

Correspondence

Extended-spectrum β -lactamase producing *Klebsiella pneumoniae* from blood cultures in Puducherry, India

Sir,

Extended-spectrum β -lactamases (ESBLs) are plasmid-mediated enzymes that confer resistance to all penicillins and cephalosporins, including the sulbactam and clavulanic acid combinations and monobactams such as aztreonam¹. ESBLs are most commonly detected in *Klebsiella pneumoniae*, which is an opportunistic pathogen associated with severe infections in hospitalized patients, including immunocompromised hosts with severe underlying diseases². ESBL producing *K. pneumoniae* was first reported in 1983 from Germany, with a steady worldwide increase in *K. pneumoniae*-mediated resistance against cephalosporins in the subsequent decades³.

Bloodstream infections associated with *K. pneumoniae* may arise as a consequence of pneumonia (community- and ventilator-acquired), the urinary tract, intra-abdominal pathologies, and central venous line-related infections⁴. However, though evidence shows that this pathogen is associated with nosocomial infections worldwide, relatively little information is currently available regarding ESBL producing *K. pneumoniae* isolates from southern India, and Puducherry in particular. Thus a molecular characterization study was performed on blood isolates of ESBL producing *K. pneumoniae* collected from a tertiary care hospital in Puducherry, India.

In this retrospective study, 39 non-repeat blood culture isolates of *K. pneumoniae* were collected during a 3 month period (June-August) in 2008. Isolates were obtained from patients admitted to 8 different wards at JIPMER (Jawaharlal Institute of Postgraduate Medical Education & Research), Puducherry, south India (Table). Blood culture was performed using biphasic medium consisting of Brain Heart Infusion (BHI) agar and BHI broth with sodium polyanethol sulphate as an anticoagulant. *K. pneumoniae* was identified

using standard microbiological procedures⁵. The antimicrobial susceptibility profiles of ampicillin (10 μ g), amikacin (30 μ g), gentamicin (10 μ g), piperacillin (100 μ g), piperacillin/tazobactam (100/10 μ g), cefoperazone/sulbactam (75/10 μ g), cefoxitin (30 μ g), cefotaxime (30 μ g), ceftazidime (30 μ g), ceftriaxone (30 μ g), ciprofloxacin (5 μ g) and meropenem (10 μ g) (Hi-Media, Mumbai) were tested by disk diffusion methods as described by the Clinical and Laboratory Standards Institute (CLSI formerly NCCLS)⁶. Phenotypic evidence of ESBL production was tested by the combination disk method⁶. *K. pneumoniae* ATCC 700603 and *Escherichia coli* ATCC 25922 were used as controls. These controls were available from the Department of Microbiology, Jawaharlal Institute of Postgraduate Medical Education and Research, Puducherry. Isolates were stabbed into semi-solid nutrient agar butts and were stored at 4°C before retrieval for further investigation.

All 39 isolates were subjected to molecular analysis, with PCR screening and sequencing being performed to identify the β -lactamase resistance genes; *bla*_{TEM}, *bla*_{SHV}, *bla*_{OXA-1} group and *bla*_{CTX-M}, as previously described⁷⁻¹⁰. Additional sequencing primers were required for *bla*_{TEM} PCR product sequencing ('Lagging strand 7' 5'-TTACTGTCATGCCATCC-3' and 'Lagging strand 3' 5'-AGAGAATTATGCAGTGC-3'). PCR primers corresponding to sequences downstream of *bla*_{CTX-M} genes were also used ('M3 int upp' 5'-TCACCCAGCCTCAACCTAAG-3' and 'ORF1 pol M3' 5'-GCACCGACACCCTCACACCT-3')¹¹. PCR products of *bla*_{CTX-M} positive isolates were subjected to sequencing using primers 'CTX-M-1 fw multi' 5'-AAAAATCACTGCGCCAGTTC-3', 'CTX-M-1 multi (REV)F seq' 5'-AACGTGGCGATGAATAAGCT-3' and 'ORF1 pol M3' 5'-GCACCGACACCCTCACACCT-3'. A PCR-based replicon typing method was performed to study the relationship between the resistance plasmids present,

