

Research Article



Antibiotic Sensitivity Pattern in *Escherichia coli* Causing Urinary Tract Infection among Inpatients at a Tertiary Care Hospital

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ABSTRACT

Urinary tract infections (UTIs) remain the common infections in outpatients as well as hospitalized patients. Recent studies suggest an increasing antimicrobial resistance among *Escherichia coli* causing urinary tract infection (UTI). Current knowledge on antimicrobial sensitivity pattern is essential for appropriate therapy. The aim of the study is to determine the changing pattern of antibiotic sensitivity in *E.coli* causing UTI. Bacterial isolates are identified from symptomatic UTI cases attending in hospital were processed for culture and antimicrobial drug susceptibility in the Microbiology lab by Kirby Bauer's disc diffusion method. Of the total 250 samples, 191 (76.4%) cases were culture-positive for *E. coli*. The majority (63.6%) of the isolates were from female. Of the total 191 *E. coli* isolates were sensitive to levofloxacin 164 (85.8%), cefoperazone + sulbactam 160 (83.7%), Amikacin 160 (83.7%), Gentamicin 152 (79.5%), Imipenem 150 (78%), meropenem 150 (78%), Piperacillin-tazobactam 120 (71%), Ceftriaxone 74 (38.7%). The isolates showed high level of resistance to Amoxicillin-clavulanic acid (100%), Ceftriaxone 117(61%), Piperacillin-tazobactam 71(37.2%), Imipenem 41 (22%), meropenem 41 (22%), Gentamicin 39 (20.5%), cefoperazone +sulbactam 31(16.3%), Amikacin 31 (16.3%), levofloxacin 27 (14.2%). The study revealed that a significant number of the urinary tract infections in our study subjects were caused by multiple drug resistant *E. coli*. The sensitivity pattern showed that currently the levofloxacin is most effective antibiotic.

Keywords: *Escherichia coli*, Urinary Tract Infection, Antibiotic Sensitivity, Resistance

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Detection of UTI causing pathogens and analysing resistance pattern of these pathogens to commonly prescribed antibiotics in the clinical practice is essential and helpful in improving the efficacy of empirical treatment⁵.

Different factors such as age, gender, immunosuppression, comorbidity and urological instrumentation can affect prevalence of UTI⁶.

UTI caused by multidrug-resistant (MDR) *E. coli* increases the cost of treatment, morbidity, and mortality, especially in developing countries like India⁷.

However, these antibiotic sensitivity patterns may vary in different geographical locations. Hence, here, we aimed to isolate *E. coli* and study the antibiotic sensitivity profile from patients with UTIs from a tertiary care teaching hospital of S.N medical college Agra (U.P) India.

An infection anywhere in the urinary tract is called a urinary tract infection (UTI). The infection may be in the urethra (urethritis), bladder (cystitis), or kidneys (pyelonephritis).

Although men also are at risk, the frequency of UTI in women is an order of magnitude higher than that in men. Even among individuals at high risk of infection, such as catheterized patients, women have a higher risk of UTI than men.

Treatment of UTI cases is often started empirically and therapy is based on information determined from the antimicrobial resistance pattern of the urinary pathogens.

INTRODUCTION

Urinary tract infection (UTI) is a severe public health problem and is caused by a range of pathogens, but most commonly by *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterococcus faecalis* and *Staphylococcus saprophyticus*¹.

High recurrence rates and increasing antimicrobial resistance among uropathogens threaten to greatly increase the economic burden of these infections.

It is the second most common bacterial infection managed in primary care, accounting for approximately 8.1 million visits to health care providers each year².

UTI affects mostly women, with an estimated two in every three Urinary tract infection (UTI) can be caused by Gram-negative bacteria such as *Escherichia coli*, *Klebsiella* species, *Enterobacter* species, and *Proteus* species.

E. coli is the most common organism causing both community as well as hospital-acquired UTI³, often leading to serious secondary health issues.⁴



Increasing multidrug resistance in bacterial uropathogens is an important and emerging public health problem.

Increasing drug resistance in UTI needs regular monitoring of the antibiotic susceptibility of uropathogens in a particular area.

Various factors such as the type of UTI (complicated or uncomplicated), gender, age, and previous history of antibiotic therapy of each UTI patient should also be considered to find out the correct global data on susceptibility.

The distribution of antimicrobial susceptibility data of UTI-causing microorganisms changes from time to time and from place to place.

