

To Study the Prevalence of Extended-Spectrum β -lactamases (ESBL) Producing Uropathogenic *Escherichia coli* (UPEC) and its Antibiotic Sensitivity Pattern around the Semi-urban Region of Gurugram

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ABSTRACT

Background: The increasing prevalence of *E.coli* causing UTIs and its resistance pattern has become a major global healthcare concern. ESBL-producing strains, in particular, pose a significant threat to public health and have complicated treatment approaches. Most reports on this issue are from urban areas, so there is a lack of data on antibiotic sensitivity patterns in semi-urban areas.

Aim and Objective: To Study the prevalence of extended-spectrum β -lactamases (ESBL) producing uropathogenic *Escherichia coli* (UPEC) and its antibiotic sensitivity pattern around the semi-urban region of Gurugram.

Material & Method: Urine samples were processed using the conventional method. As per the CLSI guidelines, confirmed *E.coli* isolates were tested for antibiotic resistance using the Kirby-Bauer disc diffusion method. All *E.coli* isolates were subjected to ESBL HiCrome agar and DDST using combination drugs as per the CLSI guidelines for the phenotypic detection.

Results: Out of 485 samples, 256 (52.78%) ESBL *E.coli* were observed. The antibiogram of the ESBL-producing isolates showed higher resistance rates to cephalosporins compared to non-cephalosporin antibiotics. There was a low degree of resistance observed against aminoglycosides and carbapenem.

Keywords: ESBL, Urinary tract Infections, *E.coli*, Antibiotic resistance, Hicrome agar, Double Disk Synergy Test

1. INTRODUCTION

In recent years, bacterial resistance has been a global health concern. By 2050, the annual mortality toll from bacterial resistance may reach 10 million (O'Neill, 2014), with approximately 90% of predicted deaths happening in Asia and Africa (Islam et al., 2019)

A recent World Health Organization (WHO) report on antimicrobial resistance (AMR) surveillance specified nine international bacteria responsible for some of the most common infections in community and hospital settings. *Escherichia coli* the pathogen most often implicated in Urinary tract infections (UTI), is listed as one of the nine.

Uropathogenic *Escherichia coli* (UPEC) strains are the primary etiological agents causing UTI and are also responsible for more than 90% of community-acquired, with the rest being hospital-acquired. Moreover, the recent emergence of multidrug-resistant (MDR) UPEC isolates in the community has become a major challenge for clinicians to start empirical therapy.

The treatment of patients with UTIs caused by ESBL-producing UPEC has become increasingly difficult because of the rapid spread of antibiotic resistance. Extended-spectrum beta-lactamase (ESBL) are plasmid-mediated enzymes that act by hydrolyzing oxyimino-cephalosporins conferring resistance to cefotaxime, ceftazidime and ceftriaxone i.e., third-generation cephalosporins as well as to monobactams, such as aztreonam. Previous studies in India have reported that the incidence of ESBLs ranges from 6 to 87%. [1-5]

Infections caused by ESBL-producing organisms are associated with higher morbidity and mortality

Hence, it is extremely imperative to determine the common etiology based on the local epidemiology and the antibiotic susceptibility testing (AST) pattern to formulate the treatment prescription for empirical therapy. Although, there are many studies describing the antimicrobial resistance (AMR) pattern. However limited data is available on the local prevalence of uropathogens. So this study aims to detect ESBL-producing *E.coli* by using rapid detection tests such as combined disk diffusion and ESBL HiCrome agar and compare the efficacy of these tests.

2. MATERIAL AND METHODS

Study design

This study was an observational, prospective, and hospital-based study that was conducted from July 2022 to February 2024 in the Department of Microbiology of S.G.T. University and Hospital, Budhera (Gurugram), Haryana, India.

Study setting and population

This study was carried out on 485 patients attending outpatient and admitted to various departments of a multispecialty hospital of S.G.T. University located in the rural belt of Haryana, providing health services mostly to the rural community.

