

Antimicrobial Sensitivity and Resistance in Urinary Isolates: ESBL Producers vs. Non-Producers

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ABSTRACT: UTIs continue to be the most prevalent bacterial infection in humans, even with the widespread availability of medications. Before the laboratory findings of urine culture and sensitivity are known, antibiotics are often administered empirically. *Klebsiella* species and *E. coli* are capable of producing enormous amounts of Extended Spectrum Beta Lactamases (ESBLs). The production of beta-lactamases is a significant issue in UTI. Oxyimino beta-lactams such as monobactam, ceftazidime, cefotaxime, and ceftriaxone are hydrolysed by enzymes known as ESBLs. ESBLs are resistant to a number of antibacterial substances. Maintaining current understanding of the organisms that cause UTIs and their drug susceptibility pattern is essential to ensuring optimal management. The best empirical treatment must be determined by continuously monitoring the local susceptibility patterns of uropathogens to new antimicrobials, as antibiotic resistance in a wide range of pathogenic organisms can fluctuate even for brief periods of time and can vary by geographic region. In order to determine the prevalence rate of UTI in patients with and without symptoms, as well as to analyse the range of uropathogens, the current study was conducted. For both indoor and outdoor UTI patients, a trend of antibiotic susceptibility was also seen. The findings indicated that girls had a considerably greater frequency of UTIs (about 70.30%) than boys. Both OPD and IPD patients had strong resistance patterns and significant ESBL production, as it was determined. In order to counteract the growing trend of antibiotic resistance in UTI infections, these data inform the doctors that they emphasise the need for updated antibiotic stewardship programs and the need of routine screening for ESBL development in clinical isolates.

Keywords: Antibiotic, ESBLs (Extended Spectrum Beta Lactamase), OPD& IPD, UTI, Spectrum of Uropathogens.

Abbreviations: *E. coli* = *Escherichia coli*, ESBL=Extended Spectrum Beta Lactamase, IPD= Inpatient Department, M:F = Male Female Ratio, OPD = Out-Patient Department, UTI = Urinary Tract Infection.

INTRODUCTION

One of the most prevalent bacterial illnesses affecting people globally, urinary tract infections (UTIs) (Flores-Mireles *et al.*, 2015; Pardeshi, 2018) greatly increase morbidity and medical expenses. Because broad-spectrum antibiotics are frequently used empirically to treat UTIs. (Tan and Chlebicki 2016) resistant bacterial strains have emerged as a result (Nicolai *et al.*, 2021). The synthesis of enzymes known as Extended Spectrum Beta Lactamases (ESBLs), (Ghafourian *et al.*, 2015) which provide resistance to a variety of beta-lactam antibiotics (Toussaint and Gallagher 2015) such as cephalosporins and penicillins, is one of the most alarming resistance mechanisms. *Escherichia coli* and *Klebsiella pneumoniae* are the two bacteria that produce ESBLs in particular.

Continuous local monitoring of uropathogen susceptibility patterns to both new and traditional antimicrobials is required to determine the best Gupta *et al.*,

empirical treatment because antibiotic resistance in a wide range of pathogenic organisms can vary, even for brief periods of time, and can also vary by geographic location (Moghadam *et al.*, 2020, Seifu and Gebissa 2018; Kumar *et al.*, 2022).

Screening and assessing the precise proportion of ESBL producers among urinary pathogens, as well as the variations in the antimicrobial susceptibility patterns of ESBL and non-ESBL producers in patients who are inside and outdoors, are further objectives of the study. This would aid in recommending the best antibiotic to give in both situations for the best treatment of such ESBL producers.

MATERIALS AND METHODS

Selection of Patients:

Sample collection. Urine samples were collected from kidney stone hospital of 45 patients in OPD and 70 patients of IPD, Patients urine were taken at the time of OPD and IPD (under clean catch method) in a

sterilized label urine container. A total of 115 clinically suspected cases of UTI were enrolled in the study. Among them 70 of them were indoor patients (20 male, 20 old age, 30 female) and 45 (16 male, 11 old age, 18 female) were from outpatient department (Fig. 1).

