










## Genomic Determinants and Antimicrobial Resistance Pattern of Clinical Isolates of Extended Spectrum Beta Lactamase (ESBL) Producing *Escherichia coli*

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## Abstract

The growing prevalence of antibiotic-resistant bacteria is a worldwide public health apprehension, and *Escherichia coli* (*E. coli*) is one of the most commonly implicated bacterial species. Among *E. coli* isolates, extended-spectrum  $\beta$ -lactamase (ESBL)-producing strains have been identified as a key contributor to antibiotic resistance. Penicillin's, cephalosporins, and monobactams are only a few of the  $\beta$ -lactam antibiotics that can be rendered inactive by ESBLs. This investigation's goals were to determine the prevalence of ESBL-producing *E. coli* isolates found in clinical samples and to analyze the distribution of the *bla*<sub>TEM</sub>, *bla*<sub>SHV</sub>, and *bla*<sub>CTX-M</sub> genes among them. Additionally, we aimed to determine the antibiotic susceptibility patterns of these isolates to other antibiotics. Clinical isolates from urine, ear swab, and wound/pus swabs were collected from patients with suspected *E. coli* bacterial infections from different regions of north India viz., SKIMS-JVC medical college and NABL accredited Dr. Qadri's Lab both from Srinagar Kashmir valley region and SMSR, SU from Greater Noida UP. Standard laboratory techniques were used to identify *E. coli* isolates, and the combined disc method and other phenotypic confirmation techniques were used to confirm ESBL formation. PCR amplification and sequencing were used to find the *bla*<sub>TEM</sub>, *bla*<sub>SHV</sub>, and *bla*<sub>CTX-M</sub> genes. The Kirby-Bauer disc diffusion method was used to test the antimicrobial susceptibility of various bacteria to different antibiotics. A total of 210 *E. coli* isolates were collected from different clinical samples and only 158 isolates showed positive results for ESBL by DDST and phenotypic confirmatory tests. Of these, 124 (78.48%) were ESBL-producing isolates. We found that *bla*<sub>TEM</sub> was the most prevalent gene (45.16%), followed by *bla*<sub>CTX-M</sub> (34.16%) and *bla*<sub>SHV</sub> (12.09%). Antimicrobial resistance profiles were assessed for each of the 120 isolates. Ampicillin and Cefepime were the most resistant drugs to ESBL-producing isolates, followed by Gentamicin, Ceftriaxone, and Cefixime.

**Keywords:** ESBL, PCR, DDST, Antimicrobial Susceptibility, Enterobacteriaceae

## INTRODUCTION

*Escherichia coli* (*E. coli*) is one of the bacterial species of particular concern as a result of the rising incidence of antibiotic-resistant bacteria, which is a global public health issue.<sup>1</sup> Both community-acquired and hospital associated infections are caused by this pathogen and have shown increasing resistance to third-generation cephalosporins, which can cause more expensive hospital stays, greater mortality and morbidity rates.<sup>2</sup> The major challenge for the physicians nowadays is treating ESBL producing Strains. Extended-spectrum beta-lactamase (ESBL)-producing strains of *E. coli* have been discovered as a significant cause of antibiotic resistance.<sup>3,4</sup> ESBLs are enzymes that render various beta-lactam antibiotics inactive including cephalosporins and monobactams. Due to the widespread usage of these antibiotics, resistance has emerged.<sup>5</sup> Occurrence of carbapenem resistance is also rapidly increasing in *E. coli* due to the production of different types of enzymes called carbapenemases. These enzymes have potential to hydrolyze almost all  $\beta$ -lactams, including carbapenems the genes

of which are located on mobile genetic element (MGE) called plasmids. Due to the majority of cross resistance between beta-lactamases produced by Enterobacteriaceae and other classes of commonly used antibiotics, such as aminoglycosides, trimethoprim sulfamethoxazole, and fluoroquinolones, only a limited number of therapeutic options are available in a hospital setting. There is very high hospital stay expenses and high mortality rates associated with these multidrug resistant organisms in contrast to non  $\beta$ -lactamase producing Enterobacteriaceae. Thus, the surveillance and monitoring of these  $\beta$ -lactamase producing Enterobacteriaceae is important for clinical care.<sup>6</sup> Three main types of ESBL's have been recognized which are *TEM*, *SHV*, and *CTX-M*. In terms of prevalence, CTX-M has surpassed SHV and TEM. CTX-M belongs to rapidly growing family that has expanded over a variety of clinically significant bacteria and vast geographic regions.<sup>7</sup> Understanding susceptibility patterns is important because of the rising rates of bacterial resistance which is a major global problem this is because prolonged hospital stays and higher mortality rates can result from the widespread and

inappropriate use of antimicrobial therapy which can be prevented by providing right treatment.<sup>8</sup> In this investigation, the prevalence of the ESBL phenotype was attempted to be ascertained, and clinical isolates were examined for the presence of the *bla*<sub>TEM</sub>, *bla*<sub>SHV</sub> and *bla*<sub>CTX-M</sub> genes.