The study population included both pediatric (0–18 years) and adult (>18 years) patients from rural and semi-urban communities residing in nearby villages, and attending various clinical departments of the hospital. Community-acquired infection (CAI) was defined by a positive bacterial culture obtained from patients attending outpatient departments (OPDs) and Hospital-acquired infection was defined within 48 hours of hospital admission from hospitalized patients without any history of hospitalization or antibiotic treatment in the past 30 days.[6,7]

Data Collection

Patient demographic details, brief clinical history, details of diagnosis (as made by the consultant-in-charge of the concerned department), the date of admission to the hospital, duration of stay in the hospital, and antibiotic treatment were taken.

Ethical Approval

Informed consent was taken from all the patients concerned, and institutional ethical committee clearance was obtained.

Sample Collection & Processing

Clean catch mid-stream urine samples collected from the patients representing symptoms of UTI were received in the microbiology department from various clinical department of the hospital. These samples were processed as per the standard bacteriological guidelines and further identification was confirmed based on Gram staining and biochemical reactions. Urine sample is streaked on CLED agar (Cystine-lactose-electrolyte deficient), which is a non-inhibitory growth medium used in the isolation and differentiation of urinary microbes. Cystine promotes the formation of cystine-dependent dwarf colonies. Bromothymol blue is the indicator used in the agar, it changes to yellow in case of acid production during fermentation of lactose or changes to deep blue in case of alkalization. Lactose-positive bacteria build yellow colonies. In case of *E.coli* growth large, elevated, yellow, opaque colonies with a center more intense yellow; yellowish medium is visible. After overnight incubation at 37°C the colonies growth were observed and colony count of a single pure organism with $\geq 10^4$ (c.f.u. ml) was regarded as significant bacteriuria. Only those isolates identified as *E.coli* were further processed.

Microscopic and Biochemical characterization

The identification of the collected UPEC isolates was preliminarily confirmed by subjecting them to microscopic and biochemical characterization. All the isolates were studied microscopically by Gram staining, and biochemical characterization was done using standard tests including indole test, methyl red, Voges-Proskauer, and Simmons citrate agar test.

Antimicrobial Susceptibility Testing

Principle: The key principle is that the size of the inhibition zone is inversely proportional to the minimum inhibitory concentration (MIC) of the antibiotic for the bacterial isolate. Susceptible bacteria have lower MICs and therefore larger inhibition zones, while resistant bacteria have higher MICs and smaller inhibition zones. By comparing the zone sizes to standardized breakpoints, the bacterial isolate can be classified as susceptible, intermediate, or resistant to each antibiotic tested. The test is used to determine the susceptibility of clinical isolates of bacteria towards the different antibiotics

Antimicrobial susceptibility testing (AST) was performed on all the *E.coli* isolates by Kirby–Bauer disc-diffusion method, in which Mueller-Hinton agar was used for the inoculation of pure *E.coli* inoculum prepared in broth and antibiotic discs are placed on the surface of the agar at the distance of 24mm overnight. After 24hrs of incubation at 35°C the results were interpreted as per the Clinical Laboratory Standard Institute (CLSI) guidelines.[8]

The following groups of antibiotic discs, commercially procured from HiMedia, Mumbai, India, were used: ampicillin (10

µg), amoxicillin-clavulanic acid (20/10 µg), piperacillin/tazobactam (100/10 µg), amikacin (30 µg), gentamicin (10 µg), cefoxitin (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), cefepime (30 µg), aztreonam (30 µg), ciprofloxacin (5 µg), ofloxacin (5 µg), chloramphenicol (30 µg), co-trimoxazole (25 µg), ertapenem (10 µg), meropenem (10 µg), imipenem (10 µg), tetracycline (30 µg), and tigecycline (15 µg). In addition, all the urine isolates were tested against the following antibiotics: nalidixic acid (30 µg) and nitrofurantoin (300 µg) as per the CLSI guidelines.[8]

