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Research Article

Assessment of Antibiotic Resistance Profile in Uropathogenic *E. coli* Isolates from Clinically Presumed UTI Patients in Telangana

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ABSTRACT

Objectives: The purpose of the current investigation is to assess the antibiotic susceptibility pattern of *Escherichia coli* (*E. coli*) isolated from urine samples of patients suspected of having UTIs. The majority among them tested positive for *E. coli* in the culture. **Results:** The Minimum inhibitory concentration of Trimethoprim/Sulfamethoxazole was highest and that of Ciprofloxacin was the least for *E. coli*. The isolated samples showed the highest level of resistance towards Ampicillin (22%), followed by Nalidixic Acid (20%), Ciprofloxacin (20%), Cefuroxime (18%), Cefuroxime axetil (18%), and Ceftriaxone (17%). The demographic statistical data show that women prone more to UTIs with a higher incidence at 21-30 years age group. Significant colony growth in different age groups in In-patient samples showing was observed. The β-lactamase-producing *E. coli* were high in both in-patient and out-patient urine samples. The nitrite test and distribution of pus cells confirmed the presence of UTI in most samples. Further, higher amounts of CTX-M type of Extended-spectrum β-lactamase producing *E. coli* were observed in urine samples of in-patients that unequivocally show the organism's resistance to different classes of antibiotic drugs. **Conclusion:** The results indicate that most cases of UTIs were caused in females which were mostly because of the multidrug-resistant *E. coli*.

Keywords: β-lactamase, *Escherichia coli*, Multi-Drug Resistance, Urinary tract infection.

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INTRODUCTION

Urinary tract infections are among the most prevalent cases of bacterial infections in the world, having a significant social and economic impact on humanity. It is estimated that UTIs represent hundreds of millions of cases every year across the globe, and a major burden on the healthcare system in terms of financial resources (Zagaglia *et al*., 2022; Zeng *et al*., 2022). In 2019, the number of incident cases of UTI worldwide was around 0.4 billion, and the number is steadily increasing (Zeng *et al*., 2022). Approximately 50-60% of women report having experienced at least one of these episodes at some point in their lives, and the reproductive age group (21-30 years old) frequently experiences the highest risk of UTI (Flores-Mireles *et al*., 2015; Medina and Castillo-Pino, 2019). Most of these infections are caused by a Gram-negative bacterium known as the *Escherichia coli* (*E. coli*), which causes up to 70-90% of all community-acquired UTIs (Flores-Mireles *et al*., 2015; Zagaglia *et al*., 2022). Although the majority of *E. coli* are non-pathogenic commensals of the gut, some Extraintestinal Pathogenic *E. coli* (ExPEC) strains are extremely virulent, and can cause extraintestinal infections, such as pyelonephritis and life threatening urosepsis. The increasing rates of the *E. coli* both in the community and hospital settings require localized and urgent monitoring at the highest level.

The Antimicrobial Resistance (AMR) is critically eroding the effectiveness of standard empirical antibiotic treatment of UTIs due to its rapid and endemic spread. The WHO has designated AMR as one of the most significant global health concerns, contributing to approximately 1.27 million deaths in the world in 2019 and to almost 5 million deaths (Medina and Castillo-Pino, 2019; Zeng *et al*., 2022). UTIs in particular cause tens of thousands of deaths and hundreds of thousands of related deaths every year, and is an unignorable health issue (Zeng *et al*., 2022). The rate of Multidrug-Resistance (MDR) in Uropathogenic *E. coli* (UPEC) is so high that it is frequently growing (Flores-Mireles *et al*., 2015; Kaye *et al*., 2021). The main first-line agents are already losing their effect; recent data indicate that the resistance to such antibiotics as Trimethoprim-Sulfamethoxazole and

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Ciprofloxacin (fluoroquinolones) is high and regularly increasing worldwide, reaching a stable level of over 20-30% in most places (Flores-Mireles *et al*., 2015; Kaye *et al*., 2021; Paterson and Bonomo, 2005). Main cause of this phenomenon is the widespread use, abuse and improper choice of these antibiotics, limiting the choice of clinicians.

Production of Extended-Spectrum β-Lactamase (ESBL) enzymes is the most worrying mechanism of resistance in *E. coli*. Such enzymes make them resistant to a range of essential β-lactam antibiotics, such as the third-generation cephalosporin (e.g., Ceftriaxone) (Paterson and Bonomo, 2005). The global prevalence of ESBL-producing version of *E. coli* has been approximated to span 42.1% in clinical isolates and Asia is one such regions where the highest occurrence has been noted (Paterson and Bonomo, 2005). In particular, the CTX-M-type enzymes have become the most prevalent worldwide and they are representing a drastic and accelerating change in resistance epidemiology (Medina and Castillo-Pino, 2019; Paterson and Bonomo, 2005). The CTX-M-type ESBL of uropathogens is a strong indicator of a multi-drug resistant profile, which frequently predetermines the extended period of hospital stay, high treatment expenses, and the need to use reserve antibiotics, including carbapenems. Constant monitoring of ESBL type is an essential infection control measure.

With the demographic conditions that predispose women of reproductive age to UTIs, as well as the emerging and increasing risk of MDR and ESBL-producing strains of *E. coli*, the current and localized antimicrobial susceptibility data is that which cannot be done without in the successful management of the issue. There should be a consistent monitoring of resistance patterns of major antibiotics such as Ampicillin, Nalidixic Acid, Ciprofloxacin and Cefepime (Ceftriaxone). Therefore, the purpose of this study was to investigate *E. coli* current trends of antibiotic susceptibility in the samples of urine of suspected patients contacting UTI. The goal of the paper is to establish the current resistance profile, which involves the rate of β-lactamase production (particularly CTX-M type), in order to offer empirical evidence-based local guidelines on antibiotic prescription.

MATERIALS AND METHODS

Sample collection

The present investigation was conducted in a multispecialty hospital, Hyderabad with 328 patients, both Inpatient (IP) and Outpatient (OP), with clinically UTI-suspected symptoms which were confirmed by the doctor. All of the patients involved in the study had given their informed consent. Men were instructed to retract the foreskin and clean it while, women were requested to separate their labia and clean them with soap and water before collecting urine after 2 sec after the start of urination. "Mid-stream clean catch" samples were collected in 30 mL sterilized, sealed, screw-top plastic containers (Clinical and Laboratory Standards Institute, 2024a). The label on sample' included the patient's age, gender, code number, time, and date of collection. Samples were brought to Microbiology Laboratory in coolers for further bacteriological investigations.

