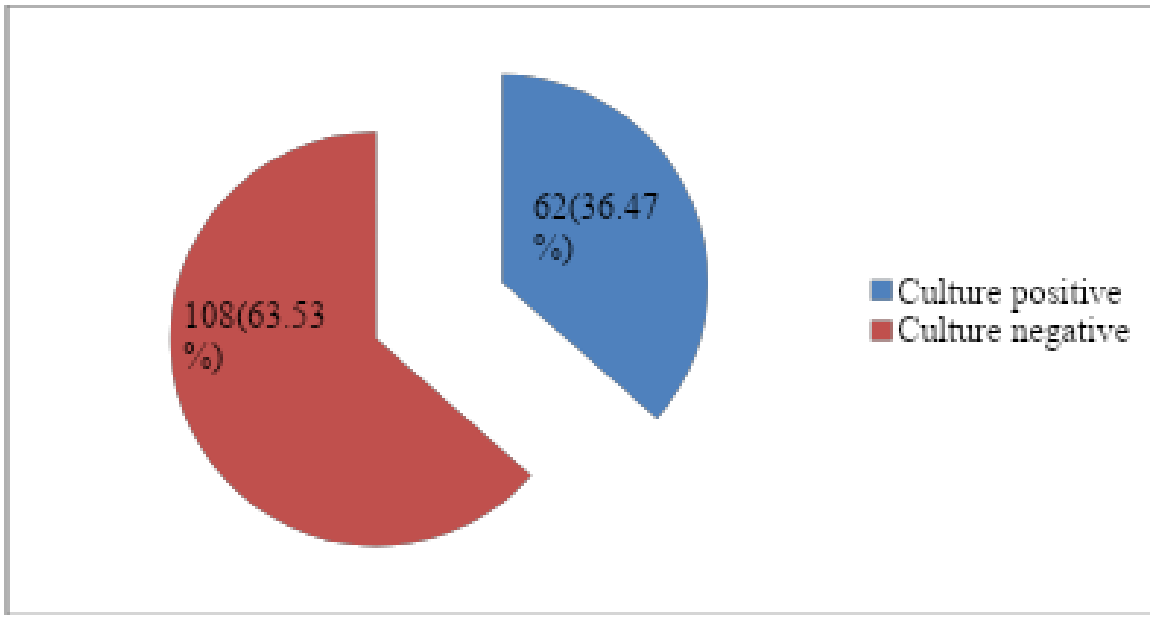


Figure 2: Rate of significant growth of urinary pathogens from urine samples

Rate of significant growth of urinary pathogens is shown in figure 2. Out of 170 urine samples, culture positive samples were 62(36.47%) and culture negative were 108(63.53%).