Detection of ESBL Production

Double-disk synergy test

Principle: This test is the observation of the interaction between the zones of inhibition created by the two antimicrobial agents. If the zones of inhibition overlap and show an increase in the size of the combined inhibition zone compared to the individual zones, it indicates a synergistic interaction between the two antimicrobial agents. The test is performed on agar with a 30-µg disk of cefotaxime (and/or ceftriaxone and/or ceftazidime and/or aztreonam) and a disk of amoxicillin clavulanate (containing 10 µg of clavulanate) positioned at a distance of 30 mm (centre to centre). The test is considered as positive when a decreased susceptibility to cefotaxime is combined with a clear-cut enhancement of the inhibition zone of cefotaxime in front of the clavulanate-containing disk, often resulting in a characteristic shape-zone referred to as ‘champagne-cork’ or ‘keyhole’. [Fig: 1]. If the zones of inhibition do not overlap or show a decreased combined inhibition zone, it suggests an antagonistic interaction between the two antimicrobial agents.

All the *E.coli* isolates showing resistance to any of the three third-generation cephalosporins were subjected to confirmatory phenotypic testing for ESBL production by double-disc synergy test (DDST) using ceftazidime (30 µg) and ceftazidime plus clavulanic acid (30 µg plus 10 µg) discs as the first pair and cefotaxime (30 µg) and cefotaxime plus clavulanic acid (30 µg plus 10 µg) discs as the second pair of antibiotic discs.[12] [fig: 1]. *E.coli* ATCC 25922 was used as a negative control strain.

ESBL HiCrome Agar

Hichrome agar is a selective and differential culture medium that is useful for the detection and presumptive identification of extended-spectrum beta-lactamase (ESBL)-producing bacteria, particularly *Escherichia coli* and *Klebsiella* species.

Principle: Hichrome agar contains chromogenic substrates that react with specific enzymes produced by different bacterial species. ESBL-producing *E.coli* will typically develop a distinct colour (i.e. pink) on Hichrome agar, allowing for their presumptive identification. This agar contains antibiotics, such as cefotaxime or ceftazidime, at concentrations that inhibit the growth of non-ESBL-producing bacteria. This selective pressure allows for the growth and differentiation of ESBL-producing isolates.

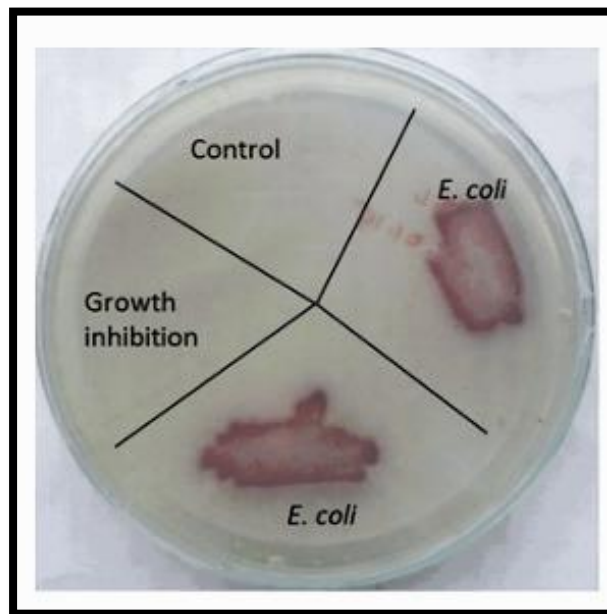
Advantages of using Hichrome agar:

- Rapid identification of the isolates through the color development on Hichrome agar enables presumptive identification of ESBL-producing bacteria, reducing the time required for further confirmatory testing.
- In addition to ESBL detection, Hichrome agar can also help differentiate between *E.coli* and *Klebsiella* species based on the specific color reactions.
- The identification of ESBL-producing bacteria on Hichrome agar can provide important information to guide the selection of appropriate antibiotic therapy, as ESBL-producing isolates are often resistant to a broader range of beta-lactam antibiotics

