

Increasing Prevalence of *Escherichia coli* and *Klebsiella pneumoniae* Producing CTX-M-Type Extended-Spectrum Beta-Lactamase, Carbapenemase, and NDM-1 in Patients from a Rural Community with Community Acquired Infections: A 3-Year Study

Abstract

Background: Increasing prevalence of community-acquired infections (CAIs) due to *Escherichia coli* and *Klebsiella pneumoniae* producing extended-spectrum beta-lactamase (ESBL), especially the Cefotaxime-Munich (CTX-M) type, carbapenemase, and New Delhi metallo- β -lactamase (NDM), has been reported globally posing a serious public health threat that has complicated treatment strategies for Gram-negative bacterial infections. While most of the reports in this regard are based on hospitalized patients from the urban community, there is a paucity of data in a rural community presenting with CAIs. **Materials and Methods:** A total of 1275 strains of *E. coli* and *K. pneumoniae* isolated over a period of 3 years from patients with CAIs were subjected to the detection of ESBL by double-disc synergy test; carbapenemase by modified Hodge test; metallo- β -lactamase by MIC test strip metallo- β -lactamase (MBL); and bla_{TEM} , bla_{SHV} , bla_{CTX-M} , and bla_{NDM} genes by polymerase chain reaction. **Results:** Among 1275 *E. coli* and *K. pneumoniae* isolated during the study period, 773 (60.6%), 102 (8%), and 28 (2.2%) isolates were detected as ESBL, carbapenemase and MBL producers, respectively. Of the 773 ESBL producers, 635 (82.1%) were found to harbor bla_{CTX-M} genes, and of the 102 carbapenemase producers, 12 (11.8%) were found to harbor bla_{NDM} genes. Gene sequencing of all the 12 NDM-positive isolates revealed bla_{NDM-1} genes. Antibiotic resistance pattern of the ESBL-positive isolates revealed a high degree of co-resistance to noncephalosporin antibiotics such as amoxycylav, co-trimoxazole, chloramphenicol, and fluoroquinolones. **Conclusion:** The present study showed the increasing the prevalence of ESBL including CTX-M variety, carbapenemase production by *E. coli* and *K. pneumoniae* isolates, and spread of NDM-1 in the patients from the rural community of North India.

Keywords: Carbapenemase, community-acquired infections, *Escherichia coli*, extended-spectrum beta-lactamase, *Klebsiella pneumoniae*, New Delhi metallo- β -lactamase-1

Introduction

Extended-spectrum-beta-lactamase (ESBL)-producing *Enterobacteriaceae* (ESBL-E) have been increasingly reported among *Escherichia coli* (ESBL-EC) and *Klebsiella pneumoniae* (ESBL-KP) strains worldwide with a major burden on the developing countries.^[1,2] ESBLs are plasmid-mediated enzymes that can hydrolyze penicillins and third generation cephalosporins and monobactams.^[1] Earlier ESBL-EC and ESBL-KP were considered to be health-care-associated pathogens. However, infections due to these bacteria have been increasingly reported in patients with community-acquired infections (CAIs) with no history of prior

contact with the health-care system.^[1,3] Cefotaxime-Munich (CTX-M) is reported to be the predominant type of ESBL carried by ESBL-EC and ESBL-KP isolated from patients with CAIs globally including India.^[4,5] Most of the reports on ESBLs are confined to patients with hospital-acquired infections (HAIs) in metropolitan cities^[6] with fewer reports from patients with CAIs, while there is hardly any report on prevalence of infections caused by ESBL producers in CAIs among the rural communities.^[7]

Steady and continued increase in the detection of ESBL producers in both HAIs and CAIs imposed clinicians

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to use carbapenems, the last resort drugs to treat serious infections caused by these bacteria leading to the emergence of carbapenemase-producing bacteria, especially carbapenemase-producing *Enterobacteriaceae* and posing a serious public health threat.^[8,9] New Delhi metallo- β -lactamase-1 (NDM-1) is a novel type of plasmid-mediated metallo- β -lactamase (MBL) among carbapenemase that was first reported in 2009 from a Swedish patient of Indian origin in *K. pneumoniae* and *E. coli* isolates. Since then, pathogens harboring this type of MBL gene have been reported across the globe among hospitalized patients and community carriers.^[8-10]

The present study was carried out to determine the prevalence of ESBL, CTX-M-type ESBL (CTX-M-ESBL), carbapenemase, and NDM-1 among *E. coli* and *K. pneumoniae* isolated in various clinical specimens from patients with CAIs and study their antimicrobial resistance (AMR) patterns over a period of 3 years in a multispecialty hospital, in district Gurugram, Haryana, India, catering to the rural population.

Materials and Methods

Study design and setting

This prospective study was conducted over a period of 3 years (July 2015–June 2018) in the department of microbiology of a multispecialty hospital located in the rural belt of Haryana, India, providing health services mostly to the rural community. The 3-year period was subdivided into year 1 (July 2015 to June 2016), year 2 (July 2016 to June 2017), and year 3 (July 2017 to June 2018). The study protocol was approved by the institutional research and ethical committee (SGTU/FMHS/MICRO/341).

Study population and demographic information

The study population included both pediatric (0–18 years) and adult (>18 years) patients with CAIs from rural community residing in nearby villages, attending various clinical departments of the hospital. CAI was defined by a positive bacterial culture obtained from patients attending outpatient departments (OPDs) or within 48 h of hospital admission from hospitalized patients without any history of hospitalization or antibiotic treatment in the past 30 days.^[9,11]

The patient information sheet was provided to each of the study participants and was explained about the purpose of the study. After obtaining consent from the study participants, demographic information was collected from them employing a predesigned proforma.

Bacteriological study of the clinical specimens

Clinical specimens such as blood, urine, pus, wound swabs, sputum, body fluids, and stool received by the microbiology department from various clinical departments were processed as per the standard bacteriological techniques, and only those isolates identified as *E. coli*

and *K. pneumoniae* based on colony morphology, Gram's staining, biochemical reactions, and by Vitek 2 system (BioMerieux, France) were further processed.^[9]

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing (AST) was performed on all the *E. coli* and *K. pneumoniae* isolates by Kirby–Bauer disc-diffusion method, and the results were interpreted as per the Clinical Laboratory Standard Institute (CLSI) guidelines 2018.^[12] The following groups of antibiotic discs, commercially procured from HiMedia, Mumbai, India, were used: ampicillin (10 μ g), amoxicillin-clavulanic acid (20/10 μ g), piperacillin/tazobactam (100/10 μ g), amikacin (30 μ g), gentamicin (10 μ g), ceftazidime (30 μ g), cefotaxime (30 μ g), ceftazidime (30 μ g), ceftriaxone (30 μ g), cefepime (30 μ g), aztreonam (30 μ g), ciprofloxacin (5 μ g), ofloxacin (5'), chloramphenicol (30 μ g), co-trimoxazole (25 μ g), ertapenem (10 μ g), meropenem (10 μ g), imipenem (10 μ g), tetracycline (30 μ g), and tigecycline (15 μ g). In addition, all the urine isolates were tested against the following antibiotics: nalidixic acid (30 μ g) and nitrofurantoin (300 μ g) as per the CLSI guidelines.^[12]

Detection of ESBL production

Double-disc synergy test

All the *E. coli* and *K. pneumoniae* 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 μ g) and ceftazidime plus clavulanic acid (30 μ g plus 10 μ g) discs as the first pair and cefotaxime (30 μ g) and cefotaxime plus clavulanic acid (30 μ g plus 10 μ g) discs as the second pair of antibiotic discs.^[12] *K. pneumoniae* ATCC 700603 and *E. coli* ATCC 25922 were used as positive and negative control strains, respectively.

Detection of *bla*_{TEM}, *bla*_{SHV}, and *bla*_{CTX-M} genes

Polymerase chain reaction (PCR) for the detection of *bla*_{TEM}, *bla*_{SHV}, and *bla*_{CTX-M} was carried out for strains showing ESBL positivity in DDST using the prepublished sequences, namely TEM primers (TEMF ATGAGTATTCAACATTTCCGTG, TEMR TTACCAATGCTTAATCAGTGAG) amplified at 840-bp fragment, while SHV primers (SHVS1 ATTTGTGCGTTCTTTACTCGC, SHVS2 TTTATGGCGTTACCTTTGACC) amplified at the 1051-bp fragment and CTX-M primers (CTX-MF TTTGCGATGTGCAGTACCAGTAA, CTX-MR CGATATCGTTGGTGGTGCCATA) amplified at 544-bp fragment.^[13]

Detection of carbapenemase production

Modified Hodge test

Isolates showing resistance to ertapenem or imipenem or meropenem in AST were subjected to the detection

of carbapenemase production by modified Hodge test (MHT)^[14] using ertapenem (10 µg) disc as described by the CLSI guidelines.^[14] *K. pneumoniae* ATCC BAA-1705 (MHT positive) and *K. pneumoniae* ATCC BAA-1706 (MHT negative) were used for quality control.