Table 1: Distribution of clinically suspected cases of UTI.

	OPD No. (%)	IPD N0(%)	Total No. (%)
MALE	16 (13.9%)	20 (17.4%)	38 (33.0%)
FEMALE	18 (15.6%)	30 (26.0%)	40 (34.8%)
OLDAGE	11(9.6%)	20 (17.4%)	37 (32.1%)
TOTAL	45(39.1%)	70(60.8%)	115(100%)

Table 2: Number of organism producing ESBL.

Organism	OPD	IPD
<i>Escherichia coli</i> *	20	35
TOTAL	20	35
Grand total of patients	55	

Of a total of 115 patients, *E. coli* (45 from OPD and 70 from IPD) was isolated. ESBL production was observed in 47.82% of *E. coli* (55/115). Among the outpatients 44.4% (20/45) of the isolates were ESBL producers while inpatients constituted 50% (35/70) isolates (Fig. 2).

Table 3: Number of Organism Non-ESBL Producing.

Organism	OPD	IPD
<i>Escherichia coli</i>	25	35
Total	25	35
Grand total of patients	60	

Of a total of 115 patients, *E. coli* (45 from OPD and 70 from IPD) was isolated. NON -ESBL production was observed in 52.18 % of *E. coli* (60/115). Among the outpatients 55.6 % (25/45) of the isolates were NON-ESBL producers while inpatients constituted 50% (35/70) isolates (Fig. 3).

Samples were collected from patients of the following departments:

- Medicine
- OPD
- Surgery
- Nephrology
- Obstetrics & Gynaecology
- Urology

Specimen Collection

In IPD and OPD

The urine culture test takes three days to complete. On the first day, the patient's urine was cultivated on culture medium, which were then stored at 37°C for twenty-four hours to check for the development of bacteria or yeast.

Microscopy was used to determine if microorganisms had grown on the culture medium on the second day. Following Gram's staining, it was determined to be either yeast or bacteria.

Bacterial growth in the culture medium was assessed on the second day. Bacterial colonies were reinoculated on Mac Conkey agar media after being mixed with distilled water or with the use of a culture swab. The plates were then stored at 37°C for a full day. A zone of inhibition was seen in the plates when the antibiotic disc-containing plates were taken out of the incubator on the third day, whether the antibiotics were effective or not was proven.

If the pure culture report has an antibiotic with a 'S' before it, it indicates that the antibiotic is sensitive and capable of killing the bacterium.

All suspected UTI OPD patients had 20 clean midstream urine samples obtained, while all suspected UTI IPD patients had 35 clean midstream urine samples collected using a sterile screw-capped container. In order to isolate the uro-pathogens later on, the urine samples were processed and cultivated.

The pure cultures that were isolated were cultivated on Mac Conkey agar. The susceptibility of the identified species was then tested by exposing them to certain antibiotics (Sedhain *et al.*, 2019; Almutawif & Eid 2023).

Culture: The urine sample was inoculated on Mac Conkey agar and Nutrient agar media by standard loop method and incubated aerobically at 37°C for 18 to 24 hours.

- **Mac Conkey Agar** is a selective and differential media used to isolate non-fastidious Gram negative rods (primarily, the family Enterobacteriaceae and the genus *Pseudomonas*) and differentiate the bacteria on their ability to ferment lactose.
- **Preparation of Mac Conkey Agar -**
 - Suspended 49.53 gm of dehydrated medium in 1000 mili litre of purified or distilled water.
 - Heated it to boiling to dissolve the medium thoroughly.
 - Sterilized it by autoclaving at 15 lbs pressure (121°C) for 15 minutes.
 - Cooled it to 45-50°C.
 - Mixed it well before pouring it into sterile petriplates.