The test organisms were inoculated onto CHROMagar using the spread-plate technique of direct streaking and then incubated at 37°C for 18–24 hours. Colonies of ESBL producers develop species-specific colors. The colony morphology on ESBL HiCrome agar is as follows [Fig: 2]



(Clavulanic acid in combination with ceftazidime inhibits the bacteria and cephalosporin becomes active and the diameter of inhibition zone becomes wider. In this figure combination disc of ceftazidime with clavulanate acid is showing particular increase in the zone of inhibition towards other cephalosporins. Hence showing the inhibition of beta lactamase production and activation of cephalosporins)



(ESBL-producing *E. coli*: pink to reddish colonies. ESBL non-producers: inhibited)

3. RESULTS

Demographic data: A total of 485 urine samples were collected of which n=256/485 (52.78%) were uropathogenic *E.coli* showing significant bacteruria, whereas n=142/256 (55.46%) were positive for ESBL-producing *E.coli*. Area-wise prevalence of UPEC and ESBL EC in rural areas n=173/256 (67.57%) & 102/142 (71.83%), urban 83/256 (32.42%) & 40/142 (28.16%) respectively. Females were predominantly infected followed by males (Table:1)

Overall, 24.60% (63/256) UPEC, 30.21% (43/142) (ESBL EC) of total female patients were found pregnant. The distribution of adults and >18 patients were observed as 96.87% (248/256) UPEC, 96.47% (137/142) ESBL EC and 3.12% (08/256) UPEC, 3.52% (5/142) ESBL EC, respectively. Among the adults, the highest prevalence was observed in the 30–50 years age group (45.31%) UPEC & (45.77%) ESBL EC, followed by 18–30 years (33.20%) UPEC & (33.69%) ESBL EC, and 50–60 years (12.10%) UPEC & (11.90%) ESBL EC. As per the data of current study 60.54% UPEC, 63.38% ESBL EC of the patients were married and 14.84%, 13.38% were unmarried. Based on occupation, majority were identified as housewives (50.39%) UPEC, (61.26%) ESBL EC followed by construction workers (17.18%) UPEC & (14.78%) ESBL EC, farmers (14.84%) UPEC & (4.29%) ESBL EC, student (8.98%) UPEC & (9.15%) ESBL EC and others (3.90%) UPEC & (2.81%) ESBL EC.

Total sample=485	Total positive uropathogenic Escherichia coli isolates=256	Total positive ESBL producing <i>E.coli</i> =142	Total percentage UPEC EC	ESBL
Urban	83	40	32.42% 28.16%	
Male	13	10	5.7%	7.4%
Female	76	37	29.68%	26.5%
Children	2	00	0.78%	00
Rural	173	102	67.57% 71.83%	
Male	34	16	13.28% 11.26%	
Female	127	79	49.60% 55.63%	
Children	4	00	01.56%	00
Age group				
>18	08	05	03.12% 03.52%	
18-30	85	45	33.20% 31.69%	
30-50	116	65	45.31% 45.77%	
50-60	31	17	12.10% 11.97%	
>60	16	10	06.25% 7.04%	

Married	155	90	60.54% 63.38%
Pregnancy	63	43	24.60% 30.28%
Unmarried	38	19	14.84% 13.38%
Occupation			
Student	23	13	8.98% 9.15%
Housewife	129	87	50.39% 61.26%
Employee	12	06	4.68% 4.22%
Farmer	38	11	14.84% 4.29%
Construction worker	44	21	17.18% 14.78%
Others	10	04	3.90% 2.81%

Table 1: Demographic Data from the UTI patients with E.coli

(Total UTI positive urine sample n=456 out of which n=256 where positive UPEC isolates and n=142 were confirmed ESBL positive *E.coli*. This table shows the prevalence and percentage of UPEC and ESBL *E.coli* among the population with taking an account of risk factors involving marital status and occupation)

