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Epidemiology and Antimicrobial Resistance of Extended-Spectrum β-Lactamase-Producing *Escherichia coli* in Urinary Tract Infections: Implications for Antibiotic Stewardship

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A B S T R A C T

Urinary tract infections (UTIs) caused by Extended-Spectrum Beta-Lactamase (ESBL)-producing *Escherichia coli* represent a growing therapeutic challenge due to their multidrug-resistant nature. The rising prevalence of ESBL-producing uropathogens, particularly in developing countries, necessitates routine detection and updated antimicrobial surveillance. This study aimed to determine the prevalence of ESBL-producing *E. coli* among UTI patients and evaluate their antimicrobial resistance profile at a tertiary care centre in Bareilly, India. A retrospective study was conducted in the Department of Microbiology, SRMS Institute of Medical Sciences, over six months (November 2023 – May 2024). One hundred urine samples (midstream, catheter, and suprapubic aspirate) from clinically suspected UTI patients were processed. Semi-quantitative culture on CLED agar, biochemical identification, and VITEK-2 automated confirmation were performed. Antibiotic susceptibility testing followed the Kirby–Bauer disk diffusion method per CLSI 2023 guidelines. ESBL detection was confirmed phenotypically using the Double Disc Synergy Test (DDST). Culture positivity was 72%. *Escherichia coli* was the predominant isolate (63.9%), of which 45.7% were confirmed ESBL producers. ESBL-positive strains showed 100% resistance to third-generation cephalosporins (cefotaxime, ceftazidime, ceftriaxone), high resistance to fluoroquinolones (ciprofloxacin 81%, levofloxacin 76%) and cotrimoxazole (85%). Carbapenems (imipenem and meropenem) retained 100% sensitivity in both ESBL and non-ESBL groups. Female patients accounted for 62% of ESBL-positive cases. ESBL-producing *E. coli* constitutes a significant proportion of UTI isolates with extensive multidrug resistance. Carbapenems remain the most reliable therapeutic option. Routine ESBL screening, antimicrobial stewardship, and continuous resistance surveillance are essential to limit further spread of resistant strains.

Introduction

Urinary tract infection (UTI) is one of the most common bacterial infections affecting individuals of all age groups, particularly infants and children. If not diagnosed and treated promptly, it may lead to serious long-term complications, including renal damage and chronic kidney disease (Ejaz *et al.,* 2011). UTIs involve any part of the urinary tract, including the urethra, bladder, ureters, and kidneys, and are more frequently observed in females due to anatomical predisposition (Lakshminarayana and Sangeetha, 2015).

Approximately 80–85% of UTIs are caused by Gram-negative bacteria, among which *Escherichia coli* is the most predominant pathogen, accounting for nearly 70–90% of cases (Lakshminarayana and Sangeetha, 2015; Nasiru *et al.,* 2020). The pathogenicity of *E. coli* is largely attributed to its ability to produce beta-lactamase enzymes, which hydrolyze beta-lactam antibiotics such as penicillins, cephalosporins, and monobactams, thereby contributing to antimicrobial resistance (Nasiru *et al.,* 2020; Ehsan *et al.,* 2023). This mechanism represents one of the most significant resistance pathways among members of the Enterobacteriaceae family.

Extended-spectrum beta-lactamases (ESBLs), first identified in 1983, are enzymes capable of hydrolyzing extended-spectrum cephalosporins (oxyiminoc-ephalosporins) and monobactams (Ejaz *et al.,* 2011; Lakshminarayana and Sangeetha, 2015; Nasiru *et al.,* 2020). These enzymes are commonly produced by uropathogens such as *Escherichia coli* and *Klebsiella pneumoniae*, leading to increased therapeutic challenges in both hospital-acquired and community-acquired infections (Ejaz *et al.,* 2011; Lakshminarayana and Sangeetha, 2015). Infections caused by ESBL-producing *E. coli* are associated with higher morbidity, mortality, and multidrug resistance, significantly limiting available treatment options (Nasiru *et al.,* 2020).

Detection of ESBL-producing organisms can be achieved through various phenotypic methods, including disc diffusion screening, double disc synergy test (DDST), inhibitor-potentiated disc diffusion test, and E-test methods (Harwalkar *et al.,* 2013). While urine culture remains the gold standard for diagnosis, it requires 48–72 hours for definitive results (Yadav and Chauhan, 2016a). Rapid diagnostic methods such as chromogenic assays have been developed to facilitate early detection and timely initiation of appropriate therapy (Yadav and Chauhan, 2016b; Yoshimura *et al.,* 2021). ESBL enzymes are primarily classified into TEM, SHV, and CTX-M types based on their molecular characteristics (Al-Jamei *et al.,* 2019).

The prevalence of UTIs caused by ESBL-producing organisms is steadily increasing worldwide, particularly among pediatric populations, posing a major public health concern (Al-Jamei *et al.,* 2019; Fitriawati *et al.,* 2021; Kayastha *et al.,* 2020). Upper urinary tract infections such as pyelonephritis can result in severe complications, including hypertension, renal insufficiency, and eventual renal failure if left untreated (Ehsan *et al.,* 2023).