Analysis of bacterial isolates in detail: The following common microbiological methods were used to analyse bacterial isolates in detail:

I. To differentiate between Gram-positive and Gram-negative bacteria, the morphology of the bacterium is studied using Gram's staining.

II. By using the "hanging drop method," locomotion was identified.

III. Colony Morphology: The colony's form, size, borders, surface, edge, elevation, colour, structure, and consistency were all noted as an eye lens was used to study it.

IV. Biochemical Tests: The capacity to proliferate on MacConkey petri dishes and nutrient agar was noted. It was noted if the glucose-topped MacConkey petri plate could ferment lactose or glucose.

The conventional Mac Conkey petri test was used to record the capacity to ferment lactose.

An isolated colony's Gram-stained smear was performed and evaluated.

The results of a routine oxidase test were conducted and examined for Gram-negative rods.

Based on the bacteria that were isolated, the following biochemical assays were performed:

For lactose fermenters that are Gram-negative:

(a) The Methyl Red Test (MR) was conducted using glucose phosphate peptone water medium. Voges-Proskauer Test (VP) was also performed.

(b) The Indole Test was conducted using peptone water.

(c) The Urease Test was conducted using Christensen's urease medium.

For Gram-negative non-lactose fermenters, *Pseudomonas* species were ruled out by first testing the bacterial isolates for Oxidase Reaction. A glucose phosphate peptone water medium for the Methyl Red Test (MR) and the Voges-Proskauer Test (VP) were used for further assessment.

(b) Indole test using peptide water culture; (c) Urease test using Christensen's urease medium; and (d) Triple Sugar Iron agar (TSI agar) test for the fermentation of glucose, lactose, and sucrose and the generation of H₂S (e) The phenylalanine deaminase assay for phenyl pyruvic acid (PPA) synthesis

• For Gram-positive organisms

These organisms, which were identified as Gram-positive bacteria or *Candida* spp., were all found to develop on nutritional agar medium but not on MacConkey agar media. They were then tested for *Staphylococcus aureus* using the Catalase and Coagulase tests.

RESULTS

The study involved the collection of urine samples from fifteen distinct UTI patients. Of the patients, 22 were boys (40%), 18 were girls (32.7%), and 15 were elderly (27%). Between 60 and 70 years old of the patients, 72.7% were male and female patients between the ages of 10 and 30. All patients (100%) were admitted to the hospital and were in the outpatient department. A 100% sterile urine sample was collected using the clean catch method (by taking a urine sample midstream) in a urine container with a label.

In 21.00%, 5.90%, 35.80%, 15.80%, 7.60%, 47.60%, and 16.90% of the half cases (42.5%), fever, dysuria, frequency, stomach discomfort, vomiting, malodorous urine, and anorexia were observed, respectively. Sonography results showed that individuals with hydronephrosis and hydroureter were about twice as likely to have bladder hypertrophy (13.70% vs. 7.80%). The most frequent risk factor for UTI in our analysis was a history of using antibiotics during the previous three months (50.50%).

The patient's history of urinary tract infections, constipation, use of immunosuppressive medications, vesicoureteral reflux, and urinary tract stones were additional risk factors for UTIs. The patient's history of urinary tract infections, constipation, use of immunosuppressive medications, vesicoureteral reflux, and urinary tract stones were additional risk factors for UTIs. According to our research, a history of prior UTI and fever are linked variables.