Table. Isolate number, patient age, ward of isolation and diagnosis of 39 patients presenting with *K. pneumoniae* blood stream infections in Puducherry between June and August 2008

Isolate No.	Age (yr)	Unit	Diagnosis
28	20	MED	Fever
83	35	CAS	Gangrene foot
74	NB	NICU	Gangrene
75	NB	NICU	Preterm with sepsis
46	NB	NICU	Sepsis
70	NB	NICU	Preterm with NEC
23	NB	NICU	MAS
30	NB	NICU	Sepsis
79	NB	NICU	Preterm
84	NB	NICU	Respiratory distress
8	NB	NICU	Sepsis
71	NB	NICU	Preterm
77	NB	NICU	Preterm
14	NB	NICU	Sepsis
56	NB	NICU	PUO
65	NB	NICU	Sepsis
52	NB	NICU	MAS
57	NB	NICU	Preterm
34	NB	NICU	HIE encephalopathy
40	NB	NICU	Preterm
43	40	SURG	Burn injury
20	7	PAED	Sepsis with DM
87	NB	PICU	Snake bite
39	26	OG	P212 with PPR
25	48	MICU	Fever
80	30	MED	Aplastic anemia
27	NB	NICU	MAS
29	NB	NICU	Preterm with NEC
24	NB	NICU	Preterm with prom
37	NB	MED	Sepsis
86	NB	NICU	Viral encephalitis
66	NB	NICU	Sepsis
13	NB	NICU	PUO
85	NB	NICU	Meningitis
31	NB	NICU	Sepsis
15	2	PAED	Meningitis
35	12	PAED	Sepsis
42	NB	NICU	Sepsis
58	70	MED	PUO

NB, newborn; PROM, premature rupture of membranes; DM, diabetes mellitus; PUO, pyrexia of unknown origin; MAS, meconium aspiration syndrome; HIE, hypoxic-ischemic encephalopathy; NEC, necrotizing enterocolitis; PPR, photo paroxysmal response

with the individual plasmid types FIA, FIB, FIIs, A/C and II replicons being screened¹². These replicon types are representative of the plasmid incompatibility groups circulating among *Enterobacteriaceae*¹². Isolate

genotyping was performed using pulsed field gel electrophoresis (PFGE) using the restriction enzyme *Xba*I. Cluster analysis was performed using the method of Dice and the unweighted pair group method with arithmetic mean (UPGMA; <http://en.wikipedia.org/wiki/UPGMA>).

Of the 39 isolates investigated, 37 (94.8%) were found to be resistant to at least one of the third generation cephalosporins. Among these 37 isolates, 36 (97.2%) were found to be ESBL positive by phenotypic testing. Antibiotic susceptibility testing revealed that the majority of these 36 isolates were multidrug resistant exhibiting 95, 87 and 92 per cent resistance to gentamicin, ciprofloxacin and ceftriaxone, respectively. Of the 39 isolates, 21 per cent showed resistance to amikacin and only 5 per cent to meropenem. By PCR, of the 39 isolates, 32 (82%) were positive for *bla*_{TEM}, 18 (46%) for *bla*_{SHV}, 36 (92%) for *bla*_{CTX-M}, and 32 (82%) for *bla*_{OXA-1} group, respectively. The sequenced amplicons of the isolates positive for *bla*_{CTX-M} revealed the presence of *bla*_{CTX-M-15} in all isolates. PCR-based replicon typing revealed that only a single isolate harboured both FIA and FIB replicons carrying *bla*_{CTX-M-15}. Plasmids with FIIs, A/C and II replicons types were not detected. PFGE analysis showed that the 39 isolates belonged to 3 (non-clonal) major genotypic clusters with no obvious association between genotype and ward.

In recent years, