ORIGINAL RESEARCH

Fecal Carriage of ESBL and AmpC Beta-lactamases Producing Escherichia coli in a Rural Community in Faridabad, Haryana

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ABSTRACT
Background and Objectives: The emergence of ESBL-producing *Escherichia coli* in the community is a major public health concern, as these strains can act as reservoirs for resistance genes and cause various infections. Additionally, plasmid-mediated AmpC beta-lactamases have further complicated treatment options by conferring resistance to broad-spectrum beta-lactam antibiotics. This study aims to assess the carriage rate of ESBL- and AmpC-producing *E. coli* among patients attending a tertiary care hospital in a rural Faridabad district.**Materials and Methods:** This prospective cross-sectional study was conducted at Al-Falah School of Medical Sciences and Research Centre, Faridabad, from May to July 2022. 102 Non-pathogenic *E.coli* isolated from 150 stool samples received in the microbiology laboratory, were included in the study. Antibiotic susceptibility testing was performed, as per the CLSI 2022 guidelines. All isolates were screened for the production of ESBL and AmpC using ceftazidime and cefotaxime for ESBL and cefoxitin for AmpC. ESBL production was confirmed using the double disk diffusion test and AmpC production was confirmed using the boronic acid and cloxacillin combination double disk test.**Results:** Of the 150 stool samples, 102 non-pathogenic *E.coli* isolates were grown. out of 102 isolates, 52.94% were ESBL producers, while 28.43% were AmpC producers. Additionally, 18.63% of the isolates produced both ESBL and AmpC, indicating a substantial burden of antimicrobial resistance. Most of these isolates were multi-drug-resistant bacteria. The least resistance was seen against carbapenems. **Conclusion:** The alarming fecal carriage rate of multi-drug resistant ESBL-producing *E. coli* in a rural community highlights the risk for resistance transmission between non-pathogenic and pathogenic bacteria.
Key words: Fecal*E.coli*, ESBL, AmpC.

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INTRODUCTION

The widespread use of antibiotics has contributed to a significant rise in multidrug resistance among gram-negative bacteria. A key mechanism of resistance in these bacteria involves extended-spectrum beta-lactamase (ESBL) enzymes, which render beta-lactam antibiotics ineffective. Although these enzymes are highly resistant to beta-lactam antibiotics, they can be inhibited in vitro by beta-lactamase inhibitors such as clavulanic acid.[1]

Enterobacteriaceae strains harboring CTX-M-type ESBLs have rapidly spread worldwide and are now recognized as leading causes of both hospital- and community-acquired infections. In recent years, the emergence of ESBL-producing *Escherichia coli* in the community has become a major public health concern.[2] Additionally, plasmid-mediated AmpC beta-lactamases have contributed to a growing resistance against broad-spectrum beta-lactam antibiotics, further complicating treatment options.[3]

The presence of ESBL and pAmpC genes on mobile genetic elements facilitates their rapid dissemination via horizontal gene transfer. *E. coli*, a common commensal of the human gut microbiome, can act as a reservoir for these resistance genes while also serving as a major pathogen responsible for urinary tract and bloodstream infections. This has led to increased morbidity and mortality across both high-income and low- to middle-income countries.[3]

ESBL- and AmpC-producing *E. coli* strains have the ability to hydrolyse penicillin, monobactams, and third-generation cephalosporins, while remaining susceptible to cephamycins and carbapenems. These resistant strains are found in significant numbers within the gut microbiota.[4]The intestinal tract plays a crucial role in the transmission of antibiotic

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resistance, as multidrug-resistant Enterobacteriaceae species are frequently present in high concentrations.[1] ESBL genes are typically located on large plasmids, which also carry resistance determinants for various antibiotic classes, including aminoglycosides, trimethoprim, sulfonamides, tetracyclines, chloramphenicol, and fluoroquinolones. Therefore, the presence of large plasmids carrying resistance genes against multiple antibiotic classes raises concerns about the increasing risk of treatment failure.[4]

Different studies have shown varied prevalence of ESBL-producing *E. coli* depending on the study population, geographical location, and research methodology used.[4] This study aims to assess the carriage rate of ESBL- and AmpC-producing *E. coli* among patients attending a tertiary care hospital in a rural area in Faridabad district.

MATERIALS & METHODS

The **Study Subjects:** A total of 150 samples received in the microbiology laboratory of the Al-Falah School of Medical Sciences and Research Centre, Faridabad, were included in the study. This was a prospective cross-sectional study conducted from May 2022 to July 2022. Ethical clearance was obtained from the Institutional Ethical Committee.

Inclusion Criteria: Patients aged between 1 - 40 years who showed willingness voluntarily (guardians in case of pediatrics) to participate in the study with their written consent were enrolled for the study.