In the current study, burning micturition was observed to be the commonest symptom (48.6%) followed by urinary frequency (31.3%), fever (30.4%), Urinary urgency (22.5%), suprapubic- tenderness (21.8%), Pain in the lower abdomen (18.3%), dysuria (10.4%), costovertebral tenderness (1.6%). Among the co-morbidities, the patients had a recent episode of UTI (36.9%), diabetes mellitus (13.4%), renal stone (1.3%), urological surgery (2.1%), recurrent UTI (4%), sexually transmitted disease (0.4%), bladder anatomy (0.1%) and hysterectomy (0.01%).

4. MICROBIOLOGICAL DATA

A total of 256 samples were positive for uropathogenic *E.coli* (52.78%) out of 485 urine specimens processed in the microbiology labs. In this study, a total number of 142 (55.46%) ESBL-producing *E.coli* isolates were detected out of 256 positive UPEC samples.

In the present study, the AMR pattern among the uropathogenic *E.coli* against the commonly prescribed drugs for urinary tract infection was found in the following manner: Ampicillin (64.45%), Amoxycylav (68.75%), Amikacin (8.20%), Ceftazidime (57%), Cefepime (44.92%), Ceftriaxzone (42.57%), Cotrimoxazole (65.23%), Ciprofloxacin (71.48%), Gentamycin (13.28%), Fosfomycin (25.56%), Tigecycline (37.5%), Nitrofurantoin (40.23%), Piperacillin/Tazobactam (10.93%), Meropenem (26.56%), Imipenem (20.31%). All the isolates resistant to carbapenem were found to be a subset of ESBL producers indicating the possible emergence of carbapenem resistance within the ESBL producers. A resistance rate of 31% (32/103) out of all positive resistant isolates was observed for ESBL-positive *E.coli*. In this study, ESBL *E.coli* were also found to exhibit resistance towards third-generation cephalosporins, and carbapenems, and resistance towards amoxycylav, ampicillin, and ciprofloxacin is the highest.

Table:2 Antibiotic resistance pattern among the UTI patients with positive *E.coli* isolates (n=256) and ESBL producing *E.coli* isolates (n=142)

[In the given table the resistance pattern of different antibiotics towards UPEC and ESBL *E.coli* is shown, where in case of UPEC Ciprofloxacin, amoxycylav, cotrimoxazole and ampicillin are highly resistance, whereas antimicrobial resistance in case of ESBL producing *E.coli* ampicillin, amoxycylav, ceftazidime, cotrimoxazole, cefepime, ciprofloxacin, ceftriaxzone are

the most resistant (drugs are mentioned in the highest to lowest order of AMR rate). Most susceptible antibiotic in case of UPEC are piperacillin/tazobactam, gentamycin, imipenem, meropenem, fosfomycin, nitrofurantoin, tigecyclin and for ESBL producing *E.coli* gentamycin, piperacillin/tazobactam, imipenem are the most sensitive along with fosfomycin, meropenem, tigecyclin, nitrofurantoin (mentioned in highest to lowest susceptibility rate). All strains are highly susceptible to gentamycin, piperacillin/tazobactam, imipenem, meropenem&fosfomycin.]

Antibiotics	Overall <i>E.coli</i> AMR rates (n=256)	ESBL <i>E.coli</i> AMR rates (n=142)
Ampicillin (10µg)	165	112
Amikacin (30µg)	21	12
Amoxyclav (30µg)	176	112
Ceftazidime (30µg)	149	102
Cefepime(30µg)	115	98
Ceftriaxzone(30µg)	109	88
Ciprofloxacin (30µg)	183	95
Cotrimoxazole (20 µg)	167	98
Fosfomycin(200µg)	68	23
Gentamycin	34	12
Imipenem	52	17
Meropenem (10µg)	68	25
PiperacillinTazobactum (100/10)	28	14
Nitrofurantoin (300µg)	103	32
Tigecycline	96	26