## MATERIALS AND METHODS

The study was carried out in department of veterinary microbiology SKUAST-K and department of microbiology SMSR, SU from Jan 2021 to Dec 2022. A total of 210 *E.coli* isolates from various samples collected from different

location in the Kashmir valley and Noida UP region (Table 1). The source of these isolates includes urine, ear swab, and wound/pus swabs. These isolates were collected from patients with suspected *E.coli* infections. The contributing centres were department of Microbiology of the Sher-e-Kashmir Institute of Medical Sciences, Jhelum Valley Medical College, Bemina, Srinagar Kashmir, India and NABL accredited Dr. Qadri's Haematology Center & Clinical Laboratory, Srinagar Kashmir, India and Sharda School of Medical Sciences & Research, Sharda Hospital, Greater Noida, UP, the distribution of which is follows:

**Table 1.** Details of isolates collected from different locations.

No.	Location	Isolates			Total
		Urine	Wound/Pus	Ear Swab	
1.	SKIMS-JVC	64	16	10	90
2.	Qadri Hematology Lab.	59	12	09	80
3.	SMSR, Sharda University	29	08	03	40
	Total	152	36	22	210

The isolates were produced from the cultures of a variety of clinical samples taken from both in-patients and out-patients who were admitted to the hospitals from the aforementioned centres. By sub-culturing on MacConkey agar, all the bacterial cultures underwent a second test for viability and purity. (Figure 1).

Preliminary phenotypic and biochemical testing were conducted to validate the identity of isolates:

### Gram staining

On a clean grease free slide, bacterial smears from 16-18 hrs were prepared, heat fixed and stained with Gram stain and observed under 100x oil immersion microscope.

### Motility test

Motility of the organism was observed using hanging drop technique

### Catalase test

On a clean grease free slide, a small amount of bacterial culture was mixed with drop

of 3% H<sub>2</sub>O<sub>2</sub>. Appearance of effervescence is an indication of catalase positive otherwise negative.

### Oxidase test

The culture was smeared on oxidase discs (Himedia) with the inoculation loop. Appearance of blue or purple color within 10 seconds in an indication or oxidase positive otherwise negative.

### Isolation of Presumptive ESBL producing *Escherichia coli*

Presumptive isolates were inoculated in nutritional broth and incubated at 37°C until the suspension met the 0.5McFarland standard (1.5x10<sup>8</sup> CFU/mL). Around 10µl of the suspension was spread using a sterile spreader on ESBL ChromoSelect Agar (Sigma-Aldrich) plates. The media was prepared by dissolving 40 grams in one liter of distilled water and heated to dissolve it completely. Sterilization of media was done by autoclaving at 15lbs pressure (121°C) for 15 minutes, cooled to 50°C and rehydrated contents of two vials of ESBL ChromoSelect Selective Supplement (Cat #61471, Sigma) was added,

mixed well and poured into sterile petri plates. From each plate two pink colonies were selected and then on nutrient agar slant colonies were streaked and then overnight incubation was done and kept at 4°C for further analysis (Figure 2).

#### Phenotypic tests for the detection of ESBLs

Use of disk diffusion test to check resistance to Cefotaxime and Cefotaxime by presumptive ESBL producing *E. coli*.

As recommended by Clinical and Laboratory Standards Institute (CLSI), the ESBL isolates underwent disc diffusion testing in order to check resistance against cefotaxime and ceftazidime (Figure 3). Each isolate was suspended in the nutrient broth and a suspension

was made whose turbidity matches with 0.5 McFarland standard ( $1.5 \times 10^8$  CFU/mL). The bacterial suspension was then spread on Mueller Hinton agar with a sterilised cotton swab to produce a uniform lawn culture. Antibiotics disc of cefotaxime and ceftazidime were placed on the surface of Muller Hinton agar plate after the plate had dried, and incubation was done for 18-24 hours at 37°C. Zone of inhibition around the discs were measured and recorded. For concluding resistance disk potency and inhibition zone diameters were used as shown in Table 2. Isolates were declared to be ESBL positive even if they show resistance to one of the antibiotic disc.