Globally, ESBL-producing *Escherichia coli* has emerged as a major multidrug-resistant pathogen associated with both community- and hospital-acquired infections (Clinical and Laboratory Standards Institute, 2023). The misuse and overuse of antibiotics, especially empirical therapy without susceptibility testing, have significantly contributed to the rising incidence of resistance (Clinical and Laboratory Standards Institute, 2023). Although *E. coli* is a normal commensal of the human gastrointestinal tract, it is widely distributed in the environment and has demonstrated increasing resistance to multiple classes of antimicrobial agents (Collee *et al.,* 2006). Multidrug resistance is defined as resistance to three or more classes of antibiotics (Collee *et al.,* 2006).

Several risk factors have been associated with ESBL-producing infections, including prolonged antibiotic use, intensive care unit (ICU) stay, urinary catheterization, and underlying comorbid conditions. Studies from India have reported ESBL prevalence rates as high as 58%, highlighting the magnitude of the problem in developing countries (Clinical and Laboratory Standards Institute, 2023; Collee *et al.,* 2006; Nandi *et al.,* 2022). Beta-lactam antibiotics act by inhibiting bacterial cell wall synthesis; however, beta-lactamase enzymes neutralize their activity, leading to therapeutic failure (Kayastha *et al.,* 2020; Abdelgader *et al.,* 2023). ESBL-producing *E. coli* typically exhibit resistance to penicillins, cephalosporins, and aztreonam, often accompanied by cross-resistance to other antibiotic groups (Abdelgader *et al.,* 2023).

The increasing prevalence of ESBL-producing *E. coli* represents a significant clinical challenge, necessitating early detection and appropriate antimicrobial therapy (Yadav and Chauhan, 2016a). UTIs continue to be

among the most frequently encountered infections in clinical practice and are diagnosed based on significant bacteriuria, defined as $\geq 10^5$ CFU/mL of urine (Willacy and Tidy, 2015). Antimicrobial resistance is a growing global threat, contributing to an estimated 700,000 deaths annually worldwide (Willacy and Tidy, 2015).

Materials and Methods

The present retrospective study was conducted in the Department of Microbiology at SRMS Institute of Medical Sciences, Bareilly, over a period of six months (November 2023 to May 2024), with a total sample size of 100. Patients presenting with symptoms suggestive of urinary tract infection (UTI), including burning micturition, urgency, frequency, hesitancy, pain during micturition, hematuria, and fever, were included. Urine samples such as clean-catch midstream urine (MSU), catheter specimen urine (CSU), and suprapubic aspirates were collected. Ethical approval was obtained from the Institutional Human Ethics Committee, and informed written consent was secured from all participants while maintaining confidentiality throughout the study.

Urine samples were collected in sterile universal containers and transported promptly to the bacteriology laboratory; in case of delay, samples were refrigerated at 4°C. All samples were processed using conventional bacteriological techniques. Semi-quantitative culture was performed using a standard loop method on CLED (Cystine Lactose Electrolyte Deficient) agar and incubated aerobically at 37°C for 18–24 hours. A loopful of approximately 0.005 mL urine yielding $\geq 10^5$ colony-forming units (CFU)/mL was considered indicative of significant bacteriuria.

Identification of uropathogens was carried out using colony characteristics, Gram staining, motility testing (hanging drop method), and biochemical tests including catalase, oxidase, indole, citrate, urease, oxidative-fermentative (OF) glucose, triple sugar iron (TSI), and sugar fermentation tests. Further confirmation was performed using the automated VITEK-2 Compact system (GN card REF-21341).

Antibiotic susceptibility testing was conducted on Mueller-Hinton agar using the Kirby–Bauer disk diffusion method in accordance with CLSI 2023 guidelines. Standard *Escherichia coli* ATCC 25922 was used as a quality control strain. The antibiotics tested included cefotaxime, ceftriaxone, cefepime, ceftazidime, cefpodoxime, cephalothin, ciprofloxacin, levofloxacin, ofloxacin, norfloxacin, amikacin, gentamicin, tobramycin, netilmicin, meropenem, imipenem, doxycycline, chloramphenicol, cotrimoxazole, cefoperazone-sulbactam, amoxicillin-clavulanic acid, piperacillin-tazobactam, aztreonam, nitrofurantoin, colistin, and polymyxin-B. Plates were inoculated to obtain a uniform lawn, antibiotic discs were applied, incubated at 37°C for 24 hours, and zones of inhibition were measured and interpreted as per CLSI standards. Minimum inhibitory concentration (MIC) of selected antibiotics was determined using the VITEK-2 Compact system (GN card 235). Phenotypic screening for ESBL production was performed using the double disc synergy test (DDST). The test strain was inoculated as a lawn and exposed to discs of third-generation cephalosporins (cefotaxime and ceftazidime) along with a co-amoxiclav disc placed at an appropriate distance. Following overnight incubation, an enhanced zone of inhibition towards the co-amoxiclav disc indicated ESBL production.

