# Molecular characterization of extended-spectrum β-lactamases among clinical isolates of *Escherichia coli* & *Klebsiella pneumoniae*: A multi-centric study from tertiary care hospitals in India

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***Background & objectives***: The increasing prevalence of extended-spectrum β-lactamases (ESBLs) has abated therapeutic options worldwide. This study was undertaken to investigate the molecular profile and resistance patterns of ESBLs among clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae* at four tertiary care centres in India.

***Methods***: Clinical isolates of *E. coli* and *K. pneumoniae* were collected from the All India Institute of Medical Sciences (AIIMS), New Delhi; the Jawaharlal Institute of Postgraduate Medical Education & Research (JIPMER), Puducherry; Postgraduate Institute of Medical Education & Research (PGIMER), Chandigarh and Christian Medical College (CMC), Vellore, over one and a half year period. Antimicrobial susceptibility was determined by Kirby-Bauer disc diffusion method. ESBLs were confirmed phenotypically, and multiplex PCR was performed to identify genes for β-lactamases ($bla_{TEM}$, $bla_{SHV}$, $bla_{OXA-1}$, $bla_{CTXM-1}$, $bla_{CTXM-2}$, $bla_{CTXM-9}$ and $bla_{CTXM-15}$).

***Results***: Among 341 *E. coli* isolates collected during the study period, 171 (50%) harboured $bla_{TEM}$, 145 (43%) $bla_{OXA-1}$, 70 (21%) $bla_{CTXM-1}$, 19 (6%) $bla_{SHV}$ and four (1%) harboured $bla_{CTXM-2}$. Phenotypically, combined disc test detected ESBL production in 98/298 (33%) *E. coli*. Among 304 *K. pneumoniae* isolates, 115 (38%), 89 (29%), 83 (27%), 64 (21%) and two (0.6%) harboured $bla_{TEM}$, $bla_{OXA-1}$, $bla_{CTXM-1}$, $bla_{SHV}$ and $bla_{CTXM-2}$, respectively. Combined disc test (CDT) detected ESBL production in 42 per cent *K. pneumoniae*. Most of the $bla_{CTXM-1}$ positive isolates were also $bla_{CTXM-15}$ positive. The carbapenem susceptibility ranged from 56 to 88 per cent for *E. coli* and from 20 to 61 per cent for *K. pneumoniae*. Antibiotic sensitivity patterns showed that colistin (CST) was the most sensitive drug for both *E. coli* (271/274, 99%) and *K. pneumoniae* (229/234, 98%).

*Interpretation & conclusions*: The prevalence of ESBL among four study centres varied, and $bla_{TEM}$, $bla_{OXA-1}$ and $bla_{CTXM-15}$ were the most common genotypes in *E. coli* and *K. pneumoniae* isolates in India. The growing carbapenem resistance and emerging colistin resistance warrant the judicious use of these antimicrobials.

Extended-spectrum β-lactamases (ESBLs) are often plasmid-encoded β-lactamases that confer resistance to penicillins, narrow- and extended-spectrum cephalosporins and aztreonam. ESBL-producing organisms are also often resistant to quinolones, trimethoprim-sulphamethoxazole and aminoglycosides. Organisms harbouring these enzymes, thus, become multidrug resistant (MDR) and cause nosocomially-acquired infections[1]. ESBLs can be classified into three main types, designated as TEM, SHV and CTX-M. The CTX-M type of ESBL can further be classified into three groups: CTX-M-1, CTX-M-2 and CTX-M-9. Previously, ESBLs were generally found in *Klebsiella pneumoniae* (TEM or SHV types) and most of the isolates were from nosocomial infections[1]. The prevalence and molecular profile of the ESBL-producing *Escherichia coli* is substantially different from that of ESBL-producing *K. pneumoniae*[2]. Further, ESBL-producing *Enterobacteriaceae* isolates have been reported from community-associated infections leading to higher mortality and treatment cost than their non-ESBL-producing counterparts[3]. There is, thus, a need for geographical surveillance of ESBL production to guide appropriate antimicrobial therapy. The challenge is more in a country like India where bacterial disease burden is highest in the world[4]. Almost all the known generations of β-lactamases have been found to be circulating in India, and particularly the tertiary care hospitals encounter massive antimicrobial resistance (AMR) with prevalence ranging from 6 to 86 per cent among *E. coli* and *K. pneumoniae* isolates[2].