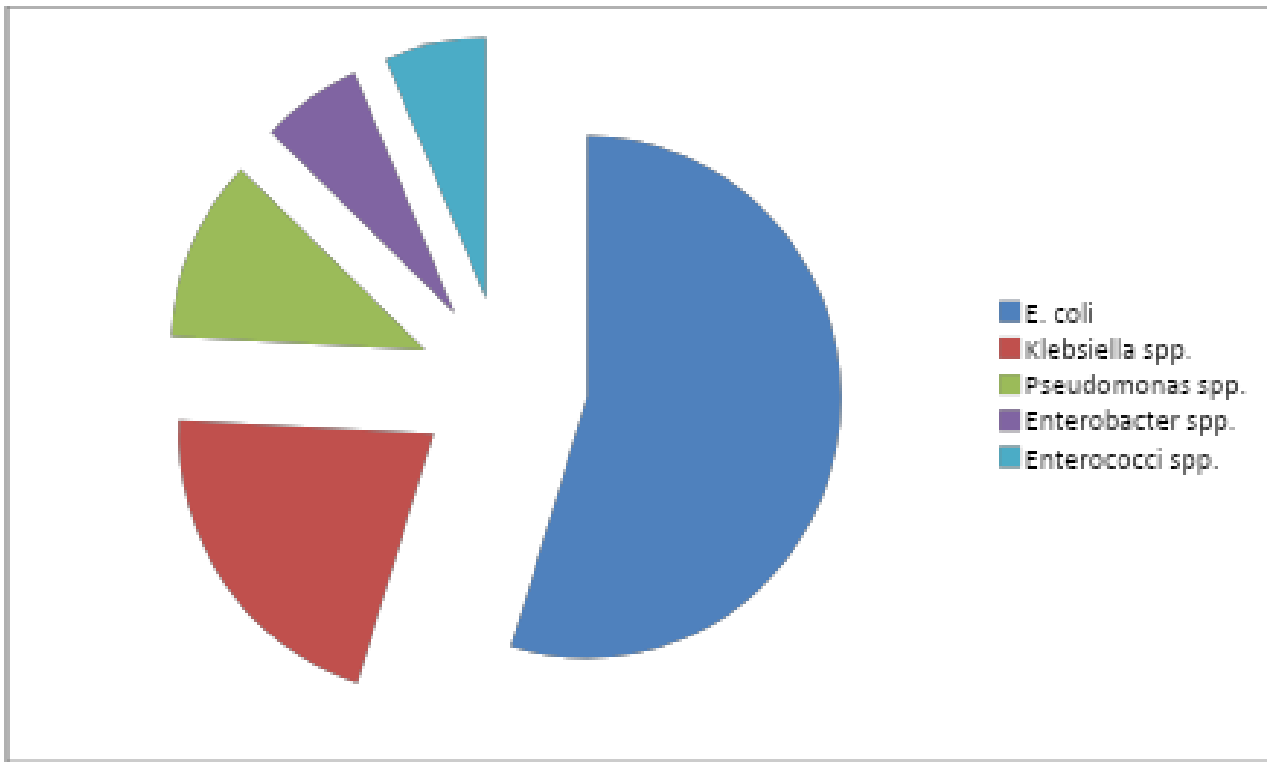


Figure 3: Distribution of bacteria isolated from culture positive cases (n=170)

Figure 3 shows distribution of bacteria isolated from culture positive cases. Out of 62 culture positive isolates, *E. coli* 34(20%) was found to be the most common pathogen followed by *Klebsiella* spp. 13(7.65%), *Pseudomonas* spp. 7(4.12%), *Enterobacter* spp. 4(2.35%) and *Enterococci* spp. 4(2.35%).

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue V May 2025

Table IV: Antibiotic susceptibility pattern of *E. coli* (n-34)