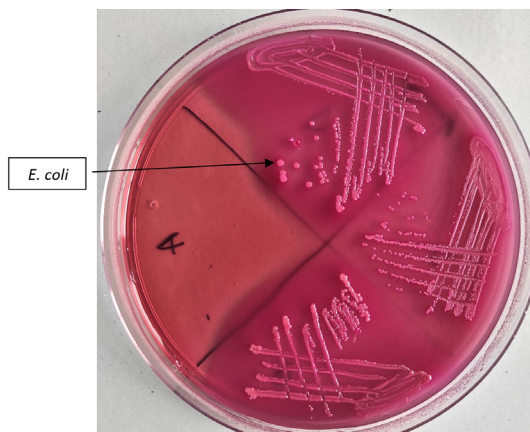
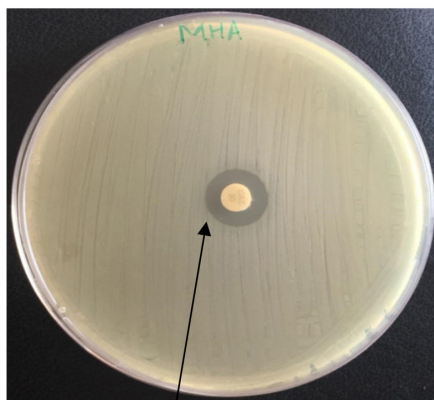


Figure 1. Isolation of *E. coli* on MacConkey's Lactose Agar



Figure 2. Isolation of ESBL producing *E. coli* on Chromogenic agar



Ceftazidime (CAZ)

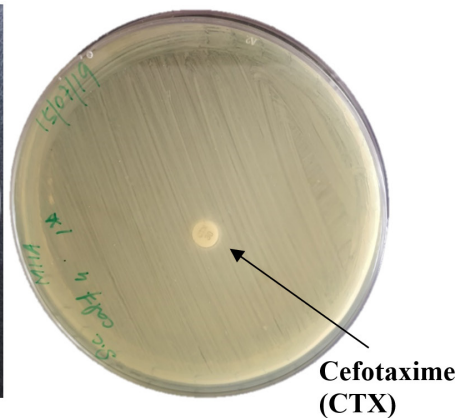


Figure 3. Screening of ESBL *E. coli* isolates for resistance to ceftazidime (CAZ) (Left) and cefotaxime (CTX) (Right) by disk diffusion method

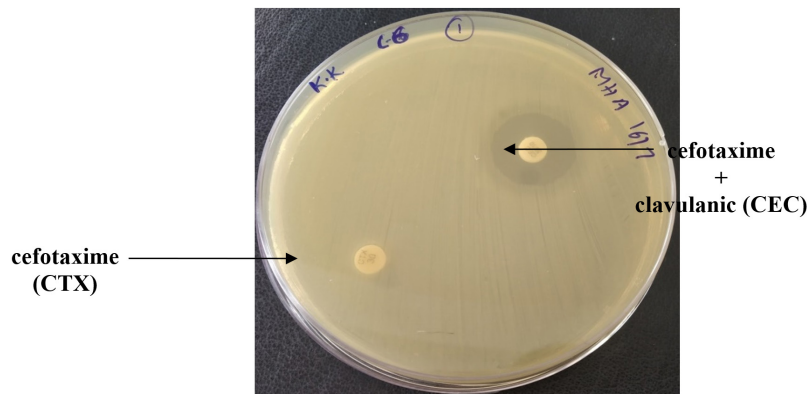
### Confirmation of ESBL producing *E. coli* by Cephalosporin/Clavulanate combination disks

Double disc synergy test was performed as per CLSI guidelines in 2010 for those isolates which were resistant to one of the antibiotics viz. Cefotaxime and Ceftazidime. The isolates were grown in nutrient broth till they matched 0.5McFarland standard ( $1.5 \times 10^8$  CFU/mL). Bacterial suspension was spread on the Muller Hinton agar plates uniformly. After the plates dry, antibiotic discs containing cefotaxime, cefotaxime+ clavulanic acid and ceftazidime, ceftazidime+clavulanic acid were placed on the surface of the plates and incubated for 18-24 hours at 37°C. After overnight incubation and visible growth, zone of inhibition around the discs were measured and recorded. Increase in zone diameter of 5mm around the antibiotic disc containing cephalosporins and clavulanic acid over