Bacteria isolation and identification

Blood agar, MacConkey agar, Eosin Methylene Blue agar, Nutrient agar, and Mannitol Salt agar (Hi Media, Bombay, India) were utilized. The samples were spotted on the agar plates and left to incubate at 37ºC after 24 hr. Bacterial growth in these cultures was then monitored. Subcultures were created on nutrient agar plates, which were then left to incubate for 24 hr. Using traditional microbiological techniques, the bacterial colonies were identified by colony emergence and coloration (Benson, 2021).

Antibiotic Stability Testing

Antibiotic Stability test was conducted as per the procedure of Nair *et al*., 2005. The stability of isolates of *E. coli* was tested by agar diffusion technique with following antibiotics disks: Amikacin, Amoxicillin/Clavulanic Acid, Ampicillin, Cefepime, Cefoperazone/Sulbactam, Ceftriaxone, Cefuroxime, Cefuroxime axetil, Ciprofloxacin, Colistin, Ertapenem, Gentamicin, Imipenem, Meropenem, Nalidixic Acid, Nitrofurantoin, Piperacillin/Tazobactam, and Trimethoprim/Sulfamethoxazole.

Multiple Antibiotic Resistance (MAR) Index

The index of Multiple Antibiotic Resistance (MAR) was calculated using the formula: MAR=x/y. The number of antibiotics to which the test isolate showed resistance was denoted by x, and y denoted the total number of antibiotics to which the test organism's sensitivity was assessed (Akinjogunla and Enabulele, 2010).

β-Lactamase production

β-Lactamase production was assayed according to Al-Hayanni and El-Shora, 2023. The broth culture was inoculated on Mueller-Hinton agar and 1% starch followed by overnight incubation at 37ºC. The cultured plates were flooded with freshly prepared phosphate-buffered saline containing potassium iodide, iodine and penicillin. The appearance of colourless zones in the region of bacterial growth indicated β-Lactamase production.

Distribution of pus cells in the urine sample

10 mL of urine were centrifuged for 5 min at 1,500 rpm, followed by removal of the supernatant. The cell pellet was resuspended in 1 mL sterile PBS and stained by the Sternheimer-Malbin Staining method (Sternheimer and Malbin, 1951). About 20 microscopic fields were examined using 40X High Power Field (HPF) for each sample, and the average number of cells per HPF was calculated.

Nitrate test

Nitrites are usually absent in urine. The test detects the presence of nitrite reductase enzyme that is produced by different species of bacteria. Dietary nitrates present in the urine are reduced by these UTI-causing-bacterial-enzymes to convert them to nitrite. All Enterobacteriaceae and most of the non-fermenters are included in this nitrite-reducing bacterial species. However, Candida and Streptococci including Enterococci do not have this characteristic. Thus, a positive result in the nitrite test is indicative of presence of UTI (McPherson and Pincus, 2017). The test was carried out by asking the patients to collect morning void midstream urine samples in sterile bottles. Dipstick urine analysis was done immediately by employing Multistix 10 SG (Bayer Corporation USA) with the nitrite test being performed following instructions by the manufacturer.

Distribution of Beta-lactamase and non-Beta-lactamase producing *E. coli*

Acidimetric method was utilized to test the existence of β-lactamase using benzylpenicillin as the substrate. Each colony was resuspended and then combined with an indicator solution. The indicator solution was prepared by the addition of 1 mL sterile distilled water and 100 µL of 1% phenol red solution into a vial containing 1 million units of sodium benzylpenicillin. 1N NaOH solution was added until the violet colour was observed (pH 8.5). Dense suspension was prepared by suspending several colonies in NaCl, 9% (w/v). Colour development was observed within 1 hr after the addition of 150 µL of penicillin phenol red solution. The presence of β-lactamase was confirmed by the change of solution to yellow colour (Jorgensen *et al*., 2020).

AST phenotypes obtained for the *E. coli* by VITEK-2 method

Minimum Inhibitory Concentration (MIC) was established using VITEK- 2 on the analysis of growth kinetics in test cards. VITEK cards were inoculated and incubated in Susceptibility Testing (AST-N020) as recommended by the manufacturer with the result interpreted as per (Clinical and Laboratory Standards Institute, 2024b).

Testing for samples with significant colonies

Urine cultures with sizable colonies were used to evaluate samples from both inpatient and outpatient departments for the presence of UTIs, and those which showed growth of 1,000 CFU/mL or more were considered positive. Of the samples that tested positive, the colony growth percentage was determined and the samples with urine colony counts of 100,000 CFU/mL or more were determined as samples with significant colony growth percentage (Hooton *et al*., 2010).

Different mechanisms of beta-lactamase production by *Escherichia coli* isolated on the ESBL and KPC media

All multi-drug resistance isolates (having resistance to two or more classes of antibiotics) were collected after antimicrobial susceptibility testing and subculturing on CHROMagar™ KPC was done to test carbapenemase production. After incubation was done for 24 hr and colonies were visualized with typical colouring characteristics to assess isolates producing carbapenemase. Carbapenemase-producing *E. coli* colonies developed dark pink to reddish colour. Similarly, *E. coli* producing Extended Spectrum Beta-Lactamase (ESBL) was detected using CHROMagar ESBL medium (Panagea *et al*., 2011). Colonies were inspected for dark pink to reddish color after a 24-hr incubation period, which was indicative of *E. coli* producing ESBL.

Statistical analysis

Statistical analysis of the data was performed on Sigmaplot software (V15.0, USA). The graphs were constructed using Graphpad Prism software (V8.0, USA).

RESULTS

The current investigation comprised 326 samples in total, of which 217 were Inpatient (IP) and 109 were Outpatient (OP).