Exclusion Criteria: Individuals with any condition like diabetes mellitus, pregnancy, immunosuppressive disorders, history of recent antibiotic consumption (3 months) from the study. Also, samples with pus cells and RBCs were excluded from the study.

Sample Collection and Processing: Participants were provided with a sterile leak-proof container to collect the stool sample. Samples were transported on the same day to the laboratory and inoculated onto MacConkey agar plates and incubated at 37° C for 24 hours under aerobic incubation.

Identification of non-pathogenic *E.coli*: Colony morphology and Gram staining were used to identify the bacteria, *E.coli*isolates were detected by standard biochemical tests such as Motility, Catalase, Indole, Urease, Citrate, Triple sugar iron, Methyl red and Voges - Proskauer tests for biochemical identification. *E. coli* isolated from stool samples that looked normal i.e. without visible blood, negative occult blood test, and no RBCs or pus cells, were considered non-pathogenic and were included in the study.

Antibiotic Sensitivity Test: Antibiotic sensitivity testing was performed by the Kirby-Bauer disk diffusion method on Mueller-Hinton agar according to the Clinical and Laboratory Standards Institute (CLSI) protocol.[5] Antibiotics namely Ceftriaxone (30 µg), Cefoxitin (30 µg), Ofloxacin (5 µg), Gentamicin (10 µg), Ceftazidime (30 µg), Ampicillin/Sulbactam (10/10 mcg), Piperacillin/Tazobactam (100/10 mcg), Meropenem (10 µg), Imipenem (10 µg) were used with *Escherichia coli* ATCC 25922 as a control. The measured zone of inhibition (mm) was interpreted as sensitive or resistant according to the CLSI guidelines.

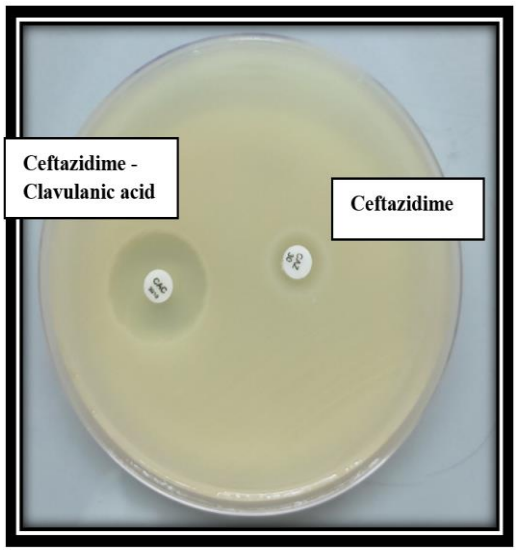

Figure 1: Phenotypic confirmation test for detection of ESBL E. Coli

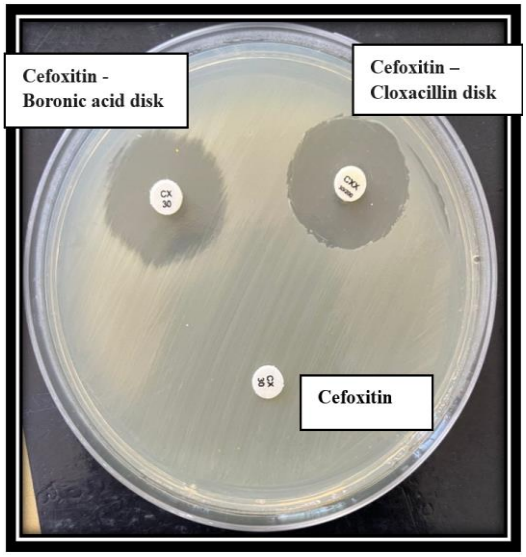

Figure 2: Phenotypic confirmation test for detection of AmpC E. Coli

ESBL detection: A 0.5 McFarland suspension of the *E.coli* isolates was swapped on Mueller-Hinton agar plates in the form of lawn culture and ceftazidime (30 µg) and ceftazidime - clavulanic acid (30/10 µg) disks (Himedia), were placed on the MHA at a distance of 30 mm. These plates were kept in the incubator for 24 hours at 37° C. *E.coli* strains showing an increase of 5 mm or more in the zone size around the ceftazidime - clavulanic acid disk compared to the ceftazidime disk was considered indicative of ESBL production. *Escherichia coli* ATCC 25922 and *K. Pneumoniae* ATCC 700603 were used as control strains.