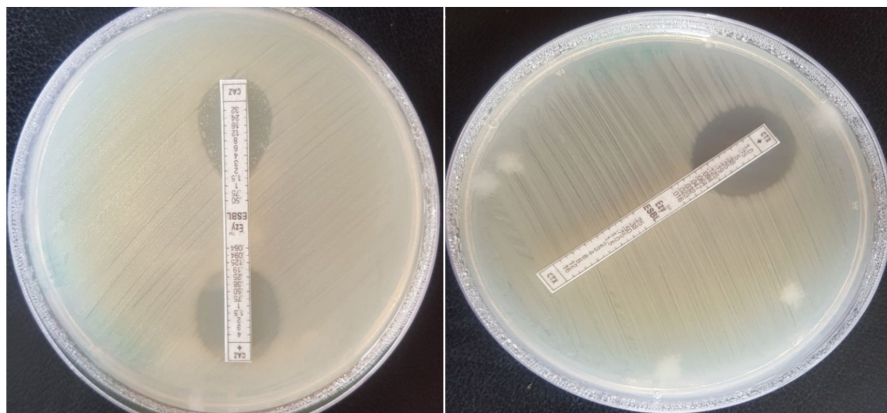
the antibiotic disc containing cephalosporin alone is an indicative of ESBL production (Figure 4).

### Etest for ESBLs

HiMedia, India, makes plastic drug-impregnated strips which has gradient of antibiotic on both the sides with one end containing ceftazidime (MIC test range 0.5 to 32 µg/mL) and other end containing ceftazidime (MIC test range 0.064 to 4 µg/mL) and a fixed concentration of clavulanate (4 µg/mL). Similar strips of cefotaxime (MIC test range 0.25 to 16 µg/mL) and cefotaxime (MIC test range 0.016 to 1µg/mL) plus a fixed concentration of clavulanate (4µg/mL) are also available. Both screening and phenotypic confirmation of ESBL production was done by these strips. Pure cultures of bacteria cultivated overnight on MacConkey agar were



**Figure 4.** Phenotypic confirmation of ESBLs production in *E. coli* isolates by disk diffusion method using cefotaxime (CTX) and cefotaxime+clavulanic (CEC) acid disks



**Figure 5.** Phenotypic confirmation of ESBLs production in *E. coli* isolates by Etest using ceftazidime/ceftazidime + clavulanic acid Strip (Left) and cefotaxime / cefotaxime+clavulanic acid strip (Right)

used to create a suspension in nutrient broth that matched the 0.5McFarland ( $1.5 \times 10^8$  CFU/mL) standard. Lawn culture was made on Muller Hinton agar with sterile cotton swab. The antibiotic impregnated strips were placed on Muller Hinton agar after the plates dry at desired position. The strips became absorbed and firmly attached to the agar surface after 60 seconds and plates were incubated for 18-24 hours at 37°C. Read the MIC at the point on the strip where the ellipse crossed the MIC scale. If the isolates displayed a 8-fold reduction in cephalosporin MICs in the presence of clavulanate, they were deemed to be phenotypically confirmed ESBLs(As per manufacturer’s instructions(HiMedia) (Figure 5).

**Molecular characterization of ESBL producing E. coli isolates**

**Extraction of bacterial DNA**

After ESBL producers were identified phenotypically, isolates were subjected to molecular analysis. A loopful of bacterial growth was obtained from nutritional agar slants and suspended in 500 microliters (µL) of sterile distilled water in a 2 mL microcentrifuge tube. The DNA was then extracted using the snap and chill technique, which involves boiling colonies suspended in distilled water for 10 minutes to release the DNA,

followed by 10 minutes of cooling on ice and 1 minute of 10,000 g centrifugation in a Table top microcentrifuge (Biofuge Stratus, Heraeus). Around 2µL of the supernatant were utilized as the template for the polymerase chain reaction (PCR).

**Detection of ESBL producing E. coli isolates**

All the isolates that were phenotypically determined to produce ESBLs were subjected to multiplex PCR (m-PCR) testing using particular primers to check for the presence of the *bla<sub>TEM</sub>*, *bla<sub>SHV</sub>*, and *bla<sub>CTX-M</sub>* genes. (Table 3). The PCR amplification was carried out in 25µL reaction volumes in 0.2 mL thin walled PCR tubes (Eppendorf, Germany). The PCR mixture consists of DNA template, Go-green Master Mix (Promega, premixed ready-to-use solution containing Taq DNA polymerase derived from bacteria, dNTPs, MgCl<sub>2</sub> and reaction buffers), specific primers and Nuclease free water. Negative template control was sterile distilled water. The following cycling parameters were used during PCR in the Mastercycler Gradient Thermal cycler (Eppendorf, Germany): an initial denaturation at 95°C for 15 min; followed by 30 cycles of amplification each consisted of 30 s of denaturation at 94°C; 90 s of annealing at 62°C and 60 s of elongation at 72°C. The mixture was subjected to final elongation at 72°C for 10 min. The DNA extracted from the positive isolate maintained by the Division of Veterinary Microbiology and Immunology, SKUAST-Kashmir was included as positive control in the PCR reaction. Sterile distilled water was used as negative control. All the nucleotide primers used throughout the study were procured from Integrated DNA Technologies, USA.