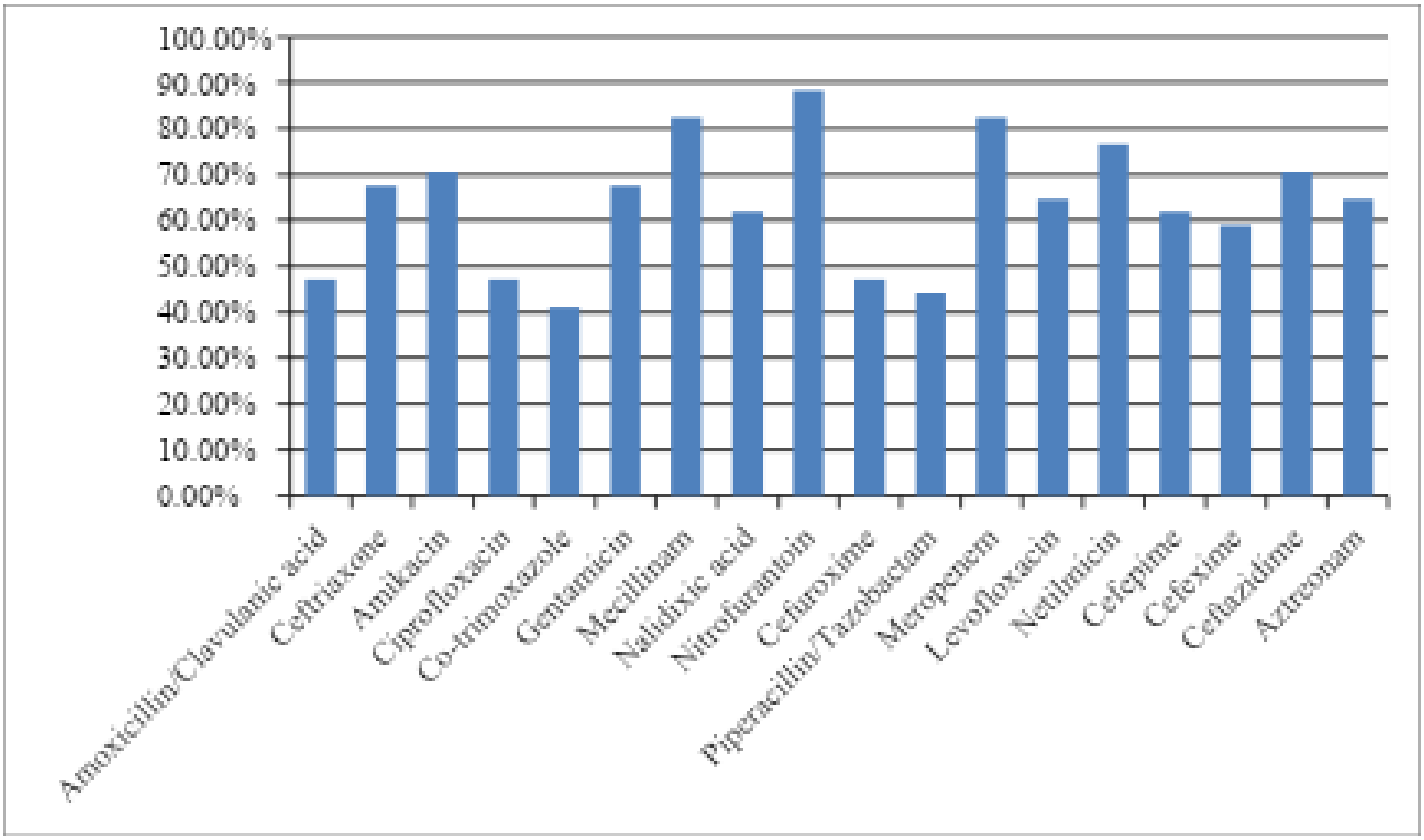


Table IV shows antibiotic susceptibility pattern of *E. coli*. *E. coli* were highly susceptible to mecillinam 28(82.35%), nitrofurantoin 30(88.23%) and meropenem 28(82.35%). *E. coli* also showed susceptibility to netilmicin 26(76.47%), ceftazidime 24(70.58%), amikacin 24 (70.58%), ceftriaxone 23(67.64%), gentamicin 23(67.64%), levofloxacin 22(64.71%), aztreonam 22 (64.71%), cefepime 21 (61.76%), nalidixic acid 21 (61.76%), cefexime 20 (58.82%), ciprofloxacin 16 (47.06%), amoxicillin/clavulanic acid 16 (47.05%), cefuroxime 16 (47.05%), piperacillin/tazobactam 15 (44.11%) and co-trimoxazole 14 (41.17%).

DISCUSSION

Every year, 150 million people worldwide suffer from urinary tract infections (UTIs), one of the most prevalent bacterial infections [16],[17]. UTI is one of the most common bacterial infections and about 50% women acquire it during their life time [18]. This can be due to the ascending movement of organisms into the bladder in women which is easier than in men because of the relatively short urethra, the absence of bactericidal prostatic secretion and the ease of contamination of the urinary tract with fecal flora [19]. In Bangladesh, the antibiogram from most of the laboratories do not reflect the whole scenario of choice of drugs in both complicated and uncomplicated UTIs. Majority of the uncomplicated UTIs are ignored due to lack of awareness and reluctance of the patients to send urine samples to laboratories. Thus, uncomplicated UTI is treated by antibiotics in both urban and rural areas without urine culture and susceptibility report. This has become a major public health concern where the chances of drug susceptibility are decreasing alarmingly. So, the aim of our study was to identify bacterial uropathogens with antibiogram of *E. coli* for better management of the UTI patients. Nearly half of adult women will experience at least one UTI episode in their lives and they are 30 times more likely than men to get one [20]. Forty percent of women generally get a UTI at some point in their lives [21]. We collected 170 urine samples from suspected patients of different age and sex with clinical evidence of one or more symptoms of UTI, such as dysuria, increased frequency, hesitation, urgency and lower abdominal pain. In figure 1, symptomatic female and male patients were 120(70.59%) and 50(29.41%) respectively. Both male and female patients 37(74%) and 86(71.67%) from OPD and 13(26%) male and 34(28.33%) female from IPD were included in our study (Table I). Of the overall number of males with symptomatic UTIs, the majority were in the old age category (70 -79 years) whereas in 120 female patients, the majority of the UTIs were reported within the age group of 19-29 years, 50-59 years and 60-69

