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Original Article

Extended-spectrum Beta-lactamases Producing *Escherichia coli* and *Klebsiella pneumoniae*: A Multi-centric Study Across Karnataka

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ABSTRACT

Background: There are sporadic reports on detection of extended-spectrum beta-lactamases (ESBL) producers from Karnataka; hence, this is a first multicentric study across Karnataka state to determine the prevalence of ESBL production among clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae*.

Aims and objectives: To determine the prevalence of ESBL producing clinical isolates of *E. coli and K. pneumoniae* from five geographically distributed centers across Karnataka, to study the susceptibility of ESBL producing isolates to other beta-lactam and beta-lactam-beta-lactamase inhibitors and to demonstrate transferability of plasmids coding for ESBL phenotype.

Materials and Methods: Two hundred isolates of *E. coli* and *K. pneumoniae* each were collected from each of the five centers (Bellary, Dharwad, Davangere, Kolar and Mangalore). They were screened for resistance to screening agents (ceftazidime, cefotaxime, ceftriaxone, aztreonam) and positive isolates were confirmed for ESBL production by test described by Clinical and Laboratory Standards Institute . Co-production of ESBL and AmpC beta-lactamase was identified by using amino-phenylboronic acid disk method. Susceptibility of ESBL producers to beta-lactam antibiotics and beta-lactamase inhibitors was performed. Transferability of plasmids was performed by conjugation experiment.

Results: Overall prevalence of ESBL production among *E. coli* and *K. pneumoniae* across five centers of the state was 57.5%. ESBL production was found to be 61.4% among *E. coli* and 46.2% among *K. pneumoniae*. ESBL production was significantly more among *E. coli* than *K. pneumoniae*. Significant variations in distribution of ESBL across the state was observed among *E. coli* isolates, but not among *K. pneumoniae* isolates. All ESBL producers demonstrated minimum inhibitory concentration levels ≥2 µg/ml towards cefotaxime, ceftazidime and ceftriaxone.

Conclusion: Overall prevalence of ESBL production among clinical isolates of *E. coli* and *K. pneumoniae* across Karnataka state was high. The prevalence of ESBL production was significantly higher with *E. coli* than *K. pneumoniae* isolates. Higher rates of resistance to ceftriaxone and cefotaxime than to ceftazidime suggests the possibility of presence of CTX-M type ESBLs. Of all the beta-lactam/beta-lactamase inhibitor combinations tested, cefepime-tazobactam demonstrated highest *in-vitro* activity against ESBL producers. There was no statistical difference in the transferability of plasmids among *E. coli* and *K. pneumoniae*.

Key words: Beta-lactamase, beta-lactamase inhibitor, extended-spectrum beta-lactamases

INTRODUCTION

Treatment of infections caused by Gram-negative bacilli is becoming increasingly difficult because of antibiotic resistance. Various mechanisms such as enzymatic inactivation of antibiotics, altered target sites, decreased porin permeability and active efflux pumps are known to produce drug resistance. One such mechanism is the production of extended-spectrum beta-lactamase (ESBL) enzymes by these bacteria. ESBLs are known to hydrolyze all penicillins, early cephalosporins, oxyimino-cephalosporins and monobactams, but they lack hydrolytic activity on cephamycins and carbapenems. ESBLs are inhibited by beta-lactamase

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Determination of Minimum inhibitory concentration values

The MIC values for cefoxitin, ceftazidime, cefotaxime and ceftriaxone against isolates identified as ESBL producers were obtained by agar dilution method using a dilution range of 128-0.25 μg/ml on Mueller Hinton agar. *K. pneumoniae* ATCC 700603 and *E. coli* ATCC 25922 were used as controls.

Additional susceptibility testing

ESBL producing isolates were further tested for susceptibility to cefepime (30 μg), cefepime-tazobactam (30/10 μg), cefepime-clavulanic acid (30/10 μg) and imipenem (10 μg) by disk diffusion method.