**Table 2.** Disk concentration and zone diameters for inferring resistance in the screening test

Antibiotic disk	Resistant, if zone diameter was
Cefotaxime-CTX (30 µg)	≤ 17 mm
Ceftazidime-CAZ (30 µg)	≤ 22 mm

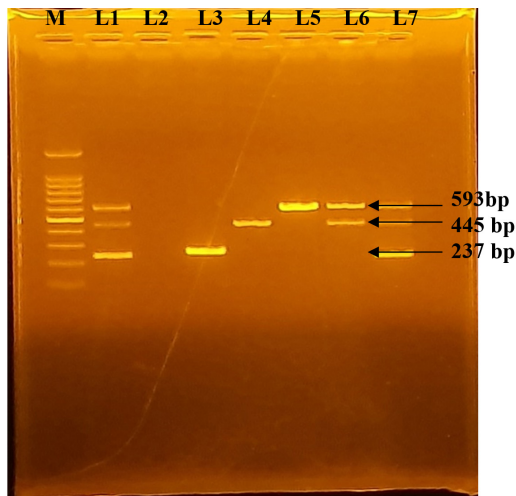
**Table 3.** Predicted amplicon length and a sequence of primers used to detect various beta-lactamase genes in multiplex PCR

Primer	Sequence (5' - 3')	Target gene	Amplicon size (bp)	Ref.
<i>blaSHV</i> -F	CTT TATCGGCCCTCACTCAA	<i>blaSHV</i>	237	[8]
<i>blaSHV</i> -R	AGGTGCTCATCATGGGAAAG			
<i>blaTEM</i> -F	CGCCGCATACACTATTCTCAGAAT GA	<i>blaTEM</i>	445	[9]
<i>blaTEM</i> -R	ACGCTCACCGGCTCCAGATTTAT			
<i>blaCTX-M</i> /F	ATGTGCAGYACCAGTAARGTKATGGC	<i>blaCTX-M</i>	593	[10]
<i>blaCTX-M</i> /R	TGGGTRAARTARGTSACCAGAAAYCAGCGG			

### Electrophoresis and documentation

Agarose gel (1.5% w/v) was made by heating the appropriate amount of agarose (Sigma Aldrich, St. Louis, USA) with 40 mL 1X Tris acetate EDTA (TAE) buffer in a 500 mL Erlenmeyer flask. The flask was cooled to 60°C and ethidium

bromide was added to the final concentration of 0.5µg/mL. The gel casting tray made of plastic was filled with warm agarose and a comb with 0.5 to 1mm wells was placed in the gel casting tray and allowed to set for around 30 minutes at room temperature. The comb was removed, and the gel was put on a Biometra electrophoresis tank that contained 1X TAE buffer. The wells in gel submerged in buffer tank were loaded with PCR products and standard molecular weight marker (Cat #DM003-R500, GeneDireX) was loaded in one well. The gel received a voltage of 5 V/cm across the ends until the yellow loading dye migrated to particular distance. The gel was removed carefully from the buffer and visualized under ultraviolet illumination and photographed with Gel Documentation System (BioDocAnalyze [BDA], Biometra, Germany) (Figure 6).