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AmpC detection: For AmpC screening, a cefoxitin disk was placed on the Mueller-Hinton agar having *E.coli* lawn culture. The cefoxitin disk (Himedia, Mumbai, India) test was used for AmpC screening by inoculating Mueller-Hinton agar by the standard disk diffusion method and placing three disks, cefoxitin 30 µg, cefoxitin disk impregnated with 300µg phenylboronic acid, and cefoxitin disk impregnated with cloxacillin on it. The plates were incubated aerobically at 35- 37°C. The isolates that showed an increase in zone diameter by $\geq$ 5 mm around the cefoxitin-boronic acid disk or cefoxitin-cloxacillin disk, as compared with the cefoxitin disk alone were considered AmpC producers.

RESULTS

Out of the 150 fecal samples collected during the study period, 102 (68%) non-pathogenic *E.coli* isolates were obtained. Almost all participants of the study were below 40 years of age with male preponderance (57%). Among *E.coli* isolates number of ESBL producers was more than AmpC producers. Maximum beta-lactamase producers were from patients less than 10 years of age. This is because maximum samples were received from the pediatrics department. Most of the isolates that produced beta-lactamases were also resistant to other classes of antimicrobials like quinolones and aminoglycosides however, resistance to carbapenem was the least. Tables 1 &2 and Figures 3, 4 & 5 explain the results of this study.

Figure 3: Fecal specimen received in Microbiology Laboratory (n = 150)

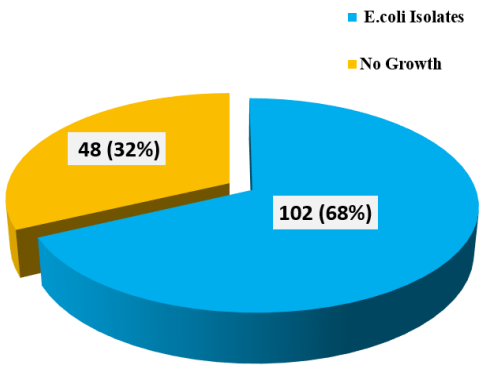

Figure 4: Gender-wise distribution of the study population (n = 102)

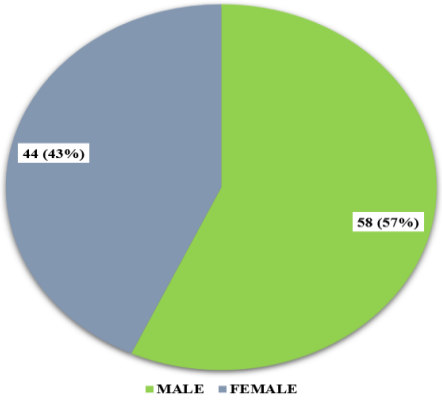

Figure 5: Antimicrobial resistance among ESBL/AmpC/ co-producer producing *E.coli*

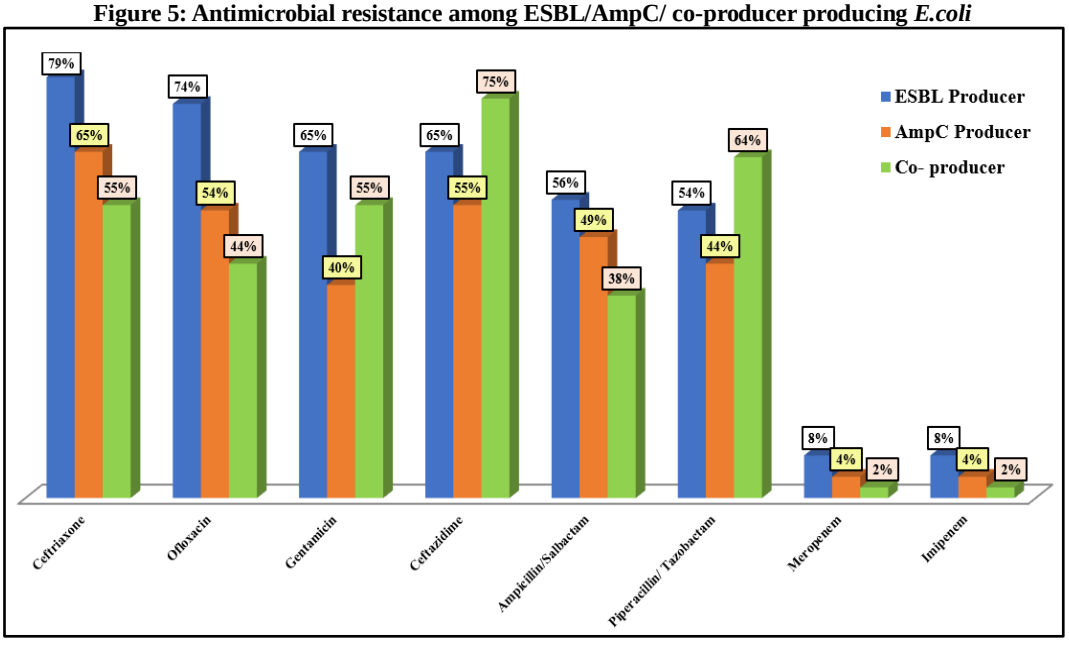

Table 1: Prevalence of ESBL and AmpC in *E. coli*

N = 102		Percentage (%)
ESBL producer	54	52.94
AmpC producer	29	28.43
Co-producer	19	18.63
Total	102	100