The Indian Council of Medical Research (ICMR) initiated a surveillance study in 2013 across the four different centres from India, including AIIMS (All India Institute of Medical Sciences, New Delhi) and PGIMER (Postgraduate Institute of Medical Education & Research, Chandigarh) from north India and JIPMER (Jawaharlal Institute of Postgraduate Medical Education & Research, Puducherry) and CMC (Christian Medical College, Vellore) from south India, to analyze the prevalence of ESBL-producing *E. coli*

and *K. pneumoniae* and to compare the molecular profile of ESBL genes isolated from these four centres. Antimicrobial susceptibility was also carried out on all isolates to determine the contemporary susceptibility profile.

## Material & Methods

*Study centres and bacterial isolates*: Four study centres (AIIMS, New Delhi; JIPMER, Puducherry; PGIMER, Chandigarh and CMC, Vellore) participated in this study. A total of 60 non-duplicate isolates from each centre, 30 consecutive isolates each of *E. coli* and *K. pneumoniae*, every six months, were included. A total of 341 *E. coli* and 304 *K. pneumoniae* isolates collected during October 2014 to March 2016 from different clinical specimens (blood, pus, sputum and body fluids) from the four centres were studied. All the isolates were stored at -80°C.

*Identification of isolates to species level*: All the isolates were subjected to MALDI-TOF MS (matrix-assisted laser desorption/ionization time of flight mass spectrometry) (Bruker Daltonics GmbH, Bremen, Germany) for identification to species level following the manufacturer's recommendations.

*Antimicrobial susceptibility testing*: Antimicrobial susceptibility was determined by Kirby-Bauer disc diffusion (KBDD) in accordance with the Clinical and Laboratory Standards Institute (CLSI)[5] guidelines using discs from HiMedia Laboratories (Mumbai) against amikacin (AMK, 30 μg), ciprofloxacin (CIP, 5 μg), chloramphenicol (CHL, 30 μg), tetracycline (TET, 30 μg), polymyxin B (PB, 300 unit), cefoperazone-sulbactam (CFS-SUL, 75/30 μg), piperacillin-tazobactam (PTZ, 100/10 μg), cefotaxime (CTZ, 30 μg), ceftazidime (CAZ, 30 μg), cefepime (CEP, 30 μg), imipenem (IMP, 10 μg), meropenem (MEM, 30 μg), cefpodoxime (30 μg) and colistin (CST, 10 μg). Quality control for disc diffusion assay was performed with *E. coli* (ATCC 25922) and *K. pneumoniae* (ATCC 700603) standard strains.

*Phenotypic detection of ESBLs*: The combined disc test (CDT) was used to detect the presence of ESBL phenotypically[6]. Briefly, discs containing 30 μg of CTZ or CAZ, with and without 10 μg of clavulanic acid (HiMedia), were placed independently, 30 mm apart (centre to centre) on a lawn culture of 0.5 McFarland opacity of the test isolate on a Mueller-Hinton agar plate and incubated for 18-24 h at 35°C. Isolates were considered ESBL positive if the inhibition zone measured around one of the combination discs was at least 5 mm larger than that of the corresponding cephalosporin disc.

*Molecular analysis of ESBLs genes*: Bacterial DNA was isolated by heat lysis method[7]. Briefly, five to six colonies of *E. coli* or *K. pneumoniae* were suspended in 1 ml of sterile distilled water in a 1.5 ml microcentrifuge tube, heated to 98°C for 20 min and centrifuged at 12,000 rpm. The supernatant was checked for concentration and purity using the spectrophotometer (NanoDrop-ND1000, Thermo Scientific, USA) and was subjected to multiplex PCR protocol for the rapid detection of ESBL ($bla_{TEM}$, $bla_{SHV}$, $bla_{CTX-M}$ and $bla_{OXA}$), as described previously[8].