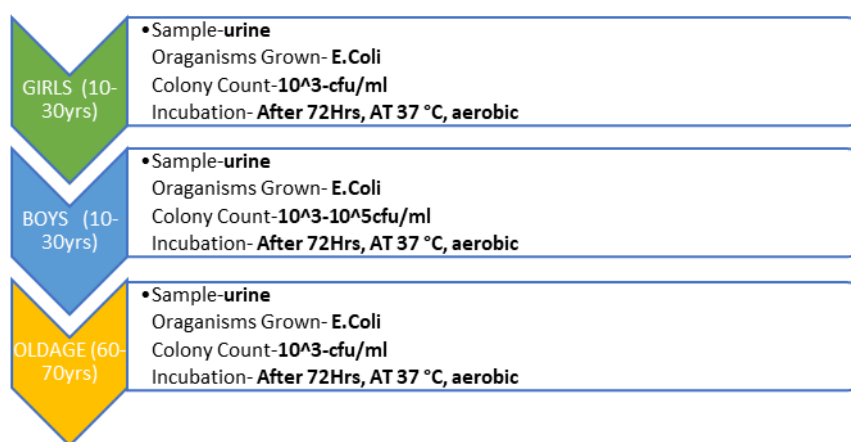


Table 4: Details the findings of the urine culture sensitivity test of different sexes as the detail given below.

Culture Sensitivity Test			
Investigation	Observed Value		
	GIRLS (10-30yr)	BOYS (10-30yr)	OLD AGE (60-70yr)
Sample	URINE	URINE	URINE
Organisms Grown	<i>E. coli</i>	<i>E. coli</i>	<i>E. coli</i>
Colony count	10 ³ cfu/ml	10 ³ -10 ⁵ cfu/ml	10 ⁵ cfu/ml
Incubation	After 72Hrs, AT 37 °C, aerobic	After 72Hrs, AT 37 °C, aerobic	After 72Hrs, AT 37 °C, aerobic
Total No. of Patient (55)	22	18	15

Source: Samples collected from Kidney Stone Hospital, Prayagraj U.P.

Urine samples from O.P.D. and I.P.D. were collected using the clean catch method in order to conduct a culture sensitivity test on boys, girls, and elderly

patients, as shown in Table 4. We discovered that *E. coli* had grown in over 55 urine samples that were collected. Additionally, as we said above, we discovered colony counts of 103 C.F.U./ml in the urine sample of females aged 10–30 years, and 10–3–10–5

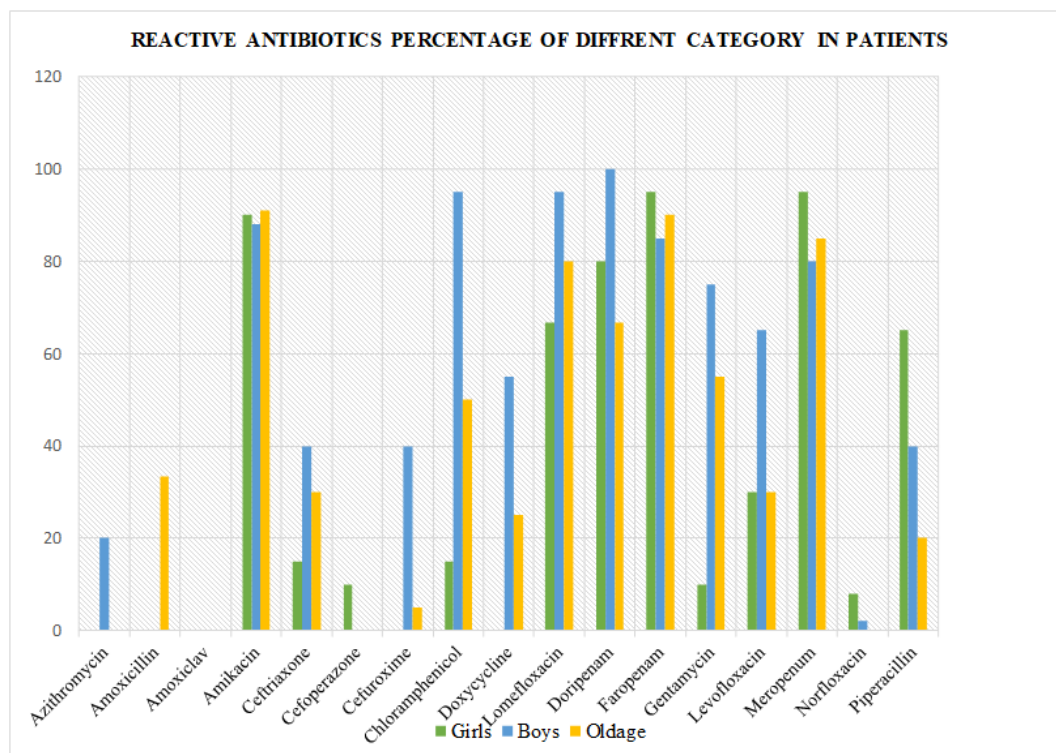
C.F.U./ml in the urine sample of boys aged 10–30 years. Additionally, urine samples from elderly individuals aged 60 to 70 years showed 10–3 C.F.U./ml. All of the samples underwent a 72-hour aerobic incubation period at 37 °C.