MIC value of Antibiotic Susceptibility of *E. coli* isolates

Antimicrobial susceptibility of *E. coli* was defined as Susceptibility breakpoints used by the authors of each of the studies included, and isolates with intermediate susceptibility to an antibiotic were considered resistant, in accordance with the accepted standards (CLSI, 2024). The results of MIC testing are given in Figures 1A and B. The data indicate that the Resistant MIC values for Piperacillin/Tazobactam (TZP>123.81) and Trimethoprim/Sulfamethoxazole (SXT>314.43) were the highest, corresponding to sensitive MIC values of 4.01±0.91 and 10.32±1.02, respectively. Moderate resistance (Resistant MIC values ranged from approximately 61.18 to 71.21) was observed for most cephalosporins (Cefuroxime (CXM), Cefuroxime axetil (CXMA), Ceftriaxone (CRO), Cefoperazone/Sulbactam (SFP), Cefepime (FEP)), Amikacin (AN), Ertapenem, and Colistin (CS). Least resistance (the lowest Resistant MIC values) was observed for Ciprofloxacin (CIP), with a sensitive MIC value of 0.27±0.07 and a Resistant MIC value of 4.26±0.51. Meropenem (MEM) and Imipenem (IPM) also showed low Resistant MIC values (14.78±0.97 and 13.45±0.99, respectively). The development of novel resistance to antibiotics, such as the evolution of extended-spectrum beta-lactamases by bacteria and the spread of genes to itinerant elements, can account for the persistent shift in the pattern of antibiotic susceptibility in *E. coli* (Figures 1A and B).

In vitro antibiotic sensitivity in isolated uropathogenic *E. coli* from OP

The data on antibiotic sensitivity analysis revealed that there were specific resistance patterns between uropathogenic *E. coli* isolates of the Outpatient (OP) and Inpatient (IP) samples, and the data is given in Table 1. The OP cohort (n=33) had the highest resistance

Table 1: Antibiotic sensitivity data from uropathogenic *E. coli* isolates from OP and IP samples.

Antimicrobial Agents	Resistant		Intermediate		Sensitive	
	OP	IP	OP	IP	OP	IP
Ampicillin	22	40	1	1	10	6
Amoxicillin/Clavulanic Acid	6	21	5	3	22	23
TZP-Piperacillin/Tazobactam	3	16	1	1	29	30
Cefuroxime	18	37	2	0	13	10
Cefuroxime Axeti	18	37	2	0	13	10
Ceftriaxone	17	37	0	0	16	10
Cefoperazone/Sulbactam	3	14	0	0	30	33
Cefepime	4	19	0	0	29	28
Entrapenem	0	10	1	0	32	37
Imipenem	0	10	1	2	32	35
Meropenem	0	9	0	0	33	38
Amikacin	0	6	0	1	33	40
Gentamicin	4	19	0	0	29	28
Nalidixic Acid	20	41	0	0	13	6
Ciprofloxacin	20	40	1	0	12	7
Nitrofurantoin	13	3	2	5	18	39
Tigecycline	0	0	0	0	33	47
Colistin	0	1	0	0	33	46
Trimethoprim/Sulfamethoxazole	11	24	0	0	22	23

rates of 66.7% Ampicillin resistant, 60.6% Nalidixic Acid and 60.6% Ciprofloxacin resistant respectively. The resistance to common third generation cephalosporins such as Cefuroxime, Cefuroxime axetil, and Ceftriaxone was also greater than 50. In comparison, the IP cohort (*n*=47) had universally more resistance burden with it showing the highest rates of Nalidixic Acid (87.2% resistant), Ciprofloxacin (85.1% resistant), and Ampicillin (85.1% resistant). Moreover, Cefuroxime, Cefuroxime axetil, and Ceftriaxone resistance was high at 78.7% in IP samples which is much greater compared to the OP group. Significantly, there was elevated sensitivity of both cohorts to newer and reserve agents including Tigecycline and Colistin and carbapenems (Meropenem, Ertapenem, and Imipenem) were also highly sensitive, especially in OP samples. These outcomes point to a major and alarming dissimilarity in the profiles of antibiotic resistance of community-acquired and hospital related UTIs due to *E. coli*.

The uropathogenic *E. coli* from samples of OP were analyzed for antibiotic sensitivity. The isolates showed the highest level of resistance towards AM-Ampicillin (22%) followed by Nalidixic Acid (20%), Ciprofloxacin (20%), Cefuroxime (18%), Cefuroxime axetil (18%), Ceftriaxone (17%). There was moderate resistance towards trimethoprim (11%) and nitrofurantoin (13%) and higher sensitivity rates towards Tigecycline, Colistin, Amikacin, Meropenem and Amikacin (33%) were observed. The results show antibiotic and antimicrobial resistance of many antibiotics as depicted in Table 1.

Analysis of samples for significant colony growth

One hundred and twenty-six urine specimens passed a test of percentage growth of colonies. The results of the analysis were that a much higher percentage of colony growth was observed in the Inpatient (IP) samples (60) as compared to the Outpatient (OP) samples (39). There was a total of 113 samples which were referred to as culture positive meaning that it contained bacteria at or above the threshold level used to define significant bacteriuria.

Frequency and distribution of bacterial isolates from UTI cases

The data on bacterial isolations of both IP and OP samples can be seen in Figure 2A. As it can be seen the frequency of isolates in IP samples (*n*=68) is greater than in OP samples (*n*=45). *E. coli* since (47 isolates or 69% of IP total) and *K. pneumoniae* (13 isolates or 19.1% of IP total) were the dominant isolates in the IP group. This distribution contrasts with the OP group, with *Escherichia coli* (33 isolates, 73% of IP total) and *Klebsiella pneumoniae* (8 isolates, 17.8% of IP total). Other percentages of *P. aeruginosa* (3), *S. aureus* (1), *Citrobacter koseri* (1), and *Proteus mirabilis* (1) were observed in the IP samples as well. In the same way, only OP samples contained *S. epidermidis* and S. *haemolyticus*

(1 isolate each, or 2.2% of OP total). *Escherichia coli*, *Klebsiella* spp., and *Proteus* spp. are unquestionably the uropathogens most frequently found in UTI patients (Flores-Mireles *et al.*, 2015).