Transfer of resistance by conjugation

Isolates phenotypically identified as ESBL producers were tested for transferability of the plasmid by conjugation (mating) experiment as described earlier.[9] Test strains and the recipient strain were grown overnight separately in Luria Bertani broth at 37°C. Cultures of test strain and recipient strains were mixed in a separate tube at 1:10 ratio and incubated at 37°C overnight. A volume of 50 μl of the mixture was placed on Mueller Hinton agar with 2 μg/ml cefotaxime and 200 μg/ml sodium azide and incubated at 37°C for up to 48 h. Growth on this medium was interpreted as successful conjugation and such colonies were confirmed for ESBL production by PCT. The recipient strain (*E. coli* J53 Az^R) was kindly provided by George Jacoby.

Statistical methods applied

Using an approximate prevalence rate of 50%, confidence interval of 95%, precision of 5% and using the formula $n = (Z_{1-\alpha})^2(P\ (1 - P)/D^2)$, a sample size of 385 was calculated. It was rounded off to 400 samples per center including 200 of *E. coli* and 200 of *K. pneumoniae*. The power of study was set at 80%. Categorical data was analyzed by Chi-square test whereas χ test for proportion was used to determine the relationship between groups. $P \leq 0.05$ was considered to be statistically significant.

Culture media, antibiotic disks, APB acid, dimethylsulphoxide, ATCC strains were procured from Hi-Media laboratories, Mumbai, India. Pure antibiotic powders for MIC determination were procured from Sigma-Aldrich, Bangalore, India.

RESULTS

Screening test

A total of 1276 isolates were considered as screen positive. Of the 1,000 *E. coli* isolates, 740 (74%) were found resistant to one or more the screening agents. Among them 738 (99.7%) were resistant to all the four screening agents. Of the 1,000 *K. pneumoniae* isolates, 536 (53.6%) were found resistant to one or more the screening agents. Among them 529 (98.7%) were resistant to all the four screening agents.

Phenotypic detection of ESBL

Of the 1276 isolates that were positive in the screening test, ESBL production was confirmed by PCT in 1076 (84.3%) isolates, which included 614 (61.4%) *E. coli* and 462 (46.2%) *K. pneumoniae*, indicating that the prevalence of ESBL production is 61.4% in *E. coli* (ESBL-EC) and 46.2% in *K. pneumoniae* (ESBL-KP) across Karnataka. Co-production of ESBL and AmpC beta-lactamase were detected in 58 (2.9%) isolates, which included 56 (5.6%) *E. coli* and 2 (0.2%) *K. pneumoniae*. AmpC production was noted in 35 (3.5%) isolates of *E. coli* and 9 (0.9%) isolates of *K. pneumoniae*. Neither AmpC nor ESBL production could be accounted for cephalosporin resistance in the remaining 35 isolates of *E. coli* and 63 isolates of *K. pneumoniae*.

Distribution of ESBL producers across the state

The distribution of ESBL producing *E. coli* and *K. pneumoniae* from various centers is as shown in Table 3 and Figure 1. The prevalence of ESBL-EC was noticed to be highest from Bellary center (83.5%) followed by Mangalore and Davangere (63.5% each) and lesser from Dharwad (49.5%) and Kolar (47%) centers. Differences in the prevalence of ESBL-EC across Karnataka was significant ($P < 0.001$); on the other hand, the prevalence of ESBL-KP across Karnataka was not significant ($P = 0.2$).

Table 3: Distribution of ESBL producing EC and KP from various centers

Location	Screening test positive *n* (%)		ESBL positive *n* (%)	
	EC	KP	EC	KP
Bellary	175 (87.5)	103 (51.5)	167 (83.5)	98 (49)
Davangere	171 (85.5)	110 (55)	127 (63.5)	95 (47.5)
Dharwad	124 (62)	102 (51)	99 (49.5)	77 (38.5)
Kolar	105 (52.5)	120 (60)	94 (47)	96 (48)
Mangalore	165 (82.5)	101 (50.5)	127 (63.5)	96 (48)
Total	740 (74)	536 (53.6)	614 (61.4)	462 (46.2)