Table 5: Details of the findings of the urine culture of antibiotic sensitivity test of different sexes and their results.

Investigation	Culture of Anti Biotic Sensitivity Test		
	Observed Value		
	GIRLS (10-30yr)	BOYS (10-30yr)	OLD AGE (60-70yr)
Azithromycin	R	++	R
Amoxicillin	R	R	+
Amoxiclav	R	R	R
Amikacin	+++	+++	+++
Ceftriaxone	+	++	+
Cefoperazone	+	R	R
Cefuroxime	R	++	+
Chloramphenicol	+	+++	++
Doxycycline	R	++	+
Lomefloxacin	++	+++	+++
Doripenam	+++	+++	++
Faropenam	+++	+++	+++
Gentamycin	R	+++	++
Levofloxacin	+	++	+
Meropenum	+++	+++	+++
Norfloxacin	R	R	R
Piperacillin	++	++	+
Total No. of Patient (55)	22	18	15

Source: Sample collected from kidney Stone hospital, Prayagraj U.P.

Note: + =10%, R = 100 %, Sensitive (S) = +, Resistance (R) = R, Low % = High sensitive, high % = Low sensitive.



Source: As per table no. 4 **Note:** + = 1 to 33.3, ++ = 33.4 to 66.6, +++ = 66.7 to 99.9, R = 0 to 10

Fig. 1.

Reactive antibiotics percentage of different category in patients Fig. 1

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The Fig. 1 above shows the precise proportion of antibiotic sensitivity in each group of sensitive patients

using various medication types. Table 5 above presents the results of the urine culture sensitivity test used to check for antibiotic resistance in males, females and old age as shown in Fig. 1 (Zúniga-Moya *et al.*, 2016) The antibiotic resistance tests for azithromycin, amoxicillin, amoxyclave, amikacin, ceftriaxone, cefoperazone, cefuroxime, chloramphenicol, doxycycline, memefloxacin, doripenam, faropenam, gentamycin, levofloxacin, Meropenum, norfloxacin, and piperacillin were examined using culture sensitivity tests.

Comparison of percentage resistance to various antimicrobial agents between ESSBL producers and ESSBL non producers.

Table 6: Comparison of Percentage Resistance to Various Antimicrobial Agent Between ESSBL Producers and ESSBL Non-Producers.

Investigation	<i>E. coli</i> N=115	
	Observed value in ESSBL producers N=55 no. of cases (% Resistance)	Observed value in ESSBL non producers N=60 no. of cases (% Resistance)
Azithromycin	44 (80%)	30 (50%)
Amoxicillin	50 (90%)	33 (55%)
Amoxclav	55 (100%)	38 (63%)
Amikacin	06 (10%)	11 (18%)
Ceftriaxone	28 (50%)	13 (22%)
Cefoperazone	50 (90%)	25 (42%)
Cefuroxime	39 (70%)	21 (35%)
Chloramphenicol	22 (40%)	15 (25%)
Doxycycline	39 (70%)	24 (40%)
Lomefloxacin	11 (20%)	11 (18%)
Doripenam	11 (20%)	09 (15%)
Faropenam	06 (10%)	05 (08%)
Gentamycin	28 (50%)	24 (40%)
Levofloxacin	33 (60%)	18 (30%)
Meropenum	06 (10%)	06 (10%)
Norfloxacin	55 (100%)	37 (62%)
Piperacilin	28 (50%)	13 (22%)

DISCUSSION

One of the leading causes of morbidity in the general population is urinary tract infections (UTIs). The only accurate way to diagnose a UTI is to show that there are a lot of bacteria using the right semi-quantitative or quantitative culture techniques. Before the definitive microbiological findings are obtained, the majority of people with a suspicion of UTI are treated empirically. This strategy's justification stems from a predicted range of UTi-causing etiological agents and their patterns of vulnerability. The doctor may be able to select the best empirical treatment with the use of area-specific monitoring studies designed to learn more about the pathogen type that causes UTIs and their susceptibility patterns. 115 clinically suspected UTI patients in all were included in our research. Seventy of them were indoor patients (20 men, 20 elderly, and 30 women), while forty-five (16 men, 11 elderly, and 18 women) were outpatients (Table 1). There were fewer samples taken from males, females and old age patients. The bulk of urine samples (40 out of 115) were taken from people aged 10 to 30, while at least some samples (15 out of 115) were from those older than 60. Table 3.

The comparison of *E. coli* that produce ESBL and those that do not for resistance to different antimicrobial drugs (Farfour *et al.*, 2022) is detailed in Table 6. It was discovered that, with the exception of amikacin, ESBL-producing *E. coli* were much more resistant to practically all antibiotics than their non-ESBL-producing counterparts. Compared to ESBL-producing *E. coli* (10%), amikacin resistance was higher in non-ESBL-producing *E. coli* (18%). Regardless of ESBL production, none of the isolates exhibited meropenem resistance (Ding *et al.*, 2021).

Of these 115 samples, 55 (47.8%) had a culture-positive UTI; 33.5% of these were significant, and 18.7% were doubtfully significant bacteriuria. The M:F ratio is 1:1.2 in the culture-positive instances.

Ten out of fifteen UTI patients with candiduria were obtained from indoor patients, and five samples were found to be grossly contaminated, indicating the importance of better patient education for sample collection. This is in proportion to the sex distribution of the samples collected, which is consistent with other studies. Several studies have reported >62% culture positivity, but our study found that the culture positivity rate was only 47.8%. This is primarily due to the history of antibiotic use prior to the patient's admission to the kidney stone hospital, which may also be the cause of increased growth of *Candida* spp. Twenty have been acquired from catheterised persons out of 115 indoor patients. Eleven of these instances of UTI were culture-positive. Out of 1205 UTsI patients, Catheters are a significant risk factor for nosocomial UTIs, according to our research.

Urinary tract infections can be caused by a wide variety of bacteria, although Gram-negative bacilli are by far the most prevalent, according to researchers in this

field. Our findings are consistent with the overall trend in epidemiology. ESBL production was found in 47.82% of the 115 *E. coli* that were isolated for our investigation (45 from OPD and 70 from IPD) (55/115). Although 50% (35/70) of the isolates were from inpatients, 44.4% (20/45) of the isolates from outpatients produced ESBL.

Following *Citrobacter* and *Klebsiella* species, *E. coli* is the most prevalent urinary pathogen in both outpatients (39.13%) and inpatients (60.86%), according to our study. While some other studies have indicated a greater incidence (85%) of *E. coli* isolates, our results are consistent with earlier studies that revealed an incidence of *E. coli* of about 50% [41, 43]. The incidence of *E. coli* has been observed to be lower in in-patients (32.4%) and out-patients (37.4%), respectively,

CONCLUSIONS

The current study examined the pattern of antimicrobial susceptibility in urine isolates, paying particular attention to those that generate Extended Spectrum Beta Lactamases (ESBLs) (Tonkic *et al.*, 2005). There were 115 clinically suspected UTI cases in all. After the investigation was finished, the following results were made: Out of the 115 urine samples that were gathered for culture, 55 (47.82%) had significant bacteriuria, whereas 60 (52.7%) had questionable significant bacteriuria. Of the remaining 115 samples, 40 were sterile, 6 had non-significant bacteriuria, 4 had severe contamination, and 10 had *Candida* spp. growth. For the sake of the investigation, these were thus thrown away.