Frequency distribution of isolated *E. coli* from OP patient

Distribution of *E. coli* isolates via all the Outpatient (OP) samples by age group and gender can be seen in Figure 2B. The findings show that the percentage overall of bacteria is greater in females (n=21) than in males (n=12). The highest concentration of *E. coli* isolates is present in patients of 21-30 (6% in females), 51-60 (3% in males), and 61-70 (4% in females). The minimum number of bacteria was registered among patients aged 81-90 years (1% among men and no percent among women). The finding that women had a higher overall incidence of UTIs than men is consistent with previous research. The primary reason for this

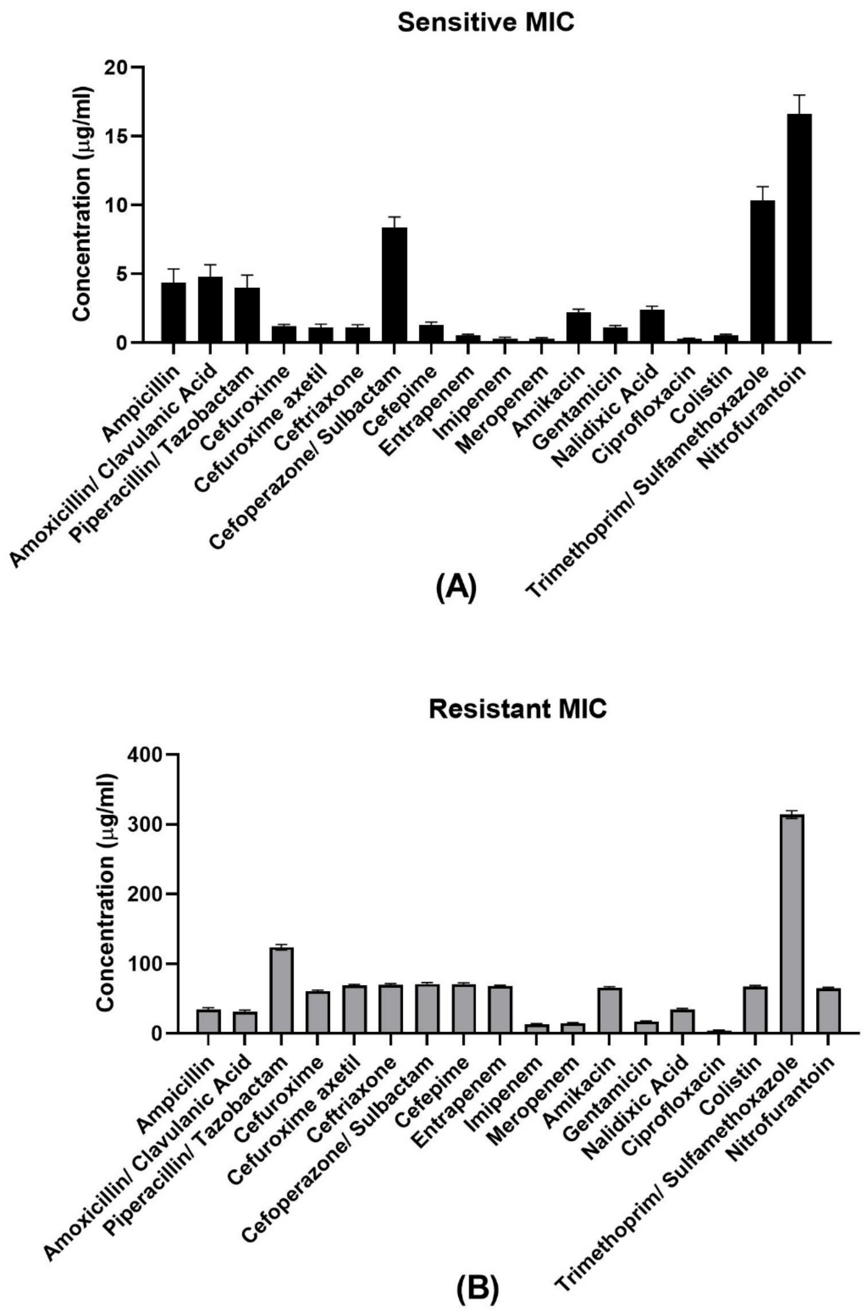

Figure 1: Antibiotic Susceptibility Profile of Uropathogenic *E. coli* (UPEC) Isolates (A) Sensitive MIC displays the MIC values (µg/mL) of 16 tested antibiotics (B) Resistant MIC displays the MIC values (µg/mL) of 16 tested antibiotics. Data presented as Mean±SE, $p<0.05$.