## Results

*Phenotypic distribution of ESBLs among E. coli and K. pneumoniae*: A total of 645 consecutive non-duplicate clinical isolates *E. coli* (341) and *K. pneumoniae* (304) from four medical centres [PGIMER, Chandigarh (131, 64 *E. coli* and 67 *K. pneumoniae*); AIIMS, New Delhi (101, 50 *E. coli* and 51 *K. pneumoniae*); CMC, Vellore (185, 103 *E. coli* and 82 *K. pneumoniae*); and JIPMER, Puducherry (228, 124 *E. coli* and 104 *K. pneumoniae*)] were included in the study. CLSI phenotypic test for ESBL production detected ESBL in 33 per cent (98/298) *E. coli* and 42 per cent (102/245) *K. pneumoniae*. Phenotypic ESBL production among *E. coli* and *K. pneumoniae* was 16 and 30 per cent, respectively, in JIPMER, Puducherry; 38 and 33 per cent, respectively, in AIIMS, New Delhi; 37 and 53 per cent, respectively, in CMC, Vellore; and 55 and 60 per cent, respectively, in PGIMER, Chandigarh.

*Genotypic profile of ESBLs among E. coli and K. pneumoniae*: Among *E. coli* isolates, $bla_{TEM}$ was the most common ESBL at AIIMS, New Delhi, and JIPMER, Puducherry, being present in 50 and 66 per cent isolates, respectively. The $bla_{OXA-1}$ was the most prevalent ESBL at PGIMER, Chandigarh and CMC, Vellore, being present in 44 and 58 per cent *E. coli* isolates, respectively. At least 8, 12.5, 5.6 and 13 per cent of *E. coli* isolates at AIIMS, New Delhi; PGIMER, Chandigarh; CMC, Vellore and JIPMER, Puducherry, respectively, harboured one of the tested ESBL genes. Among *K. pneumoniae* isolates, $bla_{TEM}$ was the most prevalent ESBL at PGIMER, Chandigarh, and JIPMER, Puducherry, and was detected in 73 and 32 per cent, respectively. The most common ESBL among *K. pneumoniae* at AIIMS, New Delhi, was $bla_{CTX-M1}$ (55%) and at CMC, Vellore, was $bla_{OXA-1}$ (37%). A minimum of 12, 16, 18 and 2 per cent of *K. pneumoniae* isolates at AIIMS, New Delhi; PGIMER, Chandigarh; CMC, Vellore; and JIPMER, Puducherry, respectively, harboured one of the tested ESBL genes. In the present study, $bla_{CTX-M-15}$ was the most common $bla_{CTX-M1}$ type ESBL in *E. coli* (59/64, 92.2%) and *K. pneumoniae* (73/83, 87.9%). Distribution of $bla_{CTXM-15}$ among $bla_{CTXM1}$ positive isolates of *E. coli* and *K. pneumoniae* is given in Table I.

**Table I.** Distribution of CTXM15 among CTXM1-positive isolates of *Escherichia coli* and *Klebsiella pneumoniae* at four centres of India

| Centre name | Organism | CTXM1 | CTXM15 | Per cent positive |
|---|---|---|---|---|
| AIIMS, New Delhi | *E. coli* | 15 | 15 | 100 |
| | *K. pneumoniae* | 28 | 25 | 89.2 |
| PGIMER, Chandigarh | *E. coli* | 8 | 8 | 100 |
| | *K. pneumoniae* | 36 | 35 | 97.2 |
| CMC, Vellore | *E. coli* | 25 | 22 | 88 |
| | *K. pneumoniae* | 15 | 12 | 80 |
| JIPMER, Puducherry | *E. coli* | 16 | 14 | 87.5 |
| | *K. pneumoniae* | 4 | 1 | 25 |

AIIMS, All India Institute of Medical Sciences; CMC, Christian Medical College; JIPMER, Jawaharlal Institute of Postgraduate Medical Education & Research; PGIMER, Postgraduate Institute of Medical Education & Research