Test for metallo-β-lactamase detection

Commercially available MIC test strip MBL (Liofilchem, www.liofilchem.net) was used to carry out presumptive screening of MBL-producing strains.^[9,15] The MBL strip had an imipenem (IMI) gradient at one end (4–256 µg/mL) and gradient of imipenem (1–64 µg/mL) plus a constant level of EDTA (4 µg/mL) at another end (IMD). The MBL production was considered positive when a strain showed MIC ratio of IMI/IMP+EDTA as ≥8.

Detection of *bla*_{NDM} gene

PCR for the detection of *bla*_{NDM} was carried out for MBL-positive stains in MIC test strip MBL using the pre-published sequences as described earlier, 5'-ACCGCCTGGACCGATGACCA-3' and reverse 5'-GCCAAAGTTGGGCGCGTTG-3' which amplified 264 bp fragment of the *bla*_{NDM} gene.^[9,15]

PCR products of all the positive strains were purified by PCR purification kit (QIAGEN, Hilden, Germany), followed by sequencing on ABI PRISM 3130XL sequencer using Big Dye terminator cycle sequencing kit (Perkin Elmer). The derived sequences were aligned with reference sequences from the database of GenBank and accession numbers were obtained after submission to the GenBank.

Statistical analysis

Chi-square test for trend was done to evaluate the statistical significance for change of discrete variables, namely demographic characteristics, the positivity rate for ESBL, CTX-M-ESBL, other ESBL genes, carbapenemase and NDM-1-MBL, and resistance rates for various antimicrobials over the 3-year period. The value of $P < 0.05$ was considered statistically significant.^[16]

Results

A total of 1275 patients with CAIs whose clinical specimens yielded *E. coli* ($n = 849$) and *K. pneumoniae* ($n = 426$) isolates during the 3-year study period were included in the study. Majority of the isolates were obtained from urine 1162/1275 (91.1%), followed by pus 63/1275 (4.9%), swabs 23/1275 (1.8%), blood 11/1275 (0.9%), stool 09/1275 (0.7%), sputum 5/1275 (0.4%), and semen 2/1275 (0.002%). Of 1275 isolates from CAI patients, 773/1275 (60.6%) were found to be ESBL producers comprising ESBL-EC (620 of 849, i.e., 73%) and ESBL-KP (153 of 426, i.e., 35.9%). Most of the ESBL producers detected were those isolated from urine samples, i.e., 693/773 (89.7%) comprising ESBL-EC ($n = 568$) and ESBL-KP ($n = 125$), while the remaining ESBL-producing

isolates, i.e., 80/773 (10.3%) comprising ESBL-EC ($n = 52$) and ESBL-KP ($n = 28$) were obtained from other samples, namely blood, pus, stool, sputum, swabs, and semen.

A significant increase in the prevalence of ESBL production among *E. coli* and *K. pneumoniae* isolated from the study participants was observed over the 3-year study period. Similarly, there was a significant increase in the proportion of CTX-M variety of ESBL among these isolates over the years [Table 1 and Figure 1].

The CAI patients yielding ESBL-EC or ESBL-KP when analyzed in relation to demographic characteristics over the 3-year period showed no difference in the relative proportion of children versus adults or male versus female among them over the 3-year period. There was no difference in ESBL positivity over the years ($P > 0.05$) among students, homemakers, and those individuals having service in a rural area. However, there was a significant decrease in the prevalence of ESBL positivity among individuals engaged in farming out of the total ESBL producers over the years (from 12% in year 1 to 6.5% in year 3). In contrast to this, there was a gradual increase over the years in the proportion of ESBL positivity among residents of rural community serving in urban sector as occupation over the years (7.8% in year 1 to 23.1% in year 3). Among the homemakers, 337 (90.8%) were engaged in collection and storage of freshly passed livestock feces.