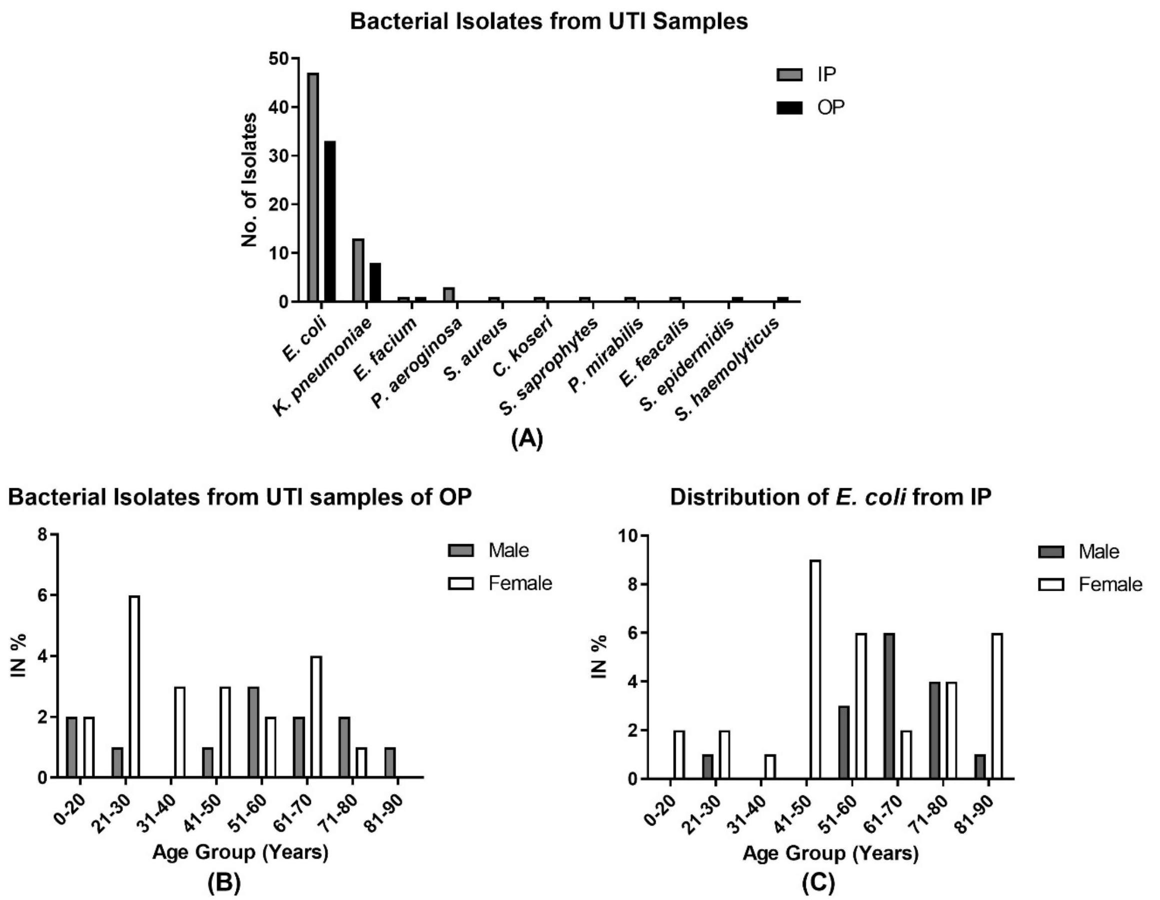

Figure 2: Analysis of Bacterial Isolates and Demographics from Urinary Tract Infection (UTI) Samples (A) Identification and distribution of bacterial isolates from UTI samples, (B) Gender- and age-specific distribution of isolates among OP, (C) Gender- and age-specific distribution of isolates among IP. IP-In Patient, IN%-Incidence Percentage, OP-Out Patient.

discrepancy is that females have shorter urethras that are closer to the vagina and anal opening (Medina and Castillo-Pino, 2019). Additionally, women are said to be more vulnerable to UTIs following menopause due to a drop in estrogen levels, which compromises the urinary tract's natural defense (Flores-Mireles *et al.*, 2015). The difference was also slight in the group of 70 and above as the distribution in the age group became more balanced.

Distribution of isolated *E. coli* from IP

Figure 2C depicts the distribution of *E. coli* isolates of all Inpatient (IP) samples based on age group and gender. The comparison showed that there were more *E. coli* isolates (*n*=32) in female IP samples compared to those in male IP samples (*n*=15). More *E. coli* was isolated in female patients within the age group of 41-50 (*n*=9), 51-60 (*n*=6), and 81-90 years (*n*=6). It is noteworthy that on the female end, the highest number of counts were recorded in the 81-180 age category (*n*=88) whereas on the male end, the highest number of isolates was recorded in the 61-70 age (*n*=6) and the 71-80 age (*n*=4) category.

Distribution of β-lactamase and non β-lactamase producing *E. coli*

Figure 3A shows the general distribution of β-lactamase and non-β-lactamase producing *E. coli* isolates in the Outpatient (OP) and Inpatient (IP) samples. Sixty-two samples were detected to be β-lactamase producing *E. coli*. This resistance phenotype was mainly present in IP samples (40 isolates) than in OP samples (22 isolates). However, 18 isolates of *E. coli* did not develop β-lactamases. This was more common in the OP than in the IP group (11 isolates and 7 isolates respectively).

Different mechanisms of β-lactamase production by *E. coli* isolated on the ESBL and KPC media

The mechanisms underlying the resistance to antibiotics specifically β-lactam are normally linked with the development of particular β-lactamase enzymes. Figure 3B displays the distribution of the isolates with Extended-Spectrum β-Lactamase (ESBL) and *Klebsiella pneumoniae* Carbapenemase (KPC) production. The general incidence of ESBL-producing *E. coli* was higher in the Inpatient (IP) samples (27 isolates) than in the Outpatient (OP) samples (2 isolates). Likewise, KPC-producing

E. coli was observed in higher frequencies in the IP samples (14 isolates) than in the OP samples (2 isolates).

The AST phenotypes obtained for *E. coli* by VITEK-2 methods

AST phenotypic analysis was performed by the use of VITEK-2 method, an inexpensive, standardized, and sensitive method, with specific attention to β-lactam and aminoglycoside resistance. The phenotype of aminoglycoside resistance was measured, and it was found that 1% (*n*=1) of the strains in the Outpatient (OP) and 6% (*n*=6) in the Inpatient (IP) were resistant to Gentamicin, Tobramycin, Amikacin, and Netilmicin (GEN, TOB, AMK, NET) (Figure 3D). The more complicated β-lactam resistance phenotypes were impermeability to CARBA + ESBL or HL + AmpC and Carbapenemase + or - ESBL, which was present in 2% (*n*=2) of OP samples and 14% (*n*=14) of IP samples, wherein 22 of the OP and 27 of the IP isolates belong to the ESBL phenotypic category of the β-lactam resistance (Figure 3C). The mechanisms that result in antibiotic resistance are usually because of the production of β-lactamase enzymes, which are present in different genotypes such as SHV, TEM, CTX-M amongst other mechanisms such as VEB, PER, BEL-1, BES-1, SFO-1, TLA and IBC (Paterson and Bonomo, 2005). The CTX-M (cefotaxime, Munich) form of the ESBL appeared in 20 OP and 27 IP urine samples but SHV1 mutant hyperproduction appeared in 2 OP urine samples only in the current study (Figure 3D). This kind of ESBL CTX-M is clinically significant since it is capable of hydrolyzing cefepime and is frequently associated with resistance to a variety of antibiotics, particularly fluoroquinolones (Paterson and Bonomo, 2005).