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Table 2: Age-wise distribution among ESBL/AmpC/ coproducer producing *E.coli*

Age - group	ESBL (n=73)	AmpC (n=54)	Co - producer (19)
0 - 10	36 (49.32%)	22 (40.74%)	9 (47.37%)
11 - 20	16 (21.92%)	11 (20.38%)	5 (26.31%)
21 - 30	13 (17.8%)	14 (25.92%)	4 (21.06%)
31 - 40	7 (9.59%)	6 (11.1%)	1 (5.26%)
> 40	1 (1.37%)	1 (1.86%)	0 (0%)
Total	73 (100%)	54 (100%)	19 (100%)

DISCUSSION

The increasing prevalence of extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* (E. coli) in both hospital and community settings is a significant public health concern.[6] In the current study, 52.94% of *E. coli* isolates from stool samples were ESBL producers, while 28.43% were AmpC producers. Additionally, 18.63% of the isolates produced both ESBL and AmpC, indicating a substantial burden of antimicrobial resistance.

Several studies have reported similar alarming trends globally. Indernath S et al., in their study conducted in South India, found that 78% of *E. coli* isolates from stool samples in rural communities were ESBL producers.[6] Similarly, a study conducted in Tanzania by Upendo O. Kibwana reported an ESBL production rate of 68.7% among fecal*E. coli* isolates.[7] In another study by EM Abdul Rahman, 71.25% of ESBL-producing isolates were identified as *E. coli* from the stool samples.[8] In a study done by Mandal D K, on stool samples of patients attending outpatient departments, in Nepal, 70.2% of ESBL-producing *E.coli* were isolated.[4] All these studies are in accordance with our study and show a very distressing level of ESBL production in fecal*E.coli*.

The presence of ESBL-producing *E. coli* in the gut is particularly concerning as it can contribute to extraintestinal infections and facilitate the transfer of antibiotic-resistance genes to other *E. coli* strains and bacterial species within the gastrointestinal tract. This increases the risk of widespread resistance transmission through human-to-human contact or environmental exposure.[9] Furthermore, individuals colonized with ESBL-producing *E. coli* pose a higher risk of infection when admitted to hospitals, potentially contributing to nosocomial outbreaks.[6]

Mandal D et al detected 18.4% of AmpC-producing *E.coli* and 13.81% of both ESBL and AmpC coproducing *E.coli* isolates in the stool samples of OPD patients.[4] Whereas, Rashid M et al., detected 14% (9 out of 61 isolates) of AmpC producing fecal*E.coli* in healthy community subjects and hospitalized patients, and only two Isolates coproduced both ESBL and AmpC , in their study in North India.[10]

Many factors contribute to the colonization and infection caused by extended-spectrum β-lactamase (ESBL)-producing bacteria. These include suboptimal antibiotic quality or inappropriate dosing, irrational antimicrobial use, unregulated self-medication, and the involvement of inadequately trained healthcare providers. Additionally, poor hygiene practices, ineffective infection control measures, and the absence of robust antimicrobial resistance (AMR) surveillance programs facilitate the dissemination of resistant bacterial strains.[11,12] Traveling to distant places has also been identified as a significant factor in the acquisition of antimicrobial-resistant bacteria.[13,14] All these factors can lead to the acquisition of resistance by gut flora as well.

Our study identified a notably high prevalence of beta-lactamase-producing organisms. This observation may be linked to socioeconomic factors prevalent in the study area, such as limited economic development and lower literacy rates. These conditions can contribute to reduced awareness and practice of hygiene measures, potentially facilitating the spread of antimicrobial-resistant bacteria within the community.

CONCLUSION

Gastrointestinal colonization by *E. coli* producing extended-spectrum beta-lactamases (ESBL) and AmpC enzymes significantly elevate the likelihood of infection. Furthermore, the horizontal transfer of resistance determinants via mobile genetic elements poses a substantial risk of widespread dissemination within the community. Hence, the presence of ESBL-producing strains complicates empirical therapy, necessitating careful antibiotic selection. The sharp rise in ESBL and AmpC-producing fecal*E. coli* over the past decade underscores the urgent need for enhanced surveillance, infection control measures, and antimicrobial stewardship to curb the spread of resistance in both hospital and community settings.

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