Results and Discussion

Distribution of Samples

A total of 100 urine samples from patients clinically suspected of urinary tract infection were processed. The majority of urine samples were midstream urine (78%), reflecting standard non-invasive collection practices commonly used in outpatient settings. Catheter specimens accounted for 18%, indicating hospitalized or catheterized patients, while suprapubic aspirates constituted a minimal proportion (4%), reserved for specific clinical indications. (table 1, chart 1)

Culture Positivity Rate

A high culture positivity rate of 72% was observed, indicating a substantial burden of urinary tract infections among the study population. The remaining 28% of samples showed no significant growth, which may be attributed to prior antibiotic use, low bacterial load, or non-bacterial causes of symptoms. (Table 2 chart 2).

Distribution of Uropathogens

Escherichia coli was the predominant uropathogen, accounting for 63.9% of isolates, confirming its primary role in urinary tract infections. Other organisms such as *Klebsiella pneumoniae*, *Proteus spp.*, and *Pseudomonas*

aeruginosa were isolated in smaller proportions, while Gram-positive organisms like *Staphylococcus saprophyticus* and *Enterococcus spp.* were comparatively less frequent. (Table 3, chart 3, figure 1)

Prevalence of ESBL Producing *E. coli*

Among the 46 *E. coli* isolates, 45.7% were identified as ESBL producers, indicating a substantial presence of resistant strains. The remaining 54.3% were non-ESBL producers, suggesting that while resistance is significant, a considerable proportion of isolates still remain susceptible to conventional antibiotics. (Table 4, chart 4, figure 2-3)

Gender-Wise Distribution of ESBL *E. Coli*

Higher ESBL prevalence was seen among female patients. (table 5)

Antibiotic Resistance Pattern of ESBL VS non-ESBL *E. coli*

ESBL-producing *E. coli* isolates exhibited markedly higher resistance to most antibiotics compared to non-ESBL strains. Complete resistance (100%) was observed against third-generation cephalosporins such as cefotaxime, ceftazidime, and ceftriaxone, while high resistance was also noted for fluoroquinolones and cotrimoxazole. In contrast, non-ESBL isolates showed relatively lower resistance across all antibiotic groups. Notably, carbapenems (imipenem and meropenem) demonstrated 0% resistance in both groups, indicating their continued effectiveness as the most reliable therapeutic agents (Table 6, figure 4)

MIC Pattern (VITEK-2)

The MIC analysis using VITEK-2 demonstrated that carbapenems, including imipenem and meropenem, showed 100% sensitivity, confirming their high efficacy against uropathogens. Amikacin also exhibited good sensitivity (78%) with relatively low resistance. In contrast, ciprofloxacin and cefotaxime showed high resistance rates of 50% and 72%, respectively, indicating reduced effectiveness of these commonly used antibiotics. Overall, the findings highlight the growing resistance to cephalosporins and fluoroquinolones. (Table 7)

The present study was undertaken to determine the prevalence of extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* among urinary tract infection (UTI) patients and to evaluate their antimicrobial resistance profile. The findings of the study reaffirm that *Escherichia coli* remains the most predominant uropathogen, accounting for 63.9% of total isolates. This observation is in close agreement with several national and international studies, including those by Taneja *et al.,* (2008) and Foxman (2002), who have consistently reported *E. coli* as the leading cause of both community-acquired and nosocomial UTIs. Similar prevalence rates ranging from 60–80% have been documented across tertiary care settings, highlighting the organism's strong uropathogenic potential and adaptability (Kothari and Sagar, 2008).

In the present study, the prevalence of ESBL-producing *E. coli* was found to be 45.7%, indicating a substantial burden of antimicrobial resistance. Comparable findings have been reported in Indian studies by Manchanda and Singh (2003) and Aggarwal *et al.,* (2009), where ESBL prevalence ranged between 40–50%. Studies conducted in other developing regions have also demonstrated similar or slightly higher rates, reflecting widespread misuse and overuse of antibiotics. In contrast, relatively lower prevalence rates have been reported in developed countries by Paterson and Bonomo (2005), suggesting that stricter antibiotic stewardship policies and effective surveillance systems contribute to reduced resistance levels. This geographic variation underscores the critical role of local prescribing practices, infection control strategies, and healthcare infrastructure in shaping antimicrobial resistance patterns.

The antimicrobial susceptibility pattern observed in the present study revealed complete resistance of ESBL-producing *E. coli* isolates to third-generation cephalosporins, including cefotaxime, ceftazidime, and ceftriaxone. This finding is consistent with earlier reports by Livermore (2008), who demonstrated that ESBL enzymes efficiently hydrolyze extended-spectrum cephalosporins, rendering them clinically ineffective. Furthermore, high resistance rates to fluoroquinolones and cotrimoxazole were observed, which is in agreement with studies by Gupta *et al.,* (2014) and De and Baveja (2013), reporting increasing multidrug resistance among uropathogens. This co-resistance phenomenon is likely due to plasmid-mediated transfer of resistance genes, where ESBL determinants are often linked with resistance to multiple antibiotic classes.