A total of 110 (14.2%) ESBL-EC and ESBL-KP were screened positive carbapenemase production using ertapenem disc, of which 102 (13.2%) isolates were confirmed phenotypically by MHT comprising ESBL-EC 56 (54.9%) and ESBL-KP 46 (45.1%). There was a significant increase in the prevalence of carbapenemase production among ESBL-EC and ESBL-KP over the 3-year study period, although the yearly prevalence rate was low. The proportion of MBL variety of carbapenemase among



Figure 1: PCR assay for the detection of *bla*_{CTX-M} gene from double-disc synergy test-positive *Escherichia coli* and *Klebsiella pneumoniae* strains. Lanes: 1 – Marker 100 bp (range 100–3000 bp) DNA ladder, 2 – Positive control, 3 – Positive for CTX-M, 4, 5 – Negative for CTX-M and 6 – Negative-control. CTX-M: Cefotaxime-Munich

the total carbapenemase-producing isolates was higher in years 2 and 3 compared to year 1 [Table 2]. NDM gene was detected in approximately half of the MBL-producing isolates. There was an identity between the partial nucleotide sequence of 12 ESBL-EC isolates and the sequence of the reference strain of *bla*_{NDM-1} [Figure 2]. The accession numbers received for the sequences deposited in GenBank are shown in Table 2.

Antimicrobial resistance

A total of 620 ESBL-EC and 153 ESBL-KP were tested against various antibiotics. There was a gradual increase in resistance rate against most of the antibiotics tested including some of the commonly used ones. However, the increasing trend in resistance was statistically significant for ESBL-EC against the following antibiotics, namely ampicillin, amoxycylav, cefoxitin, cefepime, carbapenems, chloramphenicol, co-trimoxazole, ciprofloxacin, and

ofloxacin, while ESBL-KP showed a significant increase in trend against amoxycylav, ciprofloxacin, and ofloxacin over the years [Tables 3 and 4]. Amikacin was the most effective antibiotic, followed by piperacillin/tazobactam. A high degree of resistance against ampicillin, aztreonam, amoxycylav, and co-trimoxazole was noted among ESBL-EC and ESBL-KP. Furthermore, the resistance rate to amoxycylav was higher among ESBL-KP compared to ESBL-EC, and due to intrinsic resistance to ampicillin, all the ESBL-KPs were resistant to ampicillin. Moderate degree of resistance was noted for cefoxitin (second-generation cephalosporin and cephamycin) and cefepime (fourth-generation cephalosporin) with a slight increase in its resistance rate observed over the years. Furthermore, chloramphenicol, ciprofloxacin, and ofloxacin also showed moderate activity against ESBL-EC and ESBL-KP. Low resistance rate was noted against ertapenem based on AST and thus was

Table 1: Year-wise distribution of extended-spectrum beta-lactamase and Cefotaxime-Munich-extended-spectrum beta-lactamase positivity in *Escherichia coli* and *Klebsiella pneumoniae* isolates from various clinical specimens (July 2015-June 2018)

Bacterial isolates	Resistance prevalence	Years of study			Test of significance (trend)
		Year 1	Year 2	Year 3	
<i>Escherichia coli</i>	Number of isolates	233	268	348	-
	ESBL, n (%)	142 (60.9)	198 (73.9)	280 (80.5)	<0.001
	CTX-M alone, n (%)*	112 (78.9)	160 (80.8)	255 (91.1)	<0.001
	CTX-M with other ESBL genes, n (%)*	12 (8.5)	17 (8.6)	20 (7.1)	NS (0.6)
	Other ESBL genes alone, n (%)*	18 (12.6)	21 (10.6)	5 (1.8)	<0.001
<i>Klebsiella pneumoniae</i>	Number of isolates	110	152	164	-
	ESBL, n (%)	24 (21.8)	54 (35.5)	75 (45.7)	<0.001
	CTX-M alone, n (%) [‡]	12 (50)	37 (68.5)	59 (78.7)	0.008
	CTX-M with other ESBL genes, n (%) [‡]	8 (33.3)	14 (25.9)	11 (14.6)	0.03
	Other ESBL genes alone, n (%) [‡]	4 (16.7)	3 (5.6)	5 (6.7)	NS (0.2)