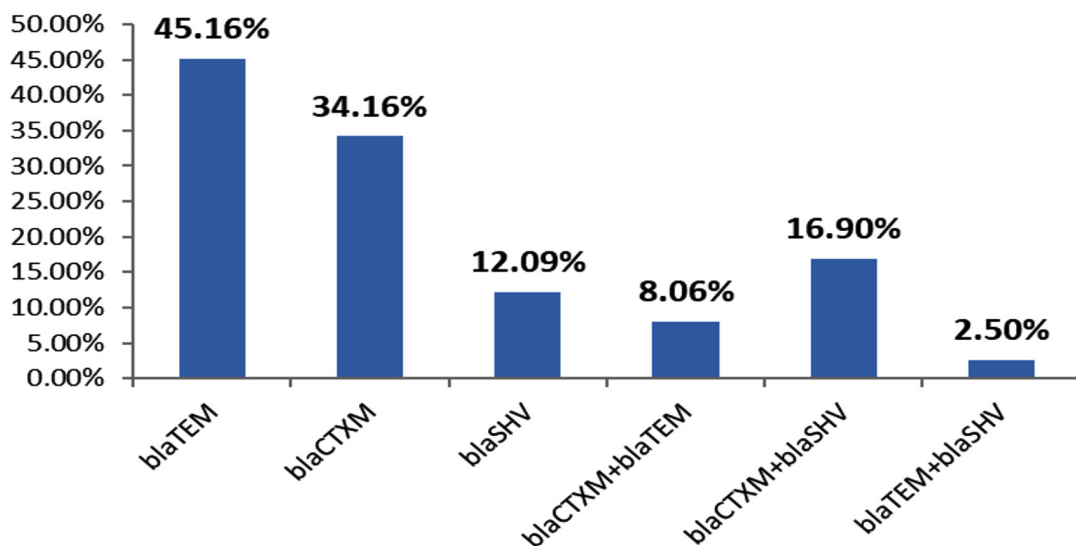
**Figure 6.** Representative *bla<sub>SHV</sub>*, *bla<sub>TEM</sub>* and *bla<sub>CTX-M</sub>* genes profile of phenotypically positive isolates using multiplex polymerase chain reaction (m-PCR):- Lane M: 100 bp DNA Ladder; Lane 1: Positive control for *bla<sub>SHV</sub>*, *bla<sub>TEM</sub>* and *bla<sub>CTX-M</sub>* genes; Lane 2: Negative control; Lane 3: *bla<sub>SHV</sub>* positive; Lane 4: *bla<sub>TEM</sub>* positive; Lane 5: *bla<sub>CTX-M</sub>* positive; Lane 6: *bla<sub>TEM</sub>* and *bla<sub>CTX-M</sub>* positive and Lane 7: *bla<sub>SHV</sub>*, *bla<sub>TEM</sub>* and *bla<sub>CTX-M</sub>* positive

### Antimicrobial susceptibility profile

Bacterial isolates found to be positive for ESBL genes by m-PCR, were tested for multi drug resistance by the Kirby Bauer disk diffusion method as per Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2010) against 20 antibiotics (Table 4).

### Statistical Analysis

Level of significance p<0.05 was done by analyzing data using students t-test.



**Figure 7.** The overall incidence of ESBL genotypes in screening positive *E. coli* isolates

**Table 4.** Antibiotic resistance pattern of *E. coli* isolates

Antibiotic	ESBL <i>E. coli</i> Isolates (N=158) Non				ESBL <i>E. coli</i> Isolates (N=52)				P -Value
	Sensitive (S)		Resistant (R)		Sensitive (S)		Resistant (R)		
	%	N	%	N	%	N	%	N	
Amikacin	91.2	144	8.8	14	80.77	42	19.23	10	0.426
Amoxyclav	16.6	26	83.4	132	55.77	29	44.23	23	<0.01
Ampicillin	0	0	100	158	11.5	6	88.5	46	<0.01
Aztreonam	21.5	34	78.5	124	59.6	31	40.4	21	<0.01
Cefepime	0	0	100	158	55.7	29	44.3	23	<0.01
Cefixime	14.6	23	85.4	135	61.5	32	38.5	20	<0.01
Ceftriaxone	13.3	21	86.7	137	73	38	27	14	<0.01
Chloramphenicol	78.5	124	21.5	34	69.2	36	30.8	16	0.444
Ciprofloxacin	97.5	154	2.5	4	78.8	41	21.2	11	0.159
Co-Trimoxazole	41.8	66	58.2	92	42.3	22	57.7	30	0.956
Doxycycline	41.8	66	58.2	92	46.1	24	53.9	28	0.646
Furazolidone	83	131	17	27	38.5	20	61.5	32	<0.01
Gentamicin	6.3	10	93.7	148	67.3	35	32.7	17	<0.01
Imipenem	100	100	0	0	78.8	41	21.2	11	0.112
Kanamycin	67.7	107	32.3	51	61.5	32	38.5	20	0.585
Nalidixic acid	78.1	76	51.9	82	44.3	23	55.7	29	0.002
Norfloxacin	50	79	50	79	78.8	41	21.2	11	0.011
Nitrofurantoin	91.2	144	8.8	14	59.6	31	40.4	21	0.010
Streptomycin	83	131	17	27	63.5	33	36.5	19	0.107
Tetracycline	33	52	67	106	67.3	35	32.7	17	<0.01