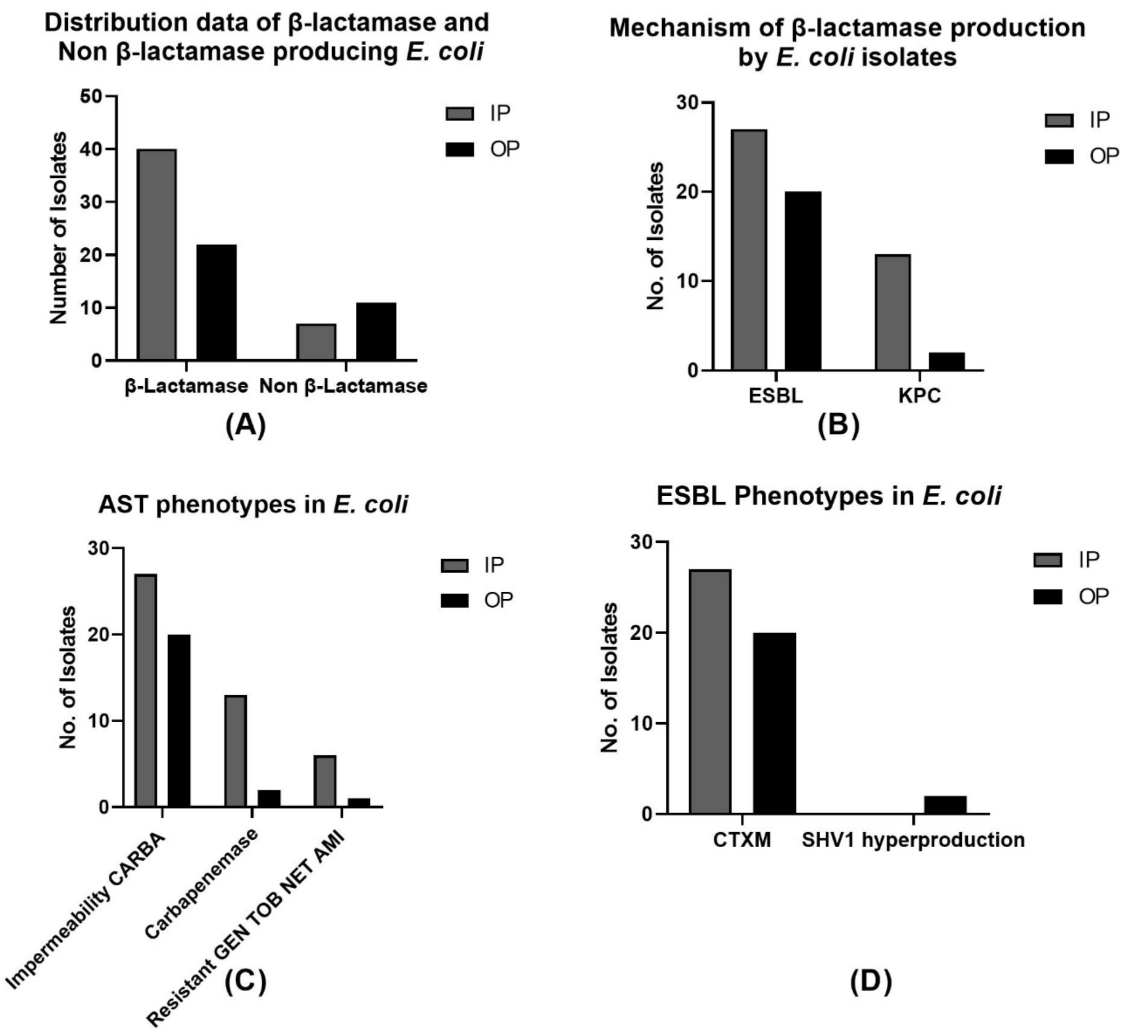

Figure 3: Distribution and Mechanisms of β-Lactamase and Antibiotic Resistance in *E. coli* Isolates (A) Distribution of β -lactamase production, (B) Mechanism of β -lactamase production by *E. coli* isolates, (C) Antibiotic Susceptibility Testing (AST) phenotypes in *E. coli*, (D) ESBL phenotypes in *E. coli*. IP-Inpatient, OP-Out Patient, ESBL -Extended-Spectrum β-Lactamase, Impermeability CARBA-Carbapenem resistance due to outer membrane impermeability, Resistant GEN TOB NET AMI-resistance to Gentamicin Tobramycin Netilmicin and Amikacin, CTXM-Cefotaxime-hydrolyzing β-lactamase, SHV1-Sulfhydryl Variable 1.

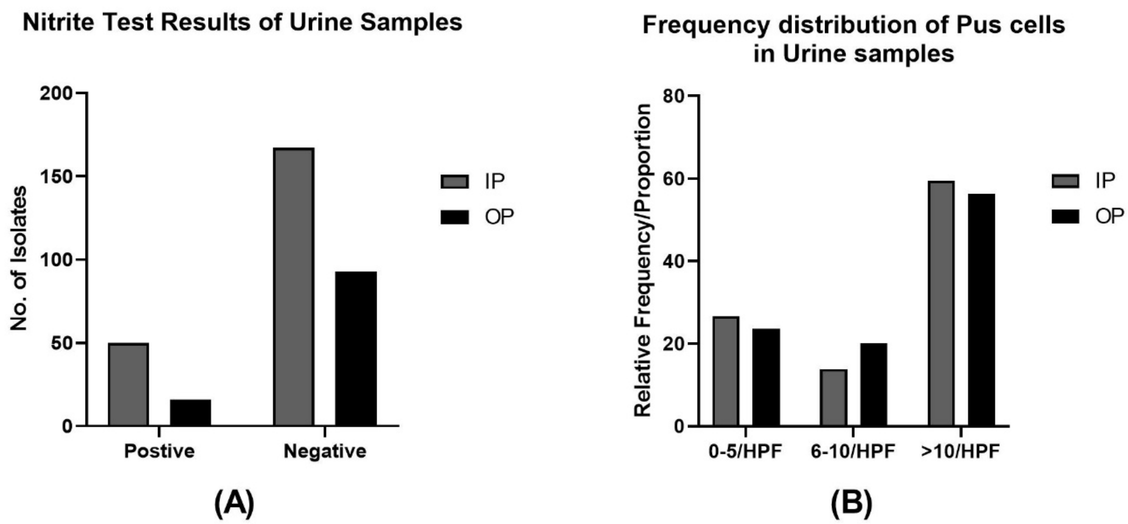

Figure 4: Analysis of Urinalysis Indicators in Inpatient (IP) and Outpatient (OP) Samples (A) Nitrite test results of urine samples (B) Frequency distribution of Pus cells in urine samples, The relative frequency (proportion) of samples is displayed across three categories of Pus cell counts per High Power Field (HPF).