Importantly, carbapenems such as imipenem and meropenem demonstrated 100% sensitivity against ESBL-producing isolates in the present study. This observation is in concordance with global findings by Vincent *et al.,* (2009) and Pfaller and Segreti (2006), who identified carbapenems as the most effective therapeutic agents for infections caused by ESBL-producing organisms. However, emerging reports of carbapenem-resistant Enterobacteriaceae (CRE) raise significant concerns, emphasizing the need for cautious and judicious use of these last-resort antibiotics.

The study also demonstrated a higher proportion of ESBL-producing *E. coli* among female patients, which is consistent with epidemiological observations by Foxman (2002). This may be attributed to anatomical predisposition, hormonal influences, and increased exposure to antibiotics in females. Recurrent infections and empirical antibiotic use further contribute to the selection and persistence of resistant strains. Overall, the findings of the present study are in concordance with numerous published reports and highlight the growing challenge of ESBL-mediated resistance in UTIs. The high prevalence of ESBL-producing *E. coli* and their multidrug-resistant nature emphasize the urgent need for routine ESBL detection, rational antibiotic use, and continuous surveillance programs (Manchanda and Singh, 2003; Aggarwal *et al.,* 2009). Strengthening antimicrobial stewardship and infection control practices is essential to curb the spread of resistant pathogens and preserve the effectiveness of existing therapeutic options (Paterson and Bonomo, 2005; Livermore, 2008).

The present study highlights the significant prevalence of extended-spectrum beta-lactamase (ESBL) producing *Escherichia coli* among patients with urinary tract infections, confirming its role as the predominant uropathogen in both community and hospital settings. The high proportion of ESBL-producing isolates observed reflects the increasing burden of antimicrobial resistance, which has become a major concern in clinical practice.

Table.1 Type of Urine Samples Collected

Sample Type	Number (n=100)	Percentage (%)
Midstream urine (MSU)	78	78%
Catheter specimen (CSU)	18	18%
Suprapubic aspirate	4	4%

Table.2 Culture Results

Result	Number	Percentage (%)
Culture Positive	72	72%
Culture Negative	28	28%

Table.3 Isolated Organisms

Organism	Number (n=72)	Percentage (%)
Escherichia coli	46	63.9%
Klebsiella pneumoniae	10	13.9%
Proteus spp.	6	8.3%
Pseudomonas aeruginosa	5	6.9%
Staphylococcus saprophyticus	3	4.2%
Enterococcus spp.	2	2.8%

Table.4 ESBL Production

Category	Number	Percentage (%)
ESBL Positive	21	45.7%
ESBL Negative	25	54.3%

Table.5 Gender-Wise Distribution of ESBL *E. coli*

Gender	ESBL Positive	Percentage (%)
Male	8	38%
Female	13	62%

Table.6 Antibiotic Resistance Pattern (%)

Antibiotic	ESBL Positive (n=21)	ESBL Negative (n=25)
Cefotaxime	100%	28%
Ceftazidime	100%	24%
Ceftriaxone	100%	32%
Cefepime	95%	20%
Ciprofloxacin	81%	36%
Levofloxacin	76%	32%
Gentamicin	62%	28%
Amikacin	38%	12%
Cotrimoxazole	85%	40%
Nitrofurantoin	24%	12%
Piperacillin-Tazobactam	19%	8%
Imipenem	0%	0%
Meropenem	0%	0%