It was discovered that the M:F ratio in 55 culture-positive cases was 1:1.2. The age range of 15–30 years old accounted for the greatest number of cases, encompassing both males (18) and girls (22). The age group of over 60 years old accounted for the smallest number of instances (15). Seventy instances came from the in-patient department, and forty-five cases were from the out-patient group. Of the 50 patients who had catheterisation, 32 (64%) had UTIs. *Escherichia coli* was the most prevalent urine pathogen in both in-patients (49.5%) and out-patients (53.3%). *Citrobacter* species, *Klebsiella* species, *Enterobacter* species, *Acinetobacter* species, *Enterococcus* species, *Pseudomonas* species, *Proteus* species, and *Staphylococcus aureus* were the next most frequent urinary pathogens, in that order. With the exception of *Pseudomonas aeruginosa*, the sensitivity pattern of Gram-negative bacilli revealed that amikacin, cefoperazone-sulbactam, and nitrofurantoin were extremely sensitive (64.1% to 88.8%) for both in-patients and out-patients; OPD isolates were more sensitive than IPD isolates. It was discovered that ampicillin, co-trimoxazole, and fluoroquinolones had very poor in vitro sensitivity (8.5% to 29.6%). The pattern of antibiotic susceptibility was not particular to any one bacterium. *Pseudomonas aeruginosa* isolates were all susceptible to cefpirome, piperacillin, meropenem, aztreonam, and piperacillin-tazobactam. Additionally, ceftazidime, amikacin, cefotaxime, and ceftazidime-clavulanic acid have demonstrated

excellent susceptibility. Linezolid, vancomycin, and teicoplanin were all effective against the Gram-positive uropathogens (Pouladfar *et al.*, 2017). There was just one Methicillin Resistant *Staphylococcus aureus* (MRSA) found in the patient cluster. *E. coli* produced ESBL in 47.82% of cases. Of the isolates in the in-patients, 63.3% were ESBL producers, compared to 36.6% in the out-patients. Therefore, a greater rate of ESBL synthesis was reported in the current investigation. Comparing *E. coli* that produced ESBL to those that did not, the former exhibited greater resistance to every antibiotic except cefoperazone-sulbactam, nitrofurantoin, and amikacin. Not a single isolate exhibited meropenem resistance. This trend was observed in both outpatients and inpatients. Indeed, this is a noteworthy discovery. Both OPD and IPD patients had a high resistance pattern and significant ESBL production, indicating that multidrug-resistant pathogens are widely distributed even within the population. In order to increase the organisms' susceptibility and ensure that the right antimicrobial drugs are chosen, urine culture and sensitivity must be made necessary. Therefore, the only way to stop bacterial infections from becoming more resistant is to utilise existing medicines sparingly and appropriately. This is a noteworthy discovery, no doubt. Both IPD and OPD patients exhibited significant ESBL production and a high resistance pattern, indicating that multidrug-resistant pathogens are widely distributed even within the population. Enforcing urine culture and sensitivity is necessary to increase organism susceptibility and ensure appropriate antimicrobial agent selection. The only way to prevent the growing resistance of bacterial infections is to utilise the existing antibiotics sparingly and appropriately.

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Data Availability: The datasets generated during and/or analyzed during the current study are available from the corresponding author(s) on reasonable request.

Declarations:

Ethical Approval: Not applicable.

Consent to Participate: Not applicable.

Consent to Publish: Not applicable.

Conflict of Interest: The authors declare no competing interests.

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