Nitrite urine dipstick screening test

The urine dipstick analysis for all culture-positive patients was performed to screen for the presence of infection, with the results are shown in Figure 4A. In the Inpatient (IP) samples, 50 (≈ 23% of IP samples) tested positive for nitrites, indicating the presence of a Urinary Tract Infection (UTI), while the majority of 167 samples tested negative. In the Outpatient (OP) urine samples, 16 (≈ 14.7% of OP samples) tested positive for the nitrite test, with 93 (≈ 85.3%) tested negative. It is significant to note that a negative test result cannot be considered conclusive proof of the absence of UTI, as many common uropathogens, such as Enterococcus species, *Staphylococcus saprophyticus*, and *Acinetobacte*r species, do not possess the necessary reductase enzyme to convert urinary nitrates to nitrites (Simerville *et al*., 2005).

Distribution of Pus Cells in Urine Specimens of IP and OP Patients

The distribution of pus cells (leukocytes) observed via urine microscopy for both Inpatient (IP) and Outpatient (OP) samples can be observed in Figure 4B. In the IP urine samples (*n*=214), the majority of samples, 126 (58.06%), showed significant pyuria, with >10 pus cells/HPF. This was followed by 58 (26.7%) samples having 0-5/HPF, and the least observed in 30 (13.82%) samples having 6-10/HPF. Similar trends were observed in the OP samples (*n*=109), where 61 (55.96%) samples showed the presence of pus cells with >10/HPF. This was followed by 26 (23.85%) samples with 0-5/HPF and 22 (20.0%) samples with 6-10/HPF. The presence of pus cells at a concentration of >10/HPF is generally considered a clinical indicator of an Urinary Tract Infection (UTI) (Figure 4B). However, the presence of pus cells is not a precise diagnostic finding for UTI, as pyuria can also be secondary to conditions such as fever, glomerulonephritis, renal stones, the use of certain drugs (e.g., cyclophosphamide), and contamination by vaginal secretions.[18]

DISCUSSION

The current cross-sectional study was conducted to gain an insight into the local incidence of Urinary Tract Infections (UTI), the bacterial pathogens associated, and to identify the current trend of antibiotic sensitivity of the involved uropathogens in Inpatient (IP) and Outpatient (OP) units.

We have substantiated the fact that Gram-negative bacteria are still the leading cause of UTIs and that the spread of infections varies significantly between care settings. Although the overall prevalence of *E. coli* was the highest, it was significantly higher in IP patients (47% vs. 33% of the patients in OP). On the other hand, *K. pneumoniae* was over-predominant in the IP group (33%) as compared to the OP group (8%). Such a gap indicates that IP UTIs are more commonly attributed to opportunistic, nosocomial, or complex infections, many of which are caused by the species such as *K. pneumoniae*. The incidence of *E. coli* is in accordance with the trends all over the world. The prevalence of *E. coli* in a study on outpatients in Sao Paulo, Brazil (Carlos *et al*., 2014) at 72% is a contributing factor to defining *E. coli* as a primary community-acquired uropathogen. In the same way, this organism is universal as the high prevalence of 80% in 402 suspected UTI samples in other study (Sabir *et al*., 2014) also reflects the importance of this organism.

According to antibiotic sensitivity study, the resistance of most of antibiotics studied is high, which is alarming, and this result is congruent with other international surveys carried out. The greatest resistance was exhibited against Ampicillin, Nalidixic

Acid, Ciprofloxacin, Cefuroxime, Cefuroxime axetil and Ceftriaxone respectively. This trend, especially, the great resistance to the third-generation cephalosporins and fluoroquinolones, is a major factor in poor empirical therapy and subsequent relapse of UTI (Bidell *et al.*, 2016). The resistance levels of the isolates of *S. typhimurium* to quinolone (21%) and fluoroquinolone (18%) are lower than the recommended level of our data, and this may mean that the local resistance is developing faster than history would suggest. Our findings indicate that in IP samples, most (60) had a significant number of colonies growing as opposed to only 39% in the OP samples, indicating that IP patients are more prone to develop the true and high-bacteria-load infection, and thus the aggressive treatment.

The molecular findings (especially the high percentage of β-lactamase-producing *E. coli* in both IP and OP samples) are strongly advocating the observed resistance (Bhandari *et al.*, 2016; Ramos *et al.*, 2020). The presence of multidrug-resistant strains was proven by high concentrations of CTX-M type of ESBLs found in the IP samples. This strong resistance system is against major antibiotics such as cefotaxime and ceftriaxone. An examination of 200 Gram-negative bacteria to produce ESBL indicated that approximately 37% of the isolates were ESBL producers (Gupta *et al.*, 2013). Our findings support this number, if not exceed it, suggesting a significant local ESBL burden. It is also emphasized by a study conducted in South India, in which 88 percent of 300 *E. coli* isolates had CTX-M genes (Manoharan *et al.*, 2019). The resemblance between this data and the fact is a strong indication that the blaCTX-M gene is the prevailing ESBL genotype in this range that has led to the resistance of cephalosporin treatment. Evidence of geographic difference in ESBL production suggests that, whereas western nations may have lower incidences because of higher hygiene and judicial utilization of antibiotics, incidences in India report 47.5% and have a global range of 36% -55% (Gharavi *et al.*, 2020; Jabeen *et al.*, 2007). Our area rate lies at the higher range of this scale, and antimicrobial stewardship programs are needed. Since CTX-M type of ESBLs is prevalent, and the resistance profile is relevant, we may conclude that first and third-generation cephalosporins should not be used empirically. Our results endorse the adoption of such agents as cefoperazone-sulbactam as a more effective antimicrobial agent in the treatment of complicated UTIs in this hospital facility.