The susceptibility data provided by regional microbiology laboratories helps to choose the empirical choice of antimicrobials to treat UTI.

Generally, the antimicrobial treatment is initiated before the laboratory results which may lead to the frequent misuse of antibiotics.

Many times, physicians' resort to prescribing broad-spectrum antibiotics over specific antibiotics in the view of resistance of the causative organism to the antibiotic.

Poor patient compliance and incomplete course of antibiotic therapy have resulted in the evolution of resistance to many of these antibiotics.

This study is planned to explore the common pathogens responsible for UTIs and to determine their antibiotic susceptibility pattern.

MATERIALS AND METHODS

Collection of urine samples

The study was performed on 250 patients complaining of burning micturition and other associated illness.

Freshly voided, clean-catch midstream urine sample is collected from each patient into sterile screw-capped universal container labelled with information on the patients age, sex, and brief clinical history.

The specimen is transported to the microbiology laboratory of S.N. medical college Agra (U.P) and processed for culture and antimicrobial drug susceptibility as per the routine microbiological techniques for processing within 2 h.

Sample processing

Culture

A calibrated sterile Nicrome wire loop for the semi-quantitative method is used for the plating. It has a 4.0 mm diameter to deliver 0.01 ml. A loopful of the well mixed urine sample is inoculated on Blood and MacConkey agar plates. The plates are then incubated at 37°C aerobically for 24 hrs. They are then examined for bacterial growth. A significant bacterial count is taken as any count equal to or in excess of 100,000 CFU /ml. A less than 10²CFU/ml is

interpreted as negative. Bacterial isolates are identified generally using conventional biochemical tests⁸.

Microscopy

The urine samples are mixed and aliquots centrifuged at 5000 rpm for 5 min. The deposits are examined using both x10 and x40 objectives. Samples with >10 white blood cells/mm³ are regarded as pyuric. A volume of the urine samples are applied to a glass microscope slide, allowed to air dry, stained with gram stain, and examined microscopically.

Bacterial isolates are identified generally using a battery of tests.

Antibiotic susceptibility testing

The sensitivity of the strains against various antibiotics is determined by using antibiotic sensitivity discs; namely imipenem (IE) 10/750 mcg, cefepime (CPM) 50 µg, piperacillin (PIT) 100/10 µg, amoxiclav (AMC) 10 µg, aztreonam (AT) 50 µg, methicillin (MET) 30 µg, penicillin G (PG) 10 units, ampicillin 10 µg, vancomycin (VA) 30 µg, bacitracin (B) 10 units, gentamicin (GEN) 50 µg, amikacin (AK) 30 µg, chloramphenicol 50 µg, , ciprofloxacin (CIP) 5 µg, ofloxacin (OF) 2 µg, norfloxacin (NX) 10 µg, co trimoxazole (COT) 25 µg, polymyxin B (PB) 50 units.

The antibiotic characteristics of the 26 isolates are analysed by Kirby Bauer disk diffusion method. The assay is conducted in triplicate for each organism to be evaluated.

Mueller Hinton Agar (MHA) by Hi media is used to evaluate each microorganism for antibiotic susceptibility test.

The Kirby–Bauer test (disc-diffusion antibiotic susceptibility test, disc-diffusion antibiotic sensitivity test, KB test), is a test of the antibiotic sensitivity of bacteria. It uses antibiotic discs to test the extent to which bacteria are affected by those antibiotics. In this test, wafers containing antibiotics are placed on an agar plate where bacteria have been placed, and the plate is left to incubate. If an antibiotic stops the bacteria from growing or kills the bacteria, there will be an area around the wafer where the bacteria have not grown enough to be visible. This is called a zone of inhibition.

The disks are allowed to settle before being inverted and placed in an incubator at 37°C. The plates are checked for susceptibility after 24 h and the zones of inhibition are recorded for all the plates.

The microorganisms are evaluated as susceptible, intermediate and resistant or resistant to each antibiotic by using the National Committee for Clinical Laboratory Standard's chart provided with the antibiotic kit by Hi media.⁹

Semi-quantitative urine culture using a calibrated loop was used to isolate bacterial pathogens on blood and MacConkey agar as per the recommendations of Kass.¹⁰



The plates were incubated at 37°C for 24 h and further incubated for 48 h in culture (growth) negative cases.

Following this, the isolates were identified by standard biochemical tests, and diagnosis of UTI was made when pathogens were present at a concentration of at least 10⁵ colony-forming unit (CFU)/ml of urine. Isolates other than *E. coli* were not considered for this study.