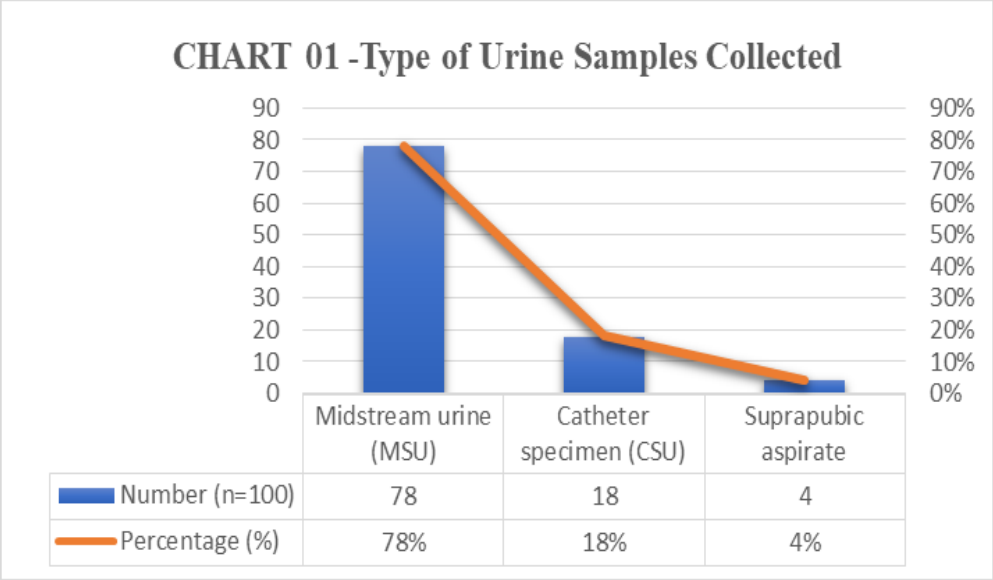
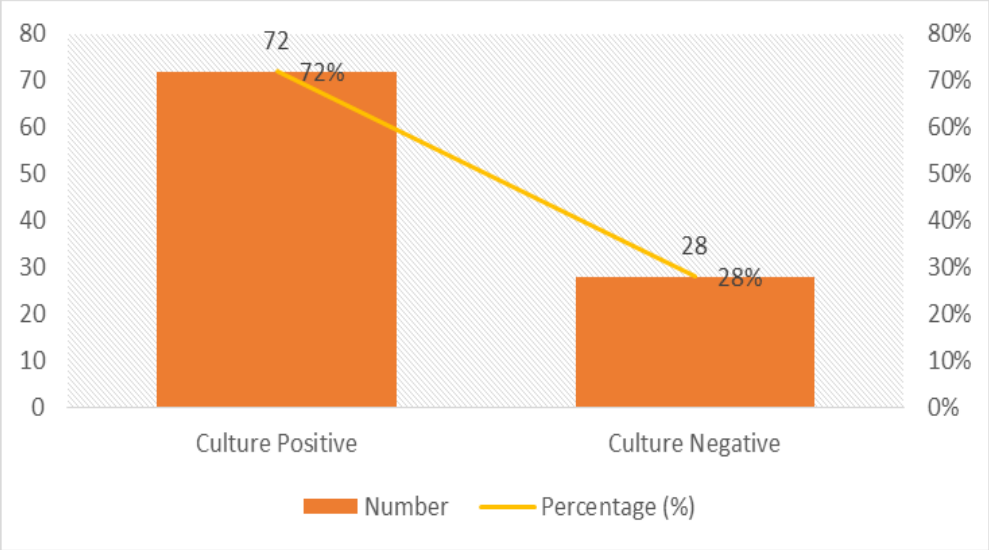
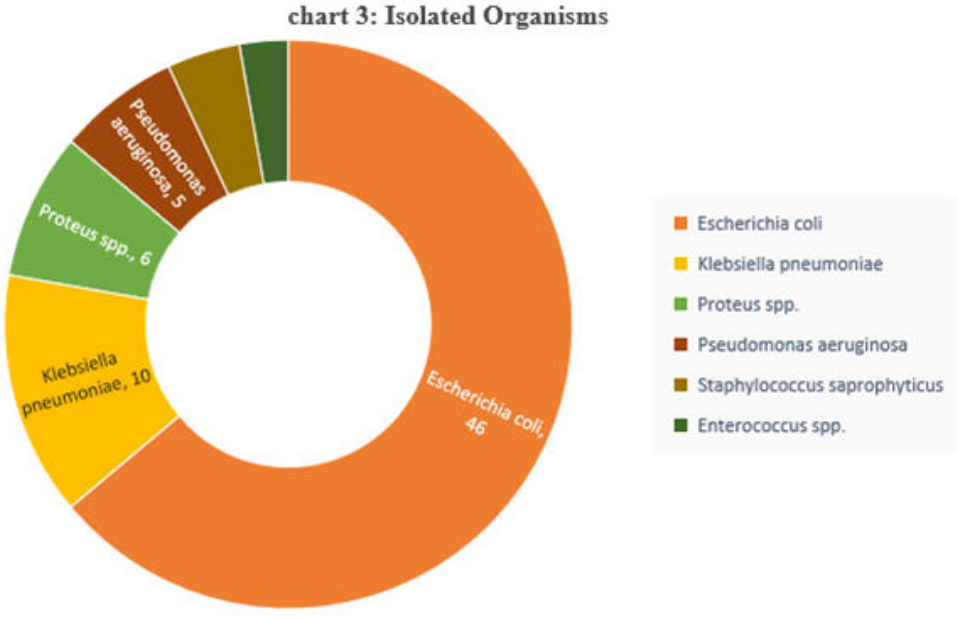


Table.7 MIC Interpretation

Antibiotic	Sensitive (%)	Intermediate (%)	Resistant (%)
Imipenem	100%	0%	0%
Meropenem	100%	0%	0%
Amikacin	78%	10%	12%
Ciprofloxacin	42%	8%	50%
Cefotaxime	22%	6%	72%