EC: *Escherichia coli*, KP: *Klebsiella pneumoniae*, ESBL: Extended-spectrum beta-lactamase

inhibitor combinations. Resistance to the combination of cefotaxime and clavulanic acid was almost the same in case of ESBL-EC (53.4%) and ESBL-KP (49.6%); however, 32.2% ESBL-KP and 9.1% of ESBL-EC were resistant to the combination of ceftazidime and clavulanic acid. This difference was found to be significant ($P < 0.001$). It was observed that 89.1% of ESBL-EC and 79.9% of ESBL-KP were resistant to cefepime. This resistance was reduced considerably (from 89.1% to 2.9%) when cefepime was combined with tazobactam or clavulanic acid. Of all the combinations of beta-lactam/beta-lactamase inhibitor tested, cefepime-tazobactam had the least resistance. Cefoxitin resistance was observed both in ESBL-EC (38.6%) and ESBL-KP (48.5%). Only 2 (0.3%) ESBL-EC and 11 (2.4%) ESBL-KP were found resistant to imipenem.

Plasmid transfer by conjugation

Plasmid mediated resistance transfer was successfully demonstrated in 362/614 (59%) isolates of ESBL-EC and 249/462 (53.9%) isolates of ESBL-KP.

DISCUSSION

In a multi-centric study conducted as part of India SENTRY surveillance, the prevalence of ESBL production was reported to be 84%.[10] Here we found that 61.4% of *E. coli* and 46.2% of *K. pneumoniae* isolates collected across five centers of Karnataka were ESBL producers. The prevalence of ESBL-EC was significantly more ($P < 0.001$) than ESBL-KP.

ESBL production among *Enterobacteriaceae* members vary widely from region to region and sometimes within the state. We found that the prevalence of ESBL producing bacteria varied across Karnataka with respect to *E. coli* but not with *K. pneumoniae*. In the case of *E. coli* it was highest at Bellary (87.5%) and lowest at Kolar (52.5%). However, it was almost the same with respect to *K. pneumoniae* expect from Dharwad. These differences could be due to varied degree exposure of these organisms to beta-lactam antibiotics or due to varied transferability of plasmids in nature among them. However, our plasmid transmission studies conducted in the laboratory did not reveal a significant difference ($P = 0.09$) in the transferability of plasmid in ESBL-EC and ESBL-KP.

Earlier studies from Karnataka found that the detection rates of ESBL-EC has varied from 23.6% (Raichur) to 86.7% (Shimoga). The detection rates of ESBL-KP from different studies across Karnataka has been reported to vary from 9.6% (Bangalore) to 81.8% (Mangalore).[11-14] Reports of ESBL detection among clinical isolates of *E. coli* range between 20% and 80.6% and those among *K. pneumoniae* ranges between 20% and 86.7% across the country; these findings are summarized in Table 6. The variation in the detection rates within and across the states could be due to the differences in the methodology used in these studies. We made our study stringent by using four beta-lactam antibiotics for screening and two beta-lactam and beta-lactamase inhibitor combination disks for confirmation of ESBL as per the CLSI guidelines, thus, bringing uniformity in testing.

Co-production of AmpC beta-lactamase and ESBL among the isolates in this study has been only minimal (2.9%), but was observed to be higher among *E. coli* (5.6%) isolates than *K. pneumoniae* (0.2%) isolates. Our findings contrasts with that reported from Bangalore and Manipal. The study from Manipal reported a co-production rate of 58.5% among *E. coli*; a study from Bangalore reported a co-production rate of 30.1% for *E. coli* and 30.3% for *Klebsiella* sps.[23,24] These variations could be due to differences in the methodologies adopted.