*Calculated out of total ESBL-producing *Escherichia coli* isolates; [‡]Calculated out of total ESBL-producing KP isolates. NS ($P>0.05$); P values for NS comparisons are shown in parenthesis. Years 1, 2, and 3 indicate periods between July 2015 and June 2016, July 2016 and June 2017, and July 2017 and June 2018, respectively. ESBL: Extended-spectrum beta-lactamase; CTX-M: Cefotaxime-Munich; NS: Not significant

Table 2: Year-wise distribution of carbapenemase and New-Delhi metallo beta-lactamase-1-producing *Escherichia coli* and *Klebsiella pneumoniae* (July 2015–June 2018)

Type of bacterial resistance	ESBL-producing <i>Escherichia coli</i> and <i>Klebsiella pneumoniae</i>			Test of significance (trend)
	Year 1 (n=166)	Year 2 (n=252)	Year (n=355)	
Carbapenemase, n (%)	14 (8.4)	29 (11.5)	59 (16.6)	<0.001
MBL*, n (%)	2 (14.3)	10 (34.5) [‡]	16 (27.1) [‡]	NS (0.6)
NDM-1* n (%)				
n (%)	1 (7.1)	3 (10.3)	8 (13.6)	NS (0.5)
Accession number	MK607951	MK607952, MK607953, MK607954	MK607955, MK607956, MK607957, MK607958, MK607959, MK607960, MK607961, MK607962	

*Calculated out of total cabapenemase producing isolates; [‡]Significantly higher prevalence in year 2 and year 3 ($P<0.05$) compared to year 1. Years 1, 2, and 3 indicate periods between July 2015 and June 2016, July 2016 and June 2017, and July 2017 and June 2018, respectively. NS ($P>0.05$); P values for NS are shown in parenthesis. n indicates the total number of bacterial isolates tested (*Escherichia coli* and KP). ESBL: Extended-spectrum beta-lactamase; NS: Not significant; MBL: Metallo- β -lactamase; NDM-1: New Delhi metallo- β -lactamase-1

Table 3: Year-wise antibiotic resistance profile of extended-spectrum beta-lactamase-producing *Escherichia coli* isolates (July 2015-June 2018)

Antibiotics (potency)	ESBL-EC resistant to various antibiotics			Test of significance (trend)
	Year 1 (n=142), n (%)	Year 2 (n=198), n (%)	Year 3 (n=280), n (%)	
AMP (10 µg)	134 (94.4)	198 (100)	280 (100)	<0.001
AMC (20/10 µg)	96 (67.6)	135 (68.2)	205 (73.2)	<0.001
PIT (100/10 µg)	7 (4.9)	13 (6.6)	26 (9.3)	NS (0.09)
AK (30 µg)	5 (3.5)	10 (5.1)	18 (6.4)	NS (0.2)
GEN (10 µg)	12 (8.5)	22 (11.1)	25 (8.9)	NS (0.98)
CX (30 µg)	50 (35.2)	72 (36.4)	150 (53.6)	<0.001
CPM (30 µg)	80 (56.3)	115 (58.1)	204 (72.9)	<0.001
ETP (10 µg)	6 (4.2)	15 (7.6)	37 (13.2)	0.002
IPM (10 µg)	9 (6.3)	22 (11.1)	42 (15)	0.009
MRP (10 µg)	11 (7.7)	26 (13.1)	46 (16.4)	0.01
C (30 µg)	48 (33.8)	75 (37.9)	130 (46.4)	0.008
COT (25 µg)	110 (77.5)	153 (77.3)	237 (84.6)	0.04
CIP (5 µg)	75 (52.8)	138 (69.7)	204 (72.9)	<0.001
OF (5 µg)	79 (55.6)	138 (69.7)	212 (75.7)	<0.001
TE (30 µg)	92 (64.8)	140 (70.7)	219 (78.2)	0.003