\*pvalue &lt;0.05 differ significantly

## RESULTS

### ESBL screening of *E. coli*

The initial screening test of ESBL production by DDST and the phenotypic confirmation test revealed that 158 (or 75%) out of the 210 *E. coli* isolates were positive. Multiplex PCR was performed on all 158 phenotypically verified *E. coli* isolates using bacterial lysate as the template. Out of 158 ESBL isolates only 124 (78.48%) isolates carried the gene/s screened for *bla*<sub>TEM</sub>, *bla*<sub>CTX-M</sub> and *bla*<sub>SHV</sub> genes. Isolates carrying *bla*<sub>SHV</sub> gene produced an amplicon of 237 bp, those carrying *bla*<sub>TEM</sub> showed an amplicon of 445 bp. The *bla*<sub>CTX-M</sub> gene produced an amplicon of 593bp. The multiplex PCR assay results indicated 45.16% *bla*<sub>TEM</sub> genes, 8.06% *bla*<sub>TEM</sub>/*bla*<sub>CTX-M</sub> genes, 34.16% *bla*<sub>CTX-M</sub>, 12.09%, *bla*<sub>SHV</sub>, 16.90% *bla*<sub>CTX-M</sub> + *bla*<sub>SHV</sub>, 2.50% *bla*<sub>TEM</sub> + *bla*<sub>SHV</sub>. The overall incidence of ESBL genotypes in *E. coli* isolates is illustrated in (Figure 7).

### Antimicrobial susceptibility profile

In total, 124 *E. coli* isolates were tested for their antimicrobial resistance profile against 20 different antimicrobial agents was tested. The prevalence of antimicrobial resistance among the ESBL positive strains isolated from various clinical isolates is shown in Table 4 against twenty antimicrobial agents and interpreted as resistant and sensitive following the guidelines of Clinical Laboratory Standard Institute Laboratory Standard Institute. Zone of inhibition were measured and the susceptibility (or resistance) of each isolate was determined (Figure 8 & 9).

## DISCUSSION

Surveillance of antimicrobial resistance is very important for keeping track of community infections in order to understand how different treatment approaches affect resistance. The emergence and dissemination of resistance are due to the non-judicious use of antibiotics and



the resistance develops either by intrinsic way or acquired way.<sup>11</sup>

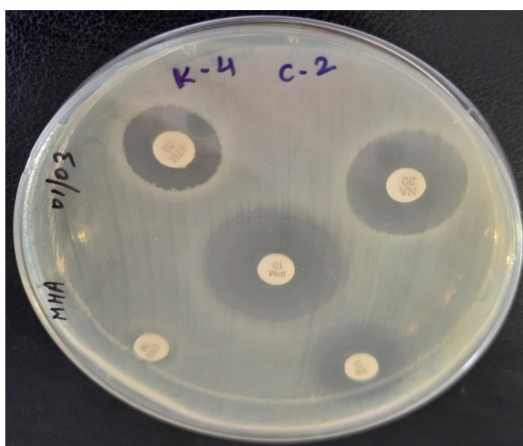
One of the eight bacterial species WHO has singled out is *E. coli* and is of particular concern for antibiotic resistance, serving as a sentinel organism which is responsible for developing resistance in urine and blood.<sup>1</sup> There are significant regional and global differences in the incidence of ESBL among clinical isolates, and these differences are evolving quickly.<sup>12</sup> The prevalence reported in our study (75%) which is in agreement with 61% by other studies Falgenhauer *et al.*<sup>13</sup>; 69.2% by Pouladfar *et al.*<sup>14</sup> and 72.9% by Mood *et al.*<sup>15</sup> and lower than reported by Ali *et al.*<sup>16</sup> (80%) and Kokabi *et al.*<sup>17</sup> (84.74%). In contrast, it is higher than those reported from Iran (26.6%) by Tabar *et al.*<sup>18</sup>; 34.6% by Naziri *et al.*<sup>19</sup>; 24.5% by Mohajeri *et al.*<sup>20</sup>; 41% by Hashemizadeh *et al.*<sup>21</sup> and 46.1% by Moosavian *et al.*<sup>22</sup>

The ESBL-positive *E. coli* isolates investigated here showed higher frequency of  $bla_{TEM}$  genes (45.16%) followed by  $bla_{CTX-M}$  (34.67%),  $bla_{SHV}$  (12.09%) and  $bla_{TEM} + bla_{CTX-M}$  genes (8.06%).