In the present study reported, none of the ESBL producing bacteria had MIC of <2 μg/ml towards ceftazidime, cefotaxime or ceftriaxone. The ESBL producers exhibited significantly higher MIC levels (≥128 μg/ml) to cefotaxime and ceftriaxone than to ceftazidime. This difference suggests

Table 5: Antibiotic resistance to other beta-lactam antibiotics and beta-lactamase inhibitors

Antibiotics tested	*Escherichia coli* n (%)	*Klebsiella pneumoniae* n (%)
Cefotaxime-clavulanic acid	328 (53.4)	229 (49.6)
Ceftazidime-clavulanic acid	56 (9.1)	149 (32.2)
Cefepime	547 (89.1)	369 (79.9)
Cefepime-clavulanic acid	53 (8.6)	94 (20.3)
Cefepime-tazobactam	18 (2.9)	48 (10.4)
Cefoxitin	237 (38.6)	224 (48.5)
Imipenem	2 (0.3)	11 (2.4)

Table 6: Reports of ESBL production in EC and KP across the country

Location	ESBL-EC %	ESBL-KP %	Reference
Mumbai	20	20	Vaidya[15]
Chandigarh	70	60	Sharma *et al.*[16]
Pondicherry	60.8	39.2	Mohamudha Parveen *et al.*[17]
Amritsar	46.4	52.3	Kaur and Aggarwal[18]
Salem	79.5	50	Priyadharsini *et al.*[19]
Guwahati	43	67	Sarma *et al.*[20]
Ujjain	69	41	Pathak *et al.*[21]
Indore	80.6	86.7	Chitnis *et al.*[22]

EC: *Escherichia coli*, KP: *Klebsiella pneumoniae*, ESBL-EC: ESBL producing *E. coli*, ESBL-KP: ESBL producing *K. pneumoniae*

Klebsiella pneumoniae: Epidemiology and control. Clin Infect Dis 2011;52:743-9.