Chart.2 Culture Positivity Rate





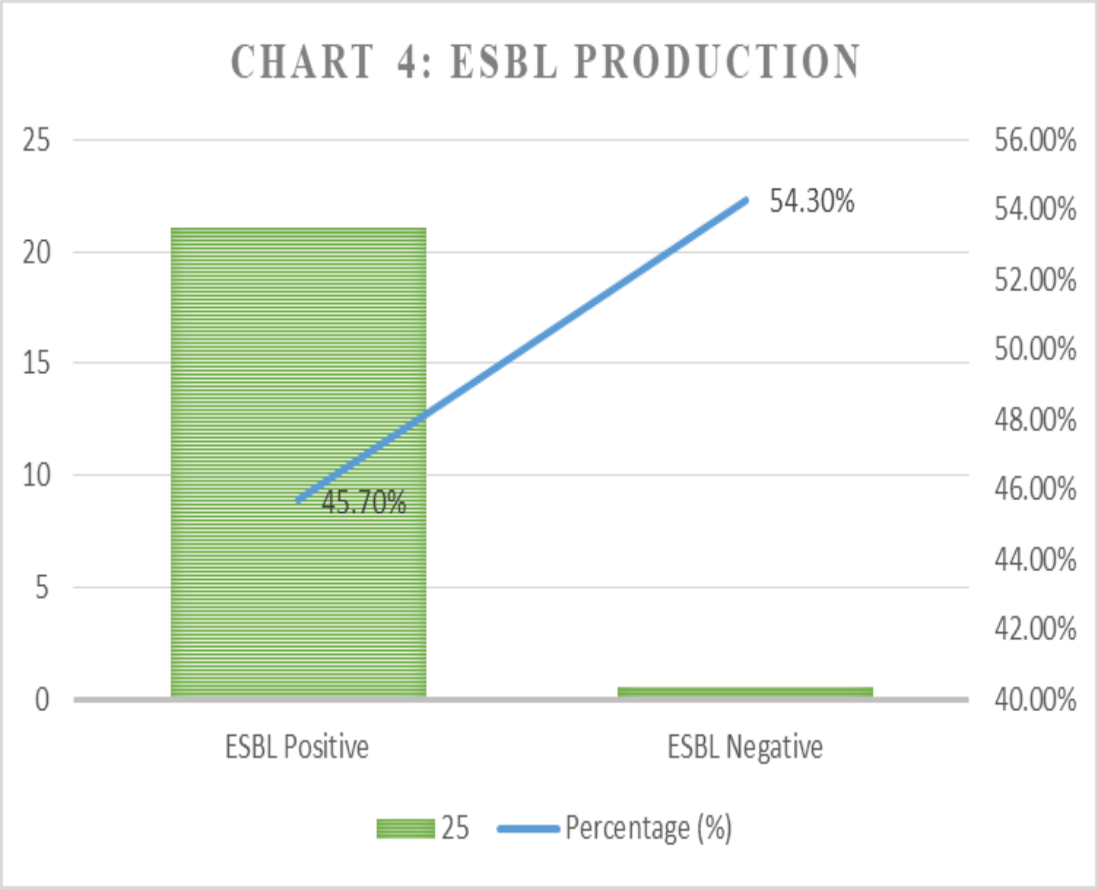


Figure.1 Spectrum of Bacterial Isolates in Urinary Tract Infections with Colony Morphology (n = 72)