In agreement with the epidemiology of general UTI, females (23.2%), but not male patients (10.3%) had a higher rate of infection due to anatomical factors including shorter urethra and positioning of the meatus near the anal orifice (Al-Badr and Al-Shaikh, 2013). Despite the fact that men have a higher risk of UTI (52%) contradicts our findings, which is the finding of some studies (Pujades-Rodriguez *et al.*, 2019; Rodriguez *et al.*, 2019). Such discrepancy could be explained by varying sampling groups (e.g. age groups, underlying comorbidities etc.) and needs to be researched on further on sex- and age-specific risk factors in our local population. The clinical applicability of conventional diagnostic markers is proved by the analysis of urine microscopy. Most positive cases were positive in nitrite test and high pus cell count (>10/HPF), which confirmed the existence of a great deal of pyuria. The small percentage of positive cultures of UTI in children (22.4% out of 85 samples) and small percentage of samples with high pus cell counts (≤4/HPF in 90% samples) is also in line with the published accounts (Lu *et al.*, 2022; Sharma *et al.*, 2017).

CONCLUSION

This cross-sectional study yields crucial findings that must guide the empirical treatment of Urinary Tract Infections (UTIs) within our facility by clearly defining the current local resistance landscape. *Escherichia coli* remains the primary etiological agent across all patient groups; however, the elevated prevalence of *Klebsiella pneumoniae* in the Inpatient (IP) setting strongly points to a significant burden of nosocomial or complicated infections. A critical and alarming level of resistance was demonstrated toward most first-line agents, including Ampicillin, Ciprofloxacin, and third-generation cephalosporins. This pervasive resistance indicates that conventional empirical therapy is increasingly unreliable, directly contributing to treatment failures and recurrence. Furthermore, this challenge is driven at the molecular level by the wide circulation of high-risk clones, evidenced by the high detection of CTX-M type Extended-Spectrum β-Lactamases (ESBLs), which is consistent with the 88% gene carriage rate observed in regional studies. Therefore, based on the documented local antimicrobial landscape, our data strongly recommend that reliance on first- and third-generation cephalosporins should be abandoned. Instead, cefoperazone-sulbactam must be adopted as a more appropriate and reliable antimicrobial option for the empirical management of complicated UTIs. Ultimately, the results underscore the urgent necessity for sustained, localized antimicrobial surveillance and robust stewardship programs to protect remaining therapeutic options. Care should also be taken to reduce the frequency of multidrug-resistant strains of *E. coli* and follow the "Reserve drugs" concept to retard the misuse of known antimicrobials.

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ABBREVIATIONS

AM: Ampicillin; **AMC:** Amoxicillin/Clavulanic acid; **AMK:** Amikacin; **AMR:** Antimicrobial Resistance; **AmpC:** AmpC β-Lactamase; **AN:** Amikacin; **AST:** Antimicrobial Susceptibility Testing; **BEL-1:** Belgian Extended-spectrum β-Lactamase; **BES-1:** Brazilian Extended-spectrum β-Lactamase; **blaCTX-M:** β-lactamase CTX-M gene; **CARBA:** Carbapenemase; **CIP:** Ciprofloxacin; **CRO:** Ceftriaxone; **CS:** Colistin; **CTX-M:** Cefotaxime-M β-Lactamase; **CXM:** Cefuroxime; **CXMA:** Cefuroxime axetil; **ESBL:** Extended-Spectrum β-Lactamase; **FEP:** Cefepime; **GEN:** Gentamicin; **HL:** High-Level; **HPF:** High Power Field; **IBC:** Inducible β-Lactamase; **IP:** Inpatient; **IPM:** Imipenem; **KPC:** *Klebsiella pneumoniae* Carbapenemase; **MAR:** Multiple Antibiotic Resistance; **MDR:** Multidrug-Resistance; **MEM:** Meropenem; **NET:** Netilmicin; **OP:** Outpatient; **PER:** *Pseudomonas* Extended Resistance; **SE:** Standard Error; **SFO-1:** *Serratia fonticola*-origin β-Lactamase; **SFP:** Cefoperazone/Sulbactam; **SHV:** Sulfhydryl Variable; **SXT:** Trimethoprim/Sulfamethoxazole; **TEM:** Temoniera; **TLA:** Tlacotalpan β-Lactamase; **TOB:** Tobramycin; **TZP:** Piperacillin/Tazobactam; **UTI:** Urinary Tract Infection; **VEB:** Vietnamese Extended-spectrum β-Lactamase; **WHO:** World Health Organization.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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FUTURE STUDY PERSPECTIVE

Further research is necessary to validate the recommended empirical shift to Cefoperazone/Sulbactam through prospective clinical outcome studies. Molecular epidemiology must track the local spread of dominant CTX-M clones. Antimicrobial Stewardship Programs (ASP) focusing on restricting Ampicillin, Ciprofloxacin, and third-generation cephalosporin use must be implemented and evaluated to measure their impact on reducing the alarming local resistance burden and protecting reserved agents like carbapenems and Colistin.

ETHICAL APPROVAL

The present study was carried out in R.B.V.R.R. Women's College, Narayanaguda, Hyderabad, Telangana, India. It does not involve the use of *in vivo* experiments. Informed consent was taken from patients for collection of urine samples.

AUTHOR'S CONTRIBUTION

Dr. J. Achyutha Devi: Planning, Reviewing, Supervision, Manuscript Writing and Editing.

Dr. S. Ravi Kiran: Experimentation, Data Analysis and Drafting the Manuscript.

G. Rahul: Experimentation, Data Analysis and Drafting the Manuscript.

SUMMARY

The study assessed the antibiotic resistance profile of Uropathogenic *E. coli* isolates from suspected UTI patients in Telangana, highlighting an alarming level of multidrug resistance, particularly in Inpatient (IP) settings. *E. coli* was the primary cause of UTIs, with a higher incidence observed in females, especially in the 21-30 age group. The highest resistance rates were found against Ampicillin, Nalidixic Acid, Ciprofloxacin, and third-generation cephalosporins (Cefuroxime, Cefuroxime axetil, and Ceftriaxone). This pervasive resistance is driven at the molecular level by a high prevalence of β-lactamase-producing *E. coli*, primarily the CTX-M type Extended-Spectrum β-Lactamases (ESBLs), which were significantly more frequent in IP samples (27 isolates) than in Outpatient (OP) samples (20 isolates). Given these findings, the study strongly recommends discontinuing the empirical use of first- and third-generation cephalosporins, and suggests adopting Cefoperazone-sulbactam as a more appropriate and reliable alternative for the empirical management of complicated UTIs.

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