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue V May 2025

years (p <0.001) (Table II). In our country another study showed highest rate of infection was observed in female age group 41-50 years [22]. These variations of different age groups in female patients of UTI may be due to frequent sexual activity, hormonal changes affecting the mucosal adherence of bacteria, use of spermicidal agents, menopause, indwelling catheterization and any immunocompromised condition like DM, malignanacy [21]. Males are protected by anatomical traits at all stages of life, yet UTIs are more common in males at both extremes of age [23]. Prostatic hypertrophy is also an increased risk factor for UTIs due to concomitant bladder stasis in males over 45 years of age [24]. UTI is also exacerbated by a number of chronic illnesses that affect men, such as diabetes, spinal cord injury, and indwelling or intermittent bladder catheterization [16]. In our study, risk factors in symptomatic group of UTI cases were observed. Out of five risk factors, the most common risk factor in female was DM 37 patients and in male HTN 20 patients (Table III).

In present study, a total of 170 samples of urine were collected from the suspected patients suffering from UTI. Out of 170 urine samples, 62(36.47%) samples showed culture positive results (figure 2). In Bangladesh, previous studies showed culture positive rate 414(55.27%) from 749 samples and 42.66% [25],[26]. Culture positive rate was 303(11.92%) out of 2541 samples identified in our previous studies [27]. In India 53.81% bacterial growth was isolated in a study [28]. The pattern of bacteria recovered from urine cultures is discussed in figure 3. Out of 62 culture positive isolates, *E. coli* 34(20%) was found to be the most common pathogen followed by *Klebsiella* spp. 13(7.65%), *Pseudomonas* spp. 7(4.12%), *Enterobacter* spp. 4(2.35%) and *Enterococci* spp. 4(2.35%). The most common uropathogen found in uncomplicated UTIs in both adults and children is *E.coli* [29]. The increased prevalence of *E. coli* and other Gram negative rods under Enterobacteriaceae family causing UTI can be imputed to some factors like adherence to the uroepithelium due to urogenital mucosa colonization via fimbriae, adhesins and P-1 blood group phenotypic receptor [30].

In this investigation we found most of the Gram negative bacteria are responsible for UTI in contrast to Gram positive bacteria. This is in accordance with other studies of the world in which *E. coli* is the commonest uropathogen frequently isolated from UTI patients [31],[32]. *E. coli* was found to be the predominant isolates 86.46% causing UTI, followed by *Pseudomonas* 3.96%, *Enterococci* 3.96%, *Klebsiella* 3.63%, *Enterobacter* 1.32%, *Proteus* 0.33% and *Staph. aureus* 0.33% in our another study in Bangladesh [27]. In Nepal, *E. coli* (81.3%) was the most prevailing organism and in India, *E. coli* was detected 31.25% followed by *Pseudomonas* 15.62%, *Proteus* 15.62% and *Klebsiella* 6.25% [33],[34]. This variation may be due to geographical variation where organism may change from place to place and time to time and also different sample size.