All the isolates were resistant to, ceftriaxone, and cefotaxime and susceptible to tigecycline. Years 1, 2, and 3 indicate periods between July 2015 and June 2016, July 2016 and June 2017, and July 2017 and June 2018, respectively. NS ($P>0.05$); *P* values NS are shown in parenthesis. AMP: Ampicillin; AMC: Amoxycylav; PIT: Piperacillin/tazobactam; AK: Amikacin; GEN: Gentamicin; CX: Cefoxitin; CPM: Cefepime; ETP: Ertapanem; IPM: Imipenem; MRP: Meropenem; C: Chloramphenicol; COT: Co-trimoxazole; CIP: Ciprofloxacin; OF: Ofloxacin; NS: Not significant; ESBL: Extended-spectrum beta-lactamase; TE: Tetracycline

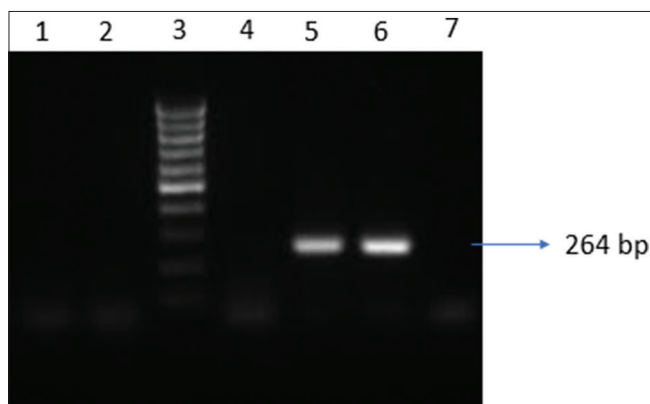


Figure 2: PCR assay for the detection of blaNDM gene for phenotypically MBL-positive *Escherichia coli* and *Klebsiella pneumoniae* strains. Lanes: 1, 2, 4 – Negative for NDM, 3 – Marker 100 bp (range 100–1000 bp) DNA ladder, 5 – Positive for NDM, 6 – Positive control, 7 – Negative control. NDM: New Delhi metallo-β-lactamase, MBL: Metallo-β-lactamase

considered as potential carbapenemase producers as per the CLSI guidelines. A higher resistance rate to this antibiotic was detected in ESBL-KP compared to ESBL-EC. All the ESBL-EC or ESBL-KP isolates were sensitive to tigecycline. Nitrofurantoin and nalidixic acid tested only for ESBL-producing uropathogens ($n = 144$, $n = 225$, and $n = 308$ isolated in year 1, year , and year 3 of the study, respectively) that showed resistance rate against nitrofurantoin ranging between 34% and 44.8% with higher resistance rate recorded against nalidixic acid, i.e., 66.7%–85.7% (data not shown in table). Higher resistance to antibiotics such as aminoglycosides, tetracycline, and co-trimoxazole, which are commonly used in veterinary

sectors, was found among homemakers and farmers compared to other groups of individuals.

Discussion

Increasing prevalence of CAIs due to ESBL-producing *E. coli* and *K. pneumoniae*, particularly of the CTX-M genotype, has been reported worldwide including India.^[4,5,17] Prior antibiotic use, history of hospitalization, underlying severe comorbid illnesses, association with health care, and old age are some of the risk factors reported to be responsible for developing CAIs due to ESBL-EC and ESBL-KP.^[5,18] A gradual decrease in the proportion of individuals engaged in farming could be due to gradual urbanization leading to relative increase of other categories of individuals at risk for the acquisition of ESBL,^[19] as evident in our study which showed a gradual increase in positivity rate for ESBL recorded among individuals engaged in service in the urban sector. Comparison of census data from the Government of India between 2001 and 2011 showed that there is an overall gradual decrease in individuals engaged in farming.^[20] Consumption of unhygienic food and water could be risk factors for the acquisition of ESBL-producing bacteria among those serving in the urban sector where the reported burden of environmental contamination rate with antimicrobial-resistant bacteria is high.^[21]

The present study conducted in a rural population showed an increasing trend in an overall prevalence rate of ESBL positivity among *E. coli* and *K. pneumoniae*. Compared to our findings, similar studies on hospital attending patients with CAI reported a lower prevalence rate of ESBL

Table 4: Yearwise antibiotic resistance profile of extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* isolates (July 2015-June 2018)