5. DISCUSSION

Urinary tract infections are the most common bacterial infections in both community and hospital settings, affecting a large number of people, with a higher incidence in females than in males. The detection of ESBL production is clinically crucial for both in-hospital and community isolates. The frequency of use of antibiotics, their reference dosages, and administration periods vary widely across different regions and even within localities, leading to significant differences in the emergence of resistant patterns. Infection control practitioners and clinicians rely on the clinical laboratory to quickly identify and characterize various types of resistant bacteria. This in turn is required to minimize the spread of these bacteria and help select appropriate antibiotics. Acquisition of efficient mobile elements results in the acceleration of the transfer of various antibiotic-resistance genes. Probably, in the future, a "superbug" resistant to almost all approved antibiotics may emerge.[9]

This prospective study aimed to investigate the prevalence and antibiotic resistance patterns of uropathogenic *Escherichia coli* (UPEC) and extended-spectrum beta-lactamase (ESBL)-producing *E.coli* causing urinary tract infections (UTIs) in the peri-urban areas of Gurugram, India. A total of 256 pathogenic *E.coli* isolates from UTI patients, of which 142 were identified as ESBL-producing strains using phenotypic methods.

A notable finding from this study was the higher susceptibility of women to UTI infections compared to men. The prevalence of both UPEC and ESBL-producing *E.coli* was particularly high among married women (60.54% and 63.38%, respectively) as well as pregnant women (24.60% and 30.28%, respectively). This gender-based disparity in UTI risk highlights the need for targeted prevention and management strategies for women, especially those in the reproductive age group.

Further analysis revealed a significant correlation between UTI incidence in females and their occupation or their partner's occupation. The highest prevalence of both UPEC and ESBL-producing *E.coli* was observed among house makers (50.39% and 61.26%, respectively), followed by construction workers (17.18% and 14.78%) and farmers (14.84% and 4.29%). This suggests that socioeconomic and environmental factors, such as exposure to potential risk factors in the workplace, may play a crucial role in the acquisition of these uropathogenic strains. [10-13]

The study also noted an increasing trend in the overall prevalence of ESBL-producing *E.coli* causing community-acquired UTIs in the peri-urban population. This is in contrast to the relatively lower prevalence rates reported from developed countries (2.2-15%) [14,15] and other developing nations (1.3-29.1%) [16,17]. The authors attribute this finding to the gradual rise in the prevalence of ESBL-producing *E.coli* in the rural population engaging in work in urban areas.

The results of this study are consistent with previous investigations conducted in rural areas and community health centers across different geographical regions of India. These findings highlight the need for continuous surveillance, improved antibiotic stewardship, and the development of targeted public health interventions to address the growing burden of ESBL-producing uropathogenic *E.coli* in peri-urban and rural communities.

While numerous studies have investigated the prevalence of ESBL-producing *E.coli* causing urinary tract infections (UTIs) in India, there appears to be a relative scarcity of data specifically from rural and peri-urban areas. The current study helps address this important gap in the literature.

Previous studies from India have reported varying prevalence rates of ESBL-producing *E.coli* causing community-acquired infections (CAIs). For instance, a study from southern India found a remarkably high prevalence of 69.2% ESBL positivity among *E.coli* isolates from outpatient UTI cases, representing true community-acquired infections [20]. In contrast, a retrospective study conducted in the USA from 2012 to 2016 reported a much lower ESBL positivity rate of 7-15% among community-acquired *E.coli* UTIs [15]. The high sensitivity (100%) of the ESBL HiCrome agar used in the current study for detecting ESBL producers is in line with previous reports, including one by Prabha et al. that found a sensitivity of 94.4% [10,24,25]. This suggests the reliability of this phenotypic method for the rapid identification of ESBL-producing *E.coli* in resource-limited settings.