The initial choice of antibiotic treatment is based on different organisms affecting patient's age group and their antimicrobial susceptibility pattern. Changing trends in antibiotic susceptibility patterns have been reported from different parts of Bangladesh. The antimicrobial susceptibility pattern of *E. coli* (Table IV) causing UTI showed highest percentage of susceptibility in case of nitrofurantoin 30(88.23%). Higher rate of susceptibility of nitrofurantoin 195(74.43%) among 262 culture positive *E. coli* isolates was observed in our previous study in Bangladesh [27]. Re-emergence of nitrofurantoin sensitivity is probably due to non-usage of this drug for a long period of time. In Nigeria, greater percentage of the isolates were sensitive to nitrofurantoin and it would be an excellent choice for empiric therapy of UTI while awaiting the result of culture and sensitivity tests [35]. Higher susceptibility of this drug may be influenced by its limited indication with narrow tissue distribution. So, it can be used as a beneficial agent for the treatment of uncomplicated UTI. In case of mecillinam, the higher percentage of susceptibility 28(82.35%) was noted in current study (Table IV) in contrary to our previous study in Bangladesh showed 197(75.19%) susceptibility [27]. Mecillinam is an oral antimicrobial agent and has a good susceptibility. Therefore clinician can use this drug for the treatment of uncomplicated UTI.

Meropenem susceptibility is decreasing day by day due to overuse of this drug in general ward and in ICU whereas it was found 100% sensitive against Gram negative rods in a study [36]. We found 28(82.35%) meropenem susceptibility (Table IV). In King Fahd Hospital, Saudi Arabia showed that meropenem was 95.8% susceptible against Gram negative rods [37]. In aminoglycoside group, *E. coli* showed susceptibility to netilmicin 26(76.47%), amikacin 24(70.58%) and gentamicin 23(67.64%) respectively in this study. We reported previously, the susceptibility towards gentamicin 70.22%, amikacin 92.36% and netilmicin 80.53%

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue V May 2025

which was higher than the current findings [27]. The decreasing sensitivity of the antibiotics may arise complications against the empirical treatment of UTI.

Regarding quinolone group, showed susceptibility to levofloxacin 22(64.71%), nalidixic acid 21(61.76%) and ciprofloxacin 16(47.06%) (Table IV). In Bangladesh a study was conducted by us where low susceptibility of nalidixic acid 11.84% and ciprofloxacin 47.71% were identified [27]. Low rate of susceptibility of fluoroquinolone against *E. coli* was noted by other studies done in India, Iran and Spain [38]-[40]. This finding is a great concern for the clinician to choose effective drugs to treat uncomplicated UTI. Ciprofloxacin was the drug of choice for both uncomplicated and complicated UTI but there is marked reduction in ciprofloxacin susceptibility which may be due to over prescription of this drug that reduced its efficacy [41]. So, empiric use of this group of drugs should be restricted.

In this current study, *E. coli* was susceptible to ceftazidime 24(70.58%), ceftriaxone 23(67.64%), cefixime 20(58.82%) and cefepime 21(61.76%) in cephalosporin group (Table IV). In Taiwan, *E. coli* showed 94.5% ceftriaxone sensitivity [42] but in India and Pakistan very low sensitivity 25.8% and 22.22% were detected [43],[44]. This low rate of susceptibility might be due to extensive use of this group of drugs in clinical practice especially cefixime which is an oral antimicrobial drug. Though ceftriaxone is an injectable drug but its susceptibility pattern is not satisfactory and this is alarming for the physicians. This study found 14(41.17%) susceptibility to co-trimoxazole, piperacillin-tazobactam 15(44.11%), aztreonam 22(64.71%) and amoxyclav 16(47.05%) (Table IV). In India, a study observed very low rate of sensitivity to co-trimoxazole [45].

CONCLUSION

We observed that *E. coli* was highly susceptible to mecillinam, nitrofurantoin and meropenem. As mecillinam and nitrofurantoin are oral antimicrobial agents, clinician can choose these drugs for the treatment of UTI. On the other hand, low susceptibility was found against cephalosporin, fluoroquinolone, aminoglycoside group and other antibiotics. Implementing the antimicrobial stewardship program will mitigate antimicrobial resistance, enhance patients' outcome, shorten hospital stay and reduce health care cost.

Limitation of the Study

Due to lack of fund, we could not conduct the research on a greater population size and perform molecular test like PCR for confirmation of the organisms. As our current research has been executed on a tertiary care teaching hospital in Dhaka city, it does not reflect the exact scenario of the antibiotic susceptibility pattern of uropathogens of the entire nation. Further research has to be carried out with a broader sample size in different areas of the country.

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