4. Doi Y, Paterson DL, Egea P, Pascual A, López-Cerero L, Navarro MD, *et al.* Extended-spectrum and CMY-type beta-lactamase-producing *Escherichia coli* in clinical samples and retail meat from Pittsburgh, USA and Seville, Spain. Clin Microbiol Infect 2010;16:33-8.
5. Shaheen BW, Nayak R, Foley SL, Kweon O, Deck J, Park M, *et al.* Molecular characterization of resistance to extended-spectrum cephalosporins in clinical *Escherichia coli* isolates from companion animals in the United States. Antimicrob Agents Chemother 2011;55:5666-75.
6. Webster DP, Young BC, Morton R, Collyer D, Batchelor B, Turton JF, *et al.* Impact of a clonal outbreak of extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* in the development and evolution of bloodstream infections by *Klebsiella pneumoniae* and *Escherichia coli*: An 11 year experience in Oxfordshire, UK. J Antimicrob Chemother 2011;66:2126-35.
7. Performance Standards for Antimicrobial Susceptibility Testing: Twenty-First Informational Supplement M100-S20. Vol. 30. In: Clinical and Laboratory Standards Institute. Wayne, PA:CLSI; 2010. p. 46.
8. Robberts FJ, Kohner PC, Patel R. Unreliable extended-spectrum beta-lactamase detection in the presence of plasmid-mediated AmpC in *Escherichia coli* clinical isolates. J Clin Microbiol 2009;47:358-61.
9. Park YJ, Park SY, Oh EJ, Park JJ, Lee KY, Woo GJ, *et al.* Occurrence of extended-spectrum beta-lactamases among chromosomal AmpC-producing *Enterobacter cloacae*, *Citrobacter freundii*, and *Serratia marcescens* in Korea and investigation of screening criteria. Diagn Microbiol Infect Dis 2005;51:265-9.
10. Manoharan A, Premalatha K, Chatterjee S, Mathai D, SARI Study Group. Correlation of TEM, SHV and CTX-M extended-spectrum beta lactamases among Enterobacteriaceae with their *in vitro* antimicrobial susceptibility. Indian J Med Microbiol 2011;29:161-4.
11. Harwalkar A, Sataraddi J, Gupta S, Yoganand R, Rao A, Srinivasa H. The detection of ESBL-producing *Escherichia coli* in patients with symptomatic urinary tract infections using different diffusion methods in a rural setting. J Infect Public Health 2013;6:108-14.
12. Bhat PV, Sarkar A. Emergence and control of multidrug resistant organisms in small cities in India: A wake up call. Indian J Med Sci 2011;65:337-43.
13. Eshwarappa M, Dosegowda R, Aprameya IV, Khan MW, Kumar PS, Kempegowda P. Clinico-microbiological profile of urinary tract infection in south India. Indian J Nephrol 2011;21:30-6.
14. Kamath S, Mallaya S, Shenoy S. Nosocomial infections in neonatal intensive care units: Profile, risk factor assessment and antibiogram. Indian J Pediatr 2010;77:37-9.
15. Vaidya VK. Horizontal transfer of antimicrobial resistance by extended-spectrum β lactamase-producing enterobacteriaceae. J Lab Physicians 2011;3:37-42.
16. Sharma J, Sharma M, Ray P. Detection of TEM and SHV genes in *Escherichia coli and Klebsiella pneumoniae* isolates in a tertiary care hospital from India. Indian J Med Res 2010;132:332-6.
17. Mohamudha Parveen R, Manivannan S, Harish BN, Parija SC. Study of CTX-M type of extended spectrum β-lactamase among nosocomial isolates of *Escherichia coli* and *Klebsiella pneumoniae* in South India. Indian J Microbiol 2012;52:35-40.
18. Kaur M, Aggarwal A. Occurrence of the CTX-M, SHV and the TEM genes among the extended spectrum β-lactamase producing isolates of enterobacteriaceae in a Tertiary Care Hospital of North India. J Clin Diagn Res 2013;7:642-5.
19. Priyadharsini RI, Kavitha A, Rajan R, Mathavi S, Rajesh KR. Prevalence of bla (CTX M) extended spectrum beta lactamase gene in enterobacteriaceae from critical care patients. J Lab Physicians 2011;3:80-3.
20. Sarma JB, Bhattacharya PK, Kalita D, Rajbangshi M. Multidrug-resistant Enterobacteriaceae including metallo-β-lactamase producers are predominant pathogens of healthcare-associated infections in an Indian teaching hospital. Indian J Med Microbiol 2011;29:22-7.
21. Pathak A, Marothi Y, Kekre V, Mahadik K, Macaden R, Lundborg CS. High prevalence of extended-spectrum β-lactamase-producing pathogens: Results of a surveillance study in two hospitals in Ujjain, India. Infect Drug Resist 2012;5:65-73.
22. Chitnis S, Katara G, Hemvani N, Chitnis DS. Augmentation in zone of inhibition of cefoperazone/cefoperazone+sulbactum compares well with the clinical laboratory standard institute standard extended spectrum beta-lactamase detection method as well as the polymerase chain reaction method. Curr Drug Saf 2011;6:155-8.
23. Rudresh SM, Nagarathnamma T. Extended spectrum β-lactamase producing Enterobacteriaceae and antibiotic co-resistance. Indian J Med Res 2011;133:116-8.
24. Shobha KL, Ramachandra L, Rao G, Majumder S, Rao SP. Extended spectrum beta-lactamases (ESBL) in gram negative bacilli at a Tertiary Care Hospital. J Clin Diagn Res 2009;3:1307-12.
25. Tzouvelekis LS, Tzelepi E, Tassios PT, Legakis NJ. CTX-M-type beta-lactamases: An emerging group of extended-spectrum enzymes. Int J Antimicrob Agents 2000;14:137-42.
26. Ananthan S, Subha A. Cefoxitin resistance mediated by loss of a porin in clinical strains of *Klebsiella pneumoniae* and *Escherichia coli*. Indian J Med Microbiol 2005;23:20-3.
27. Yu WL, Pfaller MA, Winokur PL, Jones RN. Cefepime MIC as a predictor of the extended-spectrum beta-lactamase type in *Klebsiella pneumoniae*, Taiwan. Emerg Infect Dis 2002;8:522-4.
28. Oteo J, Delgado-Iribarren A, Vega D, Bautista V, Rodríguez MC, Velasco M, *et al.* Emergence of imipenem resistance in clinical *Escherichia coli* during therapy. Int J Antimicrob Agents 2008;32:534-7.

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