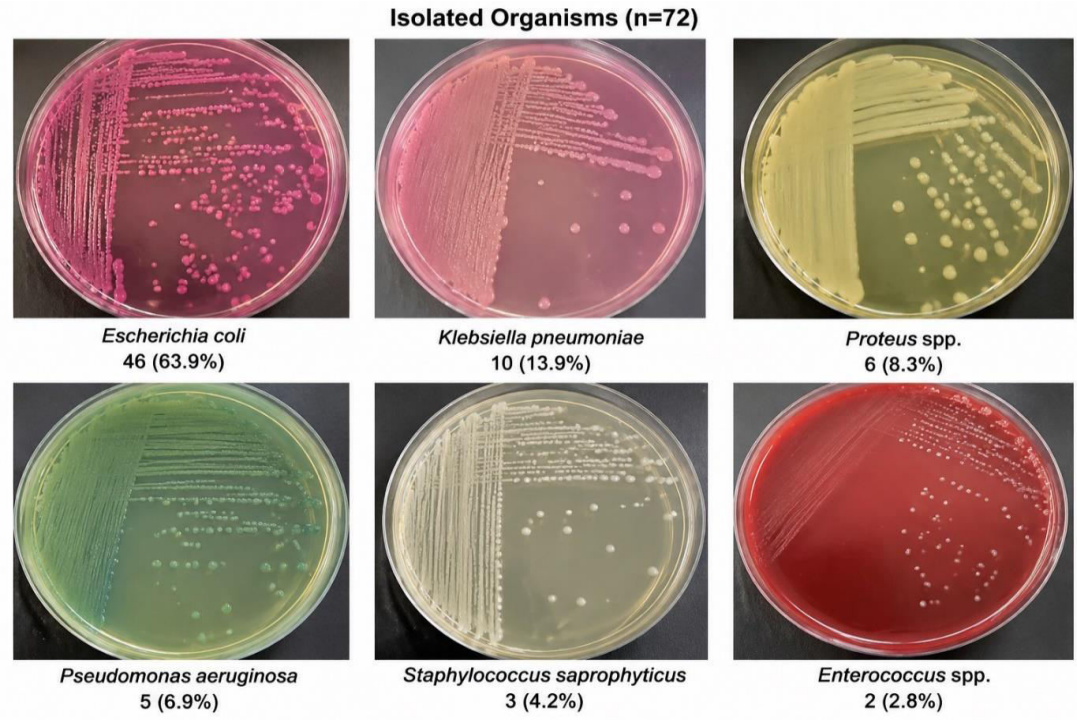


Figure.2 Phenotypic Confirmation of ESBL Production Showing Synergy (Keyhole Effect) Towards Clavulanic Acid

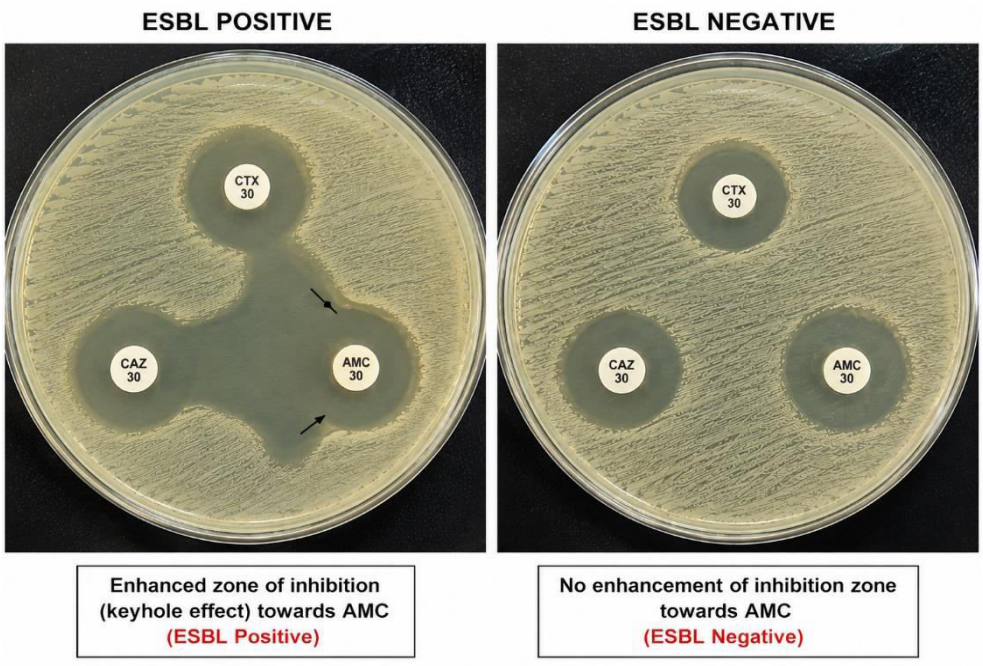


Figure.3 Double Disc Synergy Test Demonstrating ESBL Production via Clavulanate-Mediated Zone Enhancement