Regarding antibiotic resistance patterns, both the UPEC and ESBL-producing *E.coli* isolates demonstrated a high degree of multidrug resistance against commonly used antibiotics such as ampicillin, cephalosporins, fluoroquinolones, and co-trimoxazole. These findings are consistent with another study from South India that reported resistance rates ranging from 55.6% to 98.5% for these antibiotic classes among ESBL-producing *E.coli* [21].

The current study reveals low resistance towards aminoglycosides like amikacin, gentamicin, and piperacillin/tazobactam. Several studies from India and other countries have also reported a lower degree of resistance among ESBL producers against aminoglycosides, except gentamicin, with a resistance rate ranging between 4.2% and 23.5%. [16,17,21]

Extended-spectrum- β -lactamase (ESBL)-producing Enterobacterales, especially *Escherichia coli*, *Klebsiellapneumoniae*, and *Proteus mirabilis*, are highly prevalent in various parts of the world. However, the prevalence of ESBL variants in Enterobacterales from hospital-acquired (HA) and community-acquired (CA) urinary tract infections (UTI) had been infrequently reported in developing countries [26]. The high number of antibiotic-resistant and β -lactamase-producing UPEC strains necessitates further attention and consideration, particularly MBL-producing strains [27,28].

This study results corroborates previous reports from India and other countries, which have attributed the lower resistance to aminoglycosides to their infrequent use, primarily due to their injectable route of administration and potential toxicity concerns [23]. Additionally, the study highlighted the relatively lower resistance rates of UPEC and ESBL-producing *E.coli* to nitrofurantoin and fosfomycin, suggesting that the judicious use of these antibiotics may be warranted to prevent the future emergence of resistance against these crucial therapeutic options.

Overall, this study provides valuable insights into the epidemiology and antibiotic resistance patterns of ESBL-producing *E.coli* causing community-acquired UTIs in the understudied peri-urban and rural settings of India. These findings reinforce the need for continuous surveillance, optimized antibiotic stewardship, and the development of targeted interventions to combat the growing threat of multidrug-resistant uropathogenic *E.coli* in these communities.

6. CONCLUSION

The present study provides important insights into the growing prevalence of ESBL-producing *Escherichia coli* causing urinary tract infections (UTIs) in the rural and peri-urban areas of Gurugram, India. Through this study we found an alarmingly high rate of ESBL positivity, reaching up to 55.5% among the *E.coli* isolates collected from both outpatient and hospitalized UTI patients.

This worrisome trend highlights the urgent need for increased attention and action to address the spread of ESBL-producing *E.coli* within the community setting. The study also revealed high levels of antimicrobial resistance among both the uropathogenic *E.coli* (UPEC) and ESBL-producing *E.coli* isolates, with resistance observed against commonly used antibiotics such as ampicillin, cephalosporins, fluoroquinolones, and co-trimoxazole.

In contrast, the isolates displayed relatively lower resistance to aminoglycosides, as well as to nitrofurantoin and fosfomycin. This suggests that the judicious use of these antibiotics may still be an effective strategy for managing ESBL-producing *E.coli* infections, at least in the short term. Given the concerning rise in ESBL prevalence within the community, this study emphasize the critical need for enhanced local surveillance of uropathogenic bacteria and their antimicrobial resistance

patterns. This will be essential for informing appropriate empiric antibiotic treatment guidelines for community-acquired UTIs (CA-UTIs) and preventing the further spread of multidrug-resistant ESBL-producing *E.coli*.

Additionally, after the certain investigations there is an urgent recommendation of a multi-center study to gain a broader understanding of the ESBL prevalence and antimicrobial resistance trends across different geographical regions in India. Such comprehensive data will be crucial for guiding national-level interventions and policies aimed at curbing the growing threat of ESBL-producing *E.coli* in both healthcare and community settings.

7. DECLARATIONS

Conflicts of interest: There is no any conflict of interest associated with this study

Consent to participate: There is consent to participate.

Consent for publication: There is consent for the publication of this paper.

Authors' contributions: Author equally contributed the work.

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