Antibiotic sensitivity testing

Antibiotic sensitivity testing was done on Mueller-Hinton agar by Kirby–Bauer disc diffusion method¹¹ using following antibiotics discs ampicillin (AMP 10 mcg), amikacin (AK 30 mcg), amoxicillin-clavulanic acid (AMC 30 mcg), aztreonam (AT 30 mcg), ceftriaxone (CTR 30 mcg), cefuroxime (CXM 30 mcg), cefepime (CPM 30 mcg), ciprofloxacin (CIP 5 mcg), chloramphenicol (C 30 mcg), gentamicin (GEN 10 mcg), imipenem (IPM 10 mcg), nitrofurantoin (NIT 300 mcg), norfloxacin (NX 10 mcg), and piperacillin-tazobactam (PIT 100/10 mcg) as per CLSI guidelines.¹²

Statistical analysis

Statistical software package SPSS version 16 was used to analyse the data. Age, gender, organisms causing UTI, and its antibiotic sensitivity and resistance were included as variables in the model.

RESULTS

Among 250 samples tested, 76.4(191) samples showed growth of *E. coli* with colony count of over 10⁵ CFU/ml of urine. Of these 250 patients, 159 patients were female (63.6%) and 91 patients were male (36.4 %) (Table-1).

Of the total 250 samples, 191 (76.4%) cases were culture-positive for *E. coli*. These isolates were tested for antibiotic susceptibility by disk diffusion method.

Of the total 191 *E. coli* isolates were sensitive to levofloxacin 164 (85.8%), cefoperazone +sulbactam 160 (83.7%), Amikacin 160 (83.7%) %. Gentamicin 152 (79.5%). Imipenem150 (78.5%), meropenem150(78.5%), Piperacillin-tazobactam 120(62.8%), Ceftriaxone 74 (38.8%) (Table-2).

Table 1: Sex distribution of patients with urinary tract infections

| Gender | Frequency | Percentage |
|---------|-----------|------------|
| Males | 91 | 36.4% |
| Females | 159 | 63.6% |
| TOTAL | 250 | 100% |

Table 2: Antibiotic sensitivity and Resistance pattern of *E. coli* in UTI

| Antibiotic disc | <i>E. coli</i> (n=191) | |
|-------------------------------|------------------------|-------------|
| | S | R |
| Amoxicillin + clavulanic acid | 0 | 191 (100%) |
| Piperatazo | 120 (62.8%) | 71 (37.2%) |
| Meropenem | 150 (78.5%) | 41 (21.5%) |
| Imipenem | 150 (78.5%) | 41 (21.5%) |
| Ceftriaxone | 74 (38.8%) | 117 (61.2%) |
| Cefoperazone + sulbactam | 160 (83.7%) | 31 (16.3%) |
| Levofloxacin | 164 (85.8%) | 27 (14.2%) |
| Amikacin | 160 (83.7%) | 31 (16.3%) |
| gentamicin | 152 (79.5%) | 39 (20.5%) |