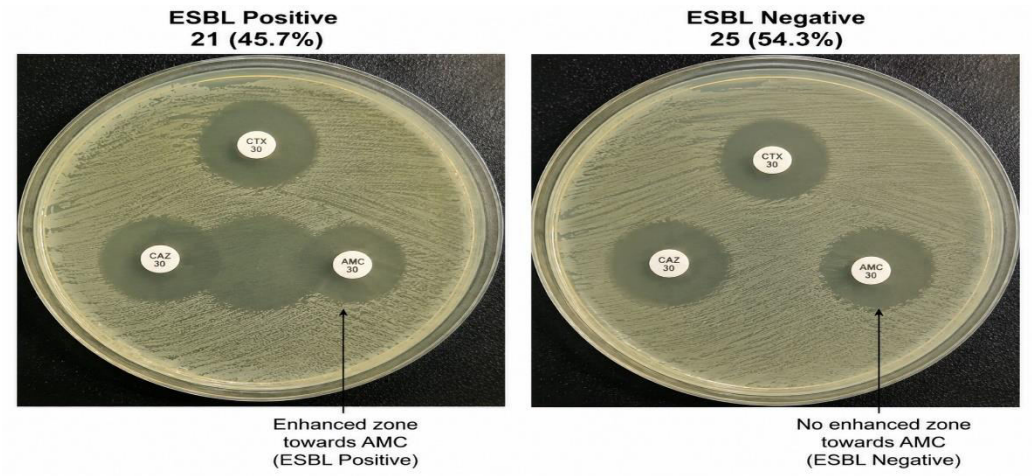
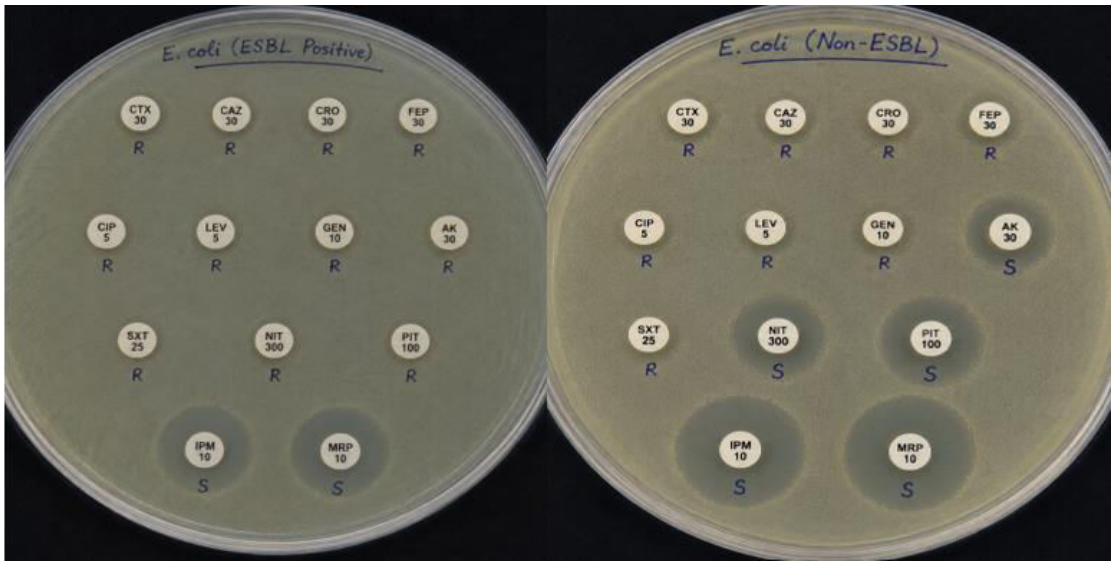


Figure.4 Antibiotic Susceptibility Profile Demonstrating Multidrug Resistance in ESBL Producing and Non-ESBL *Escherichia coli*



ESBL-producing *E. coli* demonstrated complete resistance to third-generation cephalosporins and exhibited high resistance to fluoroquinolones and cotrimoxazole, indicating a multidrug-resistant pattern. Such resistance significantly limits the effectiveness of commonly prescribed empirical therapies. In contrast, carbapenems such as imipenem and meropenem showed excellent sensitivity, reaffirming their role as the most reliable therapeutic agents against ESBL-producing organisms. The study also observed a higher prevalence of infections among female patients, consistent with known epidemiological patterns of urinary tract infections. The presence of multidrug-resistant strains further complicates treatment outcomes and increases the risk of recurrent infections, prolonged hospital stay, and healthcare costs.

In conclusion, the emergence and spread of ESBL-producing *E. coli* represent a serious clinical challenge. Early detection, appropriate antibiotic selection based on susceptibility testing, and strict infection control practices are essential to effectively manage such infections and to prevent further escalation of antimicrobial resistance.

Future Recommendations

Routine screening for ESBL production should be implemented in all clinical laboratories to ensure early detection and appropriate management of resistant infections.

Rational use of antibiotics through strict antimicrobial stewardship programs is essential to minimize the emergence of resistance. Continuous surveillance of antimicrobial susceptibility patterns should be conducted to guide empirical therapy. In addition, adherence to infection control practices in healthcare settings and increasing public awareness regarding the misuse of antibiotics are crucial steps in controlling the spread of ESBL-producing organisms. Further molecular studies are also recommended to better understand resistance mechanisms and support the development of targeted treatment strategies.

Author Contributions

Neha Sahu: Investigation, data collection, formal analysis, writing—original draft preparation. Prof. (Dr.) Nityanand Upadhyay: Conceptualization, methodology, supervision, critical review, and approval of the final manuscript. Rahul Verma: Methodology, validation, writing—review and editing. Priyanka Sharma: Formal analysis, data interpretation, writing—review and editing. Harsh Sen Yadav: Investigation, data acquisition, writing—reviewing. Gopee Yadav: Resources, investigation, data acquisition, writing—reviewing. Anjali Singh Raghav: Validation, formal analysis, writing—reviewing and editing. Raj Kumar Sharma: Data curation, methodology, writing—

reviewing and editing. Riya Srivastava: Investigation, formal analysis, manuscript drafting, writing—original draft preparation. Nandini Tiwari: Conceptualization, methodology, data curation, supervision, project administration, writing—reviewing, editing, and approval of the final manuscript. All authors have read and approved the final manuscript.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval: The study was approved by the Institutional Human Ethics Committee, SRMS Institute of Medical Sciences, Bareilly. All procedures were conducted in accordance with the ethical standards of the institution and with the 1964 Helsinki Declaration and its later amendments.

Consent to Participate: Written informed consent was obtained from all individual participants included in the study.

Consent to Publish: Not applicable.

Conflict of Interest: The authors declare no competing interests.

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