Antibiotics (potency)	ESBL-KP resistant to various antibiotics			Test of significance (Trend)
	Year 1 (n=24), n (%)	Year 2 (n=54), n (%)	Year 3 (n=75), n (%)	
AMC (20/10 µg)	20 (83.3)	48 (88.9)	72 (96)	0.04
PIT (100/10 µg)	3 (12.5)	11 (20.4)	16 (21.3)	NS (0.4)
AK (30 µg)	3 (12.5)	8 (14.8)	10 (13.3)	NS (1)
GEN (10 µg)	6 (25)	16 (29.6)	20 (26.7)	NS (1)
CX (30 µg)	10 (41.7)	24 (44.4)	39 (52)	NS (0.3)
CPM (30 µg)	12 (50)	32 (59.3)	51 (68)	NS (0.1)
ETP (10 µg)	8 (33.3)	17 (31.5)	27 (36)	NS (0.7)
IPM (10 µg)	12 (50)	18 (33.3)	32 (42.7)	NS (0.9)
MRP (10 µg)	12 (50)	21 (38.9)	33 (44)	NS (0.8)
C (30 µg)	10 (41.7)	28 (51.9)	40 (53.3)	NS (0.2)
COT (25 µg)	18 (75)	43 (79.6)	64 (85.3)	NS (0.2)
CIP (5 µg)	10 (41.7)	35 (64.8)	52 (69.3)	0.03
OF (5 µg)	10 (41.7)	36 (66.7)	54 (72)	0.01
TE (30 µg)	15 (62.5)	39 (72.2)	61 (81.3)	0.05

All the isolates were resistant to ampicillin, ceftriaxone, and cefotaxime and susceptible to tigecycline. Year 1, 2 and 3 indicate periods between July 2015 and June 2016, July 2016 and June 2017, and July 2017 and June 2018, respectively. NS ($P > 0.05$); P values NS are shown in parenthesis. AMC: Amoxycylav; PIT: Piperacillin/tazobactam; AK: Amikacin; GEN: Gentamicin; CX: Cefoxitin; CPM: Cefepime; ETP: Ertapanem; IPM: Imipenem; MRP: Meropenem; C: Chloramphenicol; COT: Co-trimoxazole; CIP: Ciprofloxacin; OF: Ofloxacin; NS: Not significant; ESBL: Extended-spectrum beta-lactamase; TE: Tetracycline

positivity from developed (2.2%–15%)^[22,23] and other developing countries (1.3%–29.1%),^[24-26] although these reports were characterized by wide variations. However, there is hardly any study on the prevalence of ESBL positivity among *E. coli* and *K. pneumoniae* from the rural population with CAIs from India except one study from rural Rajasthan which included both OPD and inpatient department patients that reported overall prevalence of 62% ESBL positivity among *E. coli* and *K. pneumoniae*.^[7] In our study, majority (89.7%) of the ESBL strains were isolated from urine samples. *E. coli* and *K. pneumoniae* are the two common agents reported to be associated with infections among patients with community-acquired urinary tract infections (CA-UTI) in India with a prevalence rate between 37.5% and % 69.2%.^[4,27] Studies in tertiary care hospitals from Southern India, i.e., Kerala and Tamil Nadu, reported the prevalence of ESBL-EC and/or ESBL-KP, as 37.5% and 40%, respectively, among children with CA-UTI.^[4,28] One of these two studies^[4] was a prospective epidemiological surveillance study in nature (over 3 years) on ESBL-EC among the pediatric population in the community settings in India. Another study from Southern India reported a higher prevalence of ESBL, i.e., 69.2% among isolates from patients of different age groups with UTI attending OPD representing CAIs.^[27] A retrospective case-control study from the USA on CA-UTI due to ESBL-EC during 2012 and 2016 reported the proportion of ESBL positivity to be 7%–15% over the 5-year study period.^[23]

It has been reported that with the increase in the prevalence of ESBL positivity over the years, there has been a shift in

the prevalent ESBL types from TEM and SHV to CTX-M type, as a result of which CTX-M type has now become the predominant ESBL type found in the community isolates.^[1,5] This is reflected in the findings of the present study showing the high prevalence of CTX-M type of ESBL detected among ESBL-EC and ESBL-KP isolated from patients with CAI. This finding is comparable to those reported in the community of other countries, namely the Republic of Korea (100%) and Morocco (85.7%).^[5,24] Studies conducted in different parts of India also reported a high prevalence of CTX-M-type ESBL, as 82.5%–93.7% among ESBL-EC and ESBL-KP isolated from clinical specimens, which is comparable to our findings.^[4,27]