Table II. Antibiotic susceptibility patterns at different study centres against *Escherichia coli* and *Klebsiella pneumoniae*

| Centre | Organism | AMK (%) | CTX (%) | CAZ (%) | FEP (%) | TZP (%) | IMP (%) | MER (%) | CFP-SUL (%) | CST (%) | TET (%) | ERT (%) | CIP (%) | CFD (%) | CHL (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AIIMS, New Delhi | *E. coli* | 90 (45/50) | 20 (10/50) | 22 (11/50) | 24 (12/50) | 64 (32/50) | 88 (44/50) | 56 (28/50) | 60 (30/50) | 100 (50/50) | 72 (36/50) | 46 (12/26) | 44 (22/50) | ND | 79 (19/24) |
| | *K. pneumoniae* | 55 (28/51) | 12 (6/51) | 12 (6/51) | 12 (6/51) | 29 (15/51) | 57 (29/51) | 41 (21/51) | 33 (17/51) | 100 (51/51) | 55 (28/51) | ND | 33 (17/51) | ND | 49 (25/51) |
| PGIMER, Chandigarh | *E. coli* | 78 (50/64) | 13 (8/64) | 9 (6/64) | 11 (7/64) | 44 (28/64) | 67 (43/64) | 56 (23/41) | 49 (20/14) | 100 (40/40) | 32 (11/34) | ND | 12 (5/41) | ND | 80 (33/41) |
| | *K. pneumoniae* | 39 (26/67) | 15 (10/67) | 13 (9/67) | 15 (10/67) | 25 (17/67) | 49 (33/67) | 20 (8/40) | 23 (9/40) | 100 (38/38) | 76 (26/34) | ND | 20 (8/40) | 12 (3/25) | 78 (31/40) |
| CMC, Vellore | *E. coli* | 82 (73/89) | 20 (18/89) | 22 (20/89) | 26 (23/89) | 56 50/89 | 71 (63/89) | 69 (61/89) | 60 (53/89) | 100 (89/89) | 36 (32/89) | ND | 24 (21/89) | 14 (6/42) | 72 (48/67) |
| | *K. pneumoniae* | 62 (51/82) | 41 (34/82) | 40 (33/82) | 40 (33/82) | 44 36/82 | 55 (45/82) | 56 (46/82) | 48 (39/82) | 94 (77/82) | 73 (60/82) | 70 (23/33) | 49 (40/82) | 24 (8/33) | 58 (34/59) |
| JIPMER, Puducherry | *E. coli* | 46 (58/125) | 47 (59/125) | 48 (60/125) | 62 (77/125) | 65 (81/125) | 66 (82/125) | 66 (82/125) | 64 (61/95) | 97 (92/95) | 57 (54/95) | 72 (39/54) | 43 (54/125) | 50 (15/30) | 89 (63/71) |
| | *K. pneumoniae* | 71 (67/95) | 56 (53/95) | 51 (48/95) | 48 (46/95) | 54 (51/95) | 59 (56/95) | 61 (58/95) | 65 (41/63) | 100 (63/63) | 60 (38/63) | 50 (15/30) | 49 (47/95) | 52 (32/62) | 94 (59/63) |

Results presented in the form of per cent sensitivity (number of sensitive/total number of isolates). AMK, amikacin; CTX, cefotaxime; CAZ, ceftazidime; FEP, cefepime; TZP, piperacillin-tazobactam; IMP, imipenem; MER, meropenem; CFS-SUL, cefoperazone-sulbactam; TET, tetracycline; ERT, ertapene; CIP, ciprofloxacin; CFD, cefpodoxime; CHL, chloramphenicol; CST, colistin; ND, not done (tested); AIIMS, All India Institute of Medical Sciences; CMC, Christian Medical College; JIPMER, Jawaharlal Institute of Postgraduate Medical Education & Research; PGIMER, Postgraduate Institute of Medical Education & Research