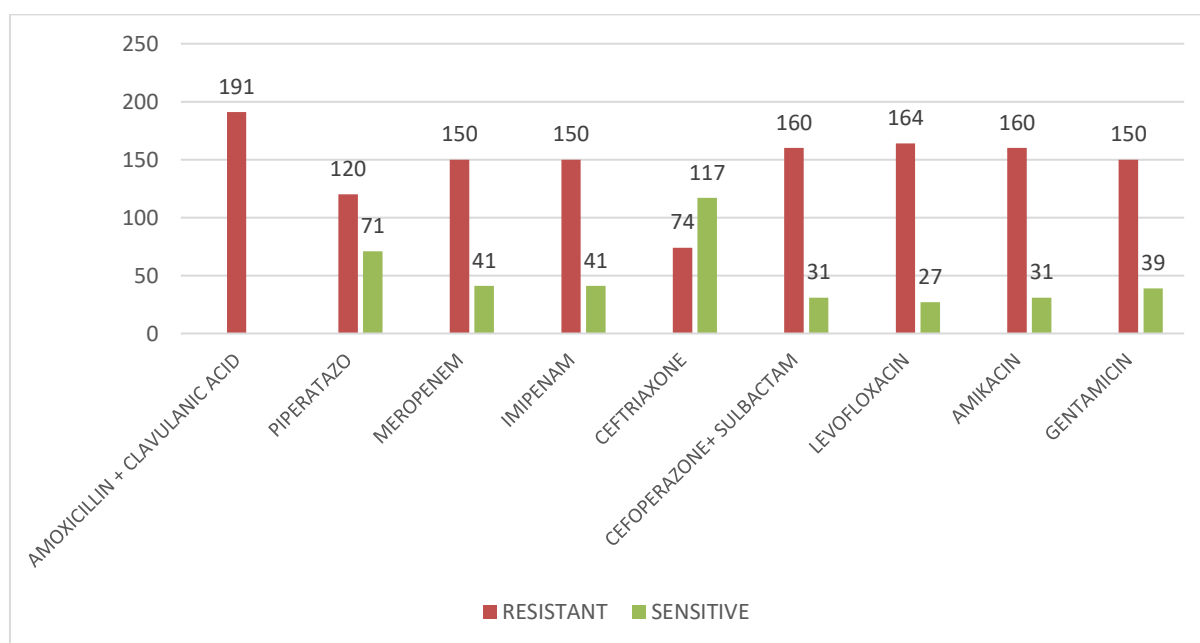


Figure 1: Antibiotic sensitivity and Resistance pattern of *E. coli* in UTI

The isolates showed high level of resistance to Amoxicillin-clavulanic acid (100%), Ceftriaxone 117(61%), Piperacillin-tazobactam 71(37.2%), Imipenem 41 (22%), meropenem 41 (22%), Gentamicin 39 (20.5%), cefoperazone +sulbactam 31 (16.3%), Amikacin 31 (16.3%), levofloxacin 27(14.2%) (Fig-1).

DISCUSSION

Our study showed a high prevalence of UTI in females (73.57%) than in males (35.14%) which correlates with other findings which revealed that the frequency of UTI is greater in females as compared to males. It is well established that female are more commonly infected with UTI than male due to anatomical position of urethra, influence of hormone and pregnancy¹³.

In this present study, *E. coli* was the most predominant species isolated in our study population. UTI caused by the MDR *E. coli* has increased in the current years probably due to irrational use of antibiotic which is available in the local market in this country and these are given without prior culture and antibiotic sensitivity pattern. In addition to that, incomplete dose is another factor.

The distribution of species and their susceptibility to antibiotics vary with time and place¹⁴.

Formation of films by bacteria inside the bladder leads to recurrent infections and also increases the possibility of MDR strain causing UTI¹⁵.

In our study maximum sensitivity was recorded for the drugs levofloxacin (85.8%), followed by cefoperazone +sulbactam (83.7%), Amikacin (83.7%), Gentamicin 152 ((79.5%), Imipenem and meropenem 150 (78.5%), Piperacillin-tazobactam (62.8%), Ceftriaxone (38.8%).

In our study *E. coli* showed high level of resistance to Amoxicillin-clavulanic acid (100%), followed by Ceftriaxone (61%), Piperacillin-tazobactam (37.2%).

Araghya Das et al. (Department of Microbiology, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India study January 2013 to September 2015) in his study observed highest resistance to Ampicillin (76.8%), ceftriaxone (100%), cephalexin (77%) cefepime (100%), Gentamicin (10.71%), Ofloxacin (66.07%), Norfloxacin (69.64%) whereas the organisms were most sensitive to piperacillin-tazobactam (100%), imipenem (100%) and amikacin (100%), gentamycin (89.29%) Nitrofurantoin (91.07 %).

The available data from a similar study from North-East India by Mukherjee et al reported a considerable rate of resistance in uropathogenic *E. coli* to Ceftriaxone (62.5%), other study supports my study result, which will be helpful in future studies.

In conclusion, the antibiotic resistance pattern of *E. coli* UTI pathogens in our study similar to that found in West Bengal, and other parts of India, as well as other parts of the globe.

Greater than 50% resistance was observed for penicillin combinations. Hence, these agents should not be used as an empiric treatment for UTI in northern India., levofloxacin appears to be the first choice of drug or a combination of the two drugs (Cefoperazone+sulbactam) followed by aminoglycosides Amikacin and gentamicin could be the most appropriate alternative in treating UTIs.

CONCLUSION

The antimicrobial resistance patterns of the causes of UTI are highly variable, and continuous surveillance of trends in resistance patterns of uropathogens is necessary. The treatment of UTI by antimicrobial agents needs to be strongly promoted by in vitro susceptibility testing to evade advance spread of antimicrobial resistance in patients and eventual development of MDR

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