In the present study, perceptible prevalence of carbapenemase production among ESBL-EC and ESBL-KP isolated from patients with CAI was noted (overall prevalence of 13.2%) during the 3-year study period with increasing prevalence over the years indicating the gradually increasing burden of carbapenemase-mediated AMR in the rural community of Northern India. Studies in urban tertiary care hospitals of Northern Indian states, namely Delhi and Haryana reported a high prevalence of carbapenemase-producing *E. coli* detected by phenotypic methods as 65.1% and 44%, respectively.^[29,30] However, there are few studies on the magnitude of the problem of CRE carbapenem resistance in the rural population reported from India. A pilot study conducted for a period of 1 year (January–December 2015) by the present laboratory reported lower prevalence of carbapenemase-producing *E. coli* and *K. pneumoniae*, i.e., 7.8%^[9] compared to studies from rural Southern India reporting 19.4%–22.1%

carbapenemase production among similar isolates.^[31,32] In the present study, the sequence analysis of 12 *bla*_{NDM} harboring isolates was found to be all positive for *bla*_{NDM-1} variety, while studies conducted in urban cities, namely Delhi and Aligarh, Uttar Pradesh, India, reported other variants of *bla*_{NDM} among *E. coli* isolates, i.e., *bla*_{NDM-4}, *bla*_{NDM-5}, *bla*_{NDM-7}, and *bla*_{NDM-8}.^[15,33] There is hardly any report of *bla*_{NDM-1} detection in CAI patients from rural community from India. A pilot study conducted earlier by our laboratory in the year 2015 reported the prevalence of *bla*_{NDM-1} to be 1.7% among *E. coli* and *K. pneumoniae* isolates from patients with CAIs.^[9]

In the present study, ESBL-EC and ESBL-KP isolates from CAIs showed a high degree of multidrug resistance against some of the commonly used antimicrobials, namely ampicillin, amoxycylav, cephalosporins, co-trimoxazole, fluoroquinolones, and nalidixic acid while moderate degree of resistance was noted for cefoxitin, chloramphenicol, and nitrofurantoin. A study from South India also reported a higher degree of resistance ranging between 55.6% and 98.5% to ampicillin, cephalosporins, ciprofloxacin (a member of fluoroquinolone group), and co-trimoxazole among ESBL-EC and ESBL-KP from CAI.^[4] Comparable findings were shown in studies from developed and developing countries where ESBL-EC isolated from CA-UTI patients showed higher degree of resistance ranging from 69.6% to 100% to most of the antibiotics, namely ampicillin, amoxycylav, cephalosporins, and ciprofloxacin.^[22,24] In the present study, a low degree of resistance was noted for aminoglycosides, namely amikacin, gentamicin, and piperacillin/tazobactam for both ESBL-EC and ESBL-KP. Studies from India and other countries also reported lower degree of resistance among CAI-ESBL producers against aminoglycosides except gentamicin, resistance rate ranging between 4.2% and 23.5%.^[24-26] Lower resistance rate to aminoglycosides could be due to their infrequent use because of injectable route of administration as well as their toxicity.^[34] However, paradoxically aminoglycoside resistance was found to be higher among homemakers and farmers in our study, possibly due to close contact with livestock and their feces coupled with unregulated use of these injectable antibiotics in veterinary sectors leading to increase in its resistance among AMR bacteria in livestock. Despite amikacin and gentamicin belonging to the same group of antibiotics, i.e., aminoglycosides, a difference in resistance rate between these antibiotics was recorded in our study that could be due to structural differences and resistance of amikacin to common enzymes that inactivate gentamicin.^[35]

Conclusion

The present study highlights an increasing trend of ESBL and carbapenemase production among *E. coli* and *K. pneumoniae* isolates from patients with CAIs and spread of NDM-1 producing strains in the rural area of Haryana,

India. This worrisome spread of ESBL and NDM-1 producers in rural community deserves an attention to promote regular antimicrobial surveillance which is the need of the hour to monitor changes in the AMR pattern. In addition, the data generated on CAI patients from rural communities are expected to complement the data on hospitalized patients from the rural community, helping in the assessment of the true magnitude of antimicrobial resistance AMR in a rural population.

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Conflicts of interest

There are no conflicts of interest.

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