*Antimicrobial susceptibility pattern ESBL-producing and-non-producing isolates of E. coli and K. pneumoniae*: Overall, CST showed the highest susceptibility with 99 per cent (271/274) ESBL-producing *E. coli* and 98 per cent (229/234) ESBL-producing *K. pneumoniae* being susceptible to it (Table II). Susceptibility to CST varied at the four centres. Chloramphenicol showed susceptibility against both *E. coli* and *K. pneumoniae* while AMK showed better susceptibility against *E. coli* than *K. pneumoniae* (Table II). Among the carbapenems, IMP susceptibility ranged from 67 to 88 per cent for *E. coli* and from 49 to 59 per cent for *K. pneumoniae*. Meropenem susceptibility ranged from 56 to 69 per cent for *E. coli* and from 20 to 61 per cent for *K. pneumoniae*. Overall, IMP showed higher susceptibility than MEM for both the organisms at all centres, except JIPMER, Puducherry, where it was similar for the two drugs. ESBL-producing isolates showed maximum resistance against cefpodoxime (although done at two centres only) (93% for *E. coli* and 94% for *K. pneumoniae*) and CTZ (59% for *E. coli* and 87% for *K. pneumoniae*). ESBL-non-producing *E. coli* and *K. pneumoniae* isolates showed maximum resistance against CAZ (65 and 60%, respectively) (Table III). Among the carbapenems, ESBL-non-producing *E. coli* had higher rates of resistance (38% for IMP and 48% for MEM) than their ESBL-producing counterparts. Carbapenem resistance in *K. pneumoniae* ranged from 44 to 54 per cent for both the drugs, irrespective of ESBL production (Table III).

## Discussion

In the third quarter of 2013, ICMR launched a surveillance network for phenotypic and genotypic characterization of antimicrobial resistance across four tertiary care centres in India to have a collective data on molecular profile of ESBL-producing isolates and their antimicrobial susceptibility pattern. Although more than 300 ESBL variants have been reported[9], $bla_{TEM}$ gene was the most common ESBL in both *E. coli* and *K. pneumoniae* in the present study followed by $bla_{OXA}$ and $bla_{CTXM-1}$. Previous report from North East India showed high rate of $bla_{SHV}$ (63.4%) followed by $bla_{CTXM}$ (60.86%) and $bla_{TEM}$ (54.3%)[10]. Goyal *et al*[11] also reported high rate of isolation of $bla_{CTXM}$ (85.4%) among ESBL isolates of north India. A study from Central India (Rajasthan) on 20 ESBL-producing *E. coli* isolates reported a high rate of $bla_{CTXM}$ (80%) followed by $bla_{TEM}$ (60%) and $bla_{SHV}$ (55%)[12]. In the present study, among north Indian *K. pneumoniae*

**Table III.** Comparison of antibiotic resistance profile of extended-spectrum β-lactamases (ESBL)-producing and non-producing *Escherichia coli* and *Klebsiella pneumoniae* identified by combined disc test method

| Antibiotics | *E. coli* | | *K. pneumoniae* | |
|---|---|---|---|---|
| | ESBLs, % (n/N)* | Non-ESBLs, % (n/N) | ESBLs, % (n/N) | Non-ESBLs, % (n/N) |
| AMK | 5 (8/167) | 12 (75/202) | 57 (56/98) | 43 (54/126) |
| CTX | 59 (99/167) | 61 (123/202) | 87 (85/98) | 57 (72/126) |
| CAZ | 58 (97/167) | 65 (132/202) | 85 (83/98) | 60 (76/126) |
| CIP | 61 (56/92) | 58 (111/191) | 59 (58/98) | 45 (57/126) |
| CFD | 93 (13/14) | 59 (32/54) | 94 (50/53) | 45 (25/56) |
| CHL | 24 (15/62) | 13 (12/90) | 14 (9/64) | 21 (16/76) |
| CFS-SUL | 14 (13/92) | 46 (49/107) | 73 (66/90) | 48 (57/119) |
| CST | 2 (2/88) | 0 | 0 | 7 (8/119) |
| ERT | 34 (10/29) | 48 (29/61) | 21 (11/53) | 43 (27/63) |
| FEP | 55 (92/167) | 64 (129/202) | 85 (83/98) | 57 (72/126) |
| IMP | 4 (7/167) | 34 (69/202) | 44 (43/98) | 48 (60/126) |
| MER | 15 (14/92) | 48 (92/193) | 53 (52/98) | 54 (68/126) |
| TET | 66 (58/88) | 50 (52/103) | 33 (29/89) | 15 (18/119) |
| TZP | 11 (18/167) | 53 (108/202) | 66 (65/98) | 51 (64/126) |