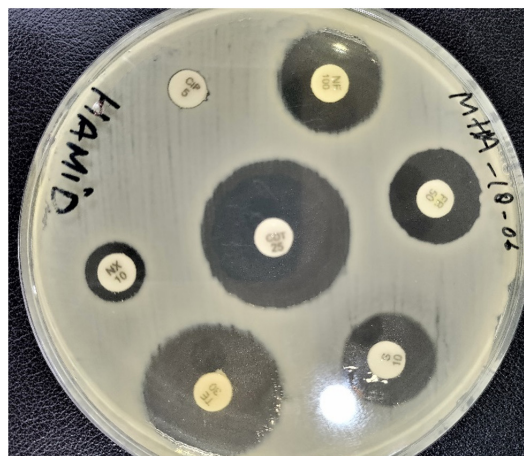
Our findings are consistent with those of Cristea *et al.*<sup>23</sup> who found 19.7% *E. coli* isolates encoded for  $bla_{SHV}$ . Furthermore, Sadeghi *et al.*<sup>24</sup> reported 38.8% isolates encoded for  $bla_{CTX-M}$  and also Habeeb *et al.*<sup>25</sup> reported 42.5% isolates and 48.1% isolates of *E. coli* encoded for  $bla_{CTX-M}$  and  $bla_{TEM}$  respectively.

In another report by Jena *et al.*,<sup>26</sup> frequency of  $bla_{TEM}$  was reported more than  $bla_{CTX-M}$  followed by  $bla_{SHV}$  which is in close conformity to our findings.

Antibiotic resistance in *E. coli* has been seen to rise across multiple drug classes in many investigations, and resistance to  $\beta$ -lactam, cephalosporin, and carbapenem antibiotics is becoming more well-documented.<sup>11,27,28,29</sup> The ESBL production is alarming. Our study showed a susceptibility of 100% for imipenem, this frequency was similar with other studies in which susceptibility varied from 95 up to 100% for imipenem.<sup>18,20,25,30,31,36</sup> We have few options for treating severe infections because of the significant rise in  $\beta$ -lactamase production, which includes the high level of ESBL producers. In this study, the ESBL strains showed high resistance to ampicillin (100%), aztreonam (78.5%), cefepime (100%), ceftriaxone (86.6%), tetracycline (66.66%), norfloxacin (50%) and nitrofurantoin (8.33%), which is consistent with a claim made by Cristea *et al* in Romania.<sup>23</sup> and also observed in other studies.<sup>31-34</sup> Most of the therapeutic problem are associated with ESBL strains due to their resistant against broad spectrum antibiotics including third generation cephalosporins. These strains also have the potential for plasmid-mediated quinolone (PMQR) and carbapenem resistance. MDR Multiple Drug Resistance is also responsible



**Figure 8.** Antimicrobial sensitivity pattern of ESBL isolates against aztreonam (ATM 30 $\mu$ g); ceftriaxone (CRO 30 $\mu$ g); chloramphenicol (C 30 $\mu$ g); imipenem (IMP 10 $\mu$ g) and nalidixic acid (NA 30  $\mu$ g)



**Figure 9.** Antimicrobial sensitivity pattern of ESBL isolates against tetracycline (TE 30 $\mu$ g); norfloxacin (NX 10 $\mu$ g); ciprofloxacin (C 5 $\mu$ g); streptomycin (S 10 $\mu$ g), furazolidone (FR 50  $\mu$ g) and co-trimoxazole (COT 25 $\mu$ g)

for creating complications in level of resistance leading to overprescribing of antibiotics in healthcare settings without prior microbiologic testing.<sup>35,36</sup>

## CONCLUSION

The high prevalence of ESBL-producing *E. coli* isolates in clinical samples indicates the urgent need for appropriate infection control restrictions. The identification of *bla*<sub>TEM</sub> as the most prevalent gene in these isolates highlights the need for further research to determine the molecular epidemiology of ESBL-producing *E. coli*. The high levels of resistance to commonly used antibiotics, such as ampicillin, doxycycline, cefixime and gentamicin, underscore the importance of prudent use of antibiotics to reduce the selection pressure on resistant strains. The findings of this study provide valuable insights into the prevailing trends of ESBL-producing *E. coli* in the region and can help in the development of effective prevention and control strategies.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## AUTHORS' CONTRIBUTION

HAS, MAB, DKK conceptualized the study. SF, ASY, ASH helped in research conduction and

sampling. SQ, BN and AT wrote the manuscript. All authors read and approved the final manuscript for publication.

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## DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

## ETHICS STATEMENT

Not applicable.

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