*% (n/N), per cent (number of resistant isolate/total number of isolate). AMK, amikacin; CTX, cefotaxime; CAZ, ceftazidime; FEP, Cefepime; TZP, piperacillin-tazobactam; IMP, imipenem; MER, meropenem, CFS-SUL, cefoperazone-sulbactam, TET, tetracycline; ERT, ertapenem; CIP, ciprofloxacin; CFD, cefpodoxime; CHL, chloramphenicol; CST, colistin

isolates, $bla_{TEM}$ (73%) and $bla_{CTXM-1}$ (55%) were the predominant ESBL genes in PGIMER and AIIMS, respectively. The finding of $bla_{TEM}$ was in accordance with that of an earlier study which reported 75 per cent $bla_{TEM}$ among ESBL-producing *K. pneumoniae* isolates from Lucknow[13].

Among the centres of south India, the predominant ESBL in *E. coli* was $bla_{OXA}$ (58%) at CMC, Vellore, and $bla_{TEM}$ (66%) at JIPMER, Puducherry. Our findings differed from those reported earlier from CMC, Vellore, wherein higher rates of $bla_{TEM}$ (75%), $bla_{SHV}$ (66%) and $bla_{CTXM}$ (71%) were noted among 138 ESBL-producing *E. coli*[14]. With respect to *K. pneumoniae* isolates from south India, the two prevalent ESBLs were $bla_{TEM}$ (24 and 32%) and $bla_{OXA-1}$ (37 and 14%) at the two centres. At JIPMER, Puducherry, no $bla_{SHV}$ gene was noted among *K. pneumoniae* isolates. In a previous study from CMC, Vellore[14], $bla_{TEM}$ was reported in 67 per cent isolates. These differences in the prevalence of different ESBL genes among *E. coli* and *K. pneumoniae* may be due to differences in sample size, time period and geographical location of the studies.

In the present study, a high prevalence of $bla_{CTX-M-15}$ gene among $bla_{CTXM-1}$ positive *E. coli* (97.6%) and *K. pneumoniae* isolates (87.9%) was observed. Ensor

et al[15] studied 47 isolates of *Klebsiella* spp. and *E. coli* collected from six study centres using PCR and DNA sequencing, and 37 were found to carry $bla_{CTX-M-15}$ only. Other $bla_{CTXM}$ were lacking in their study[15]. $bla_{CTX-M-15}$ was also identified from two of the *K. pneumoniae* isolates collected during 2002-2003 in Coimbatore in south India[16]. $bla_{CTX-M-15}$ appeared to be the predominant $bla_{CTXM-1}$ type in India whereas $bla_{CTXM-14}$ in other Asian countries[17].

The presence of multiple ESBL genes in a single isolate has been reported[18] and the same has been reflected in the present study. However, the combination of ESBL genes differed between study centres. This highlights the emerging complexity of antibacterial resistance repertoire and warrants further studies.

The rate of ESBL in the major tertiary care hospitals of India has been reported to be as high as 87 per cent[19]. Interestingly, CDT in the present study detected ESBL in only 33 per cent of *E. coli* and 42 per cent of *K. pneumoniae* isolates. Highest prevalence of ESBL was noted at PGIMER, Chandigarh, followed by AIIMS, New Delhi; CMC, Vellore; and the least from JIPMER, Puducherry. Similarly, among *K. pneumoniae*, highest numbers were observed at PGIMER, Chandigarh, followed by CMC, Vellore; AIIMS, New Delhi; and the least in JIPMER, Puducherry. Higher

rate of ESBL among *K. pneumoniae* (52.7%) than *E. coli* (46.43%) has been reported from north India earlier also[20]. The documented rate from Mumbai, however, differed wherein ESBL-producing *E. coli* outnumbered ESBL-producing *K. pneumoniae*[21]. The variation in the prevalence among the four centres in the present study could be attributed to factors such as antibiotic policy, antibiotic stewardship practices, carriage rate among the hospital personnel and the type of disinfection and other infection control practices being followed especially in the intensive care units (ICU)[22].

In our study, 40.8 per cent of PCR-positive ESBL was phenotypically undetectable by CDT method. Yazdi *et al*[23] from Turkey also reported that 13.8 per cent of *E. coli* isolates were ESBL positive by PCR and negative by CDT. Phenotypic identification of ESBL is based on the inhibition of enzyme by clavulanic acid, and if inhibitory action of clavulanic acid is masked by co-existence of multiple enzymes, CDT may not be able to detect ESBL phenotypically[24]. In addition, co-existence of AmpC type enzymes in ESBL producers may alter the pores of the cell membranes, thereby reducing the affinity for β-lactamase inhibitors for enzymes such as TEM and SHV[25]. Production of different types of β-lactamases (TEM, SHV, CTX-M and OXA) by the same microorganism can lead to erroneous phenotypic conclusions[26]. Tofteland *et al*[6] reported that hyper-production of SHV-1 or SHV-11 could also be the cause of failure in ESBL detection when the combined disc method was used. In the present study, eight per cent phenotypically ESBL producers and genotypically negative may be explained by presence of ESBL genes other than those targeted.

Aminoglycosides are being used relatively infrequently not only in the community practice (due to injectable route) but also in hospitalized patients (due to associated toxicity and availability of safer drugs), showing lesser resistance rates as previously reported[27]. In this study, all *E. coli* isolates showed moderate-to-high resistance to cephalosporins, irrespective of ESBL production. On the contrary, in *K. pneumoniae*, cephalosporin resistance was higher in ESBL-producing than ESBL-non-producing isolates. A possible explanation could be the expression of ESBL genes which was more predominant in *K. pneumoniae* than *E. coli*. In this study, PTZ and CFS-SUL showed less resistance towards ESBL-producing *E. coli* and *K. pneumoniae* than towards ESBL-non-producing *E. coli* and *K. pneumoniae*. Low resistance may be due to lesser use of these antibiotics for the treatment of community-acquired infections.

The results of carbapenem susceptibility in this study showed a higher resistance for MEM compared to IMP in both types of isolates (ESBL or non ESBL). Gupta *et al*[28] also reported similar results in 2006 wherein IMP showed better susceptibility than MEM. CST has been considered the last resort drug against MDR *Enterobacteriaceae*. Among *E. coli*, two per cent of ESBL-positive isolates were CST resistant while no CST resistance was observed among ESBL-negative isolates. Similarly, among *K. pneumoniae*, seven per cent of isolates, negative for the ESBL genes tested for, were CST resistant while no CST resistance was observed among ESBL-positive *K. pneumoniae*. All *E. coli* and *K. pneumoniae* isolates showed susceptibility against chloramphenicol. In contrast, Shilpa *et al*[29] reported that 66 per cent of their ESBL producers were resistant to chloramphenicol[29]. Our study indicated a high rate of ESBL production by *K. pneumoniae* which may be due to the selective pressure imposed by extensive use of antimicrobials.

Our study had a few limitations. First, it might appear that the data presented were one and a half to two years old and that *E. coli* and *K. pneumoniae* might have moved on to higher levels of resistance since then. It is worth noting that not only the prevalence of ESBL varied from 2 to 73 per cent in the present study but also there were large variations among the prevalent genes over time in the same geographical areas. With such flux going on in ESBL production, it is important to have a strong surveillance network that can guide judicious use of cephalosporins in susceptible isolates, thus safe-keeping higher agents such as carbapenems and CST. Secondly, CST susceptibility was looked for by KBDD method in this study. The gold standard for CST susceptibility testing is broth microdilution, but the CLSI had zone diameter breakpoints for CST when the study isolates were evaluated. Cefoperazone-sulbactam zone diameters were not available with the CLSI and we followed previously published criteria for the same[30]. Thirdly, since the study centres cater primarily to northern and southern parts of India; the results of the present study may not represent the overall situation of the whole country.

In conclusion, our data showed that the prevalence of ESBL among the four study centres was variable and their molecular profile revealed $bla_{TEM}$, $bla_{OXA-1}$ and $bla_{CTXM-15}$ to be the most common ESBL genes in India,

during the study period. The antimicrobial agent with the highest susceptibility was CST. Further molecular characterization of isolates over geographically widespread areas along with studies on carbapanem resistance progressing to CST-resistance are needed for better surveillance.

*Conflicts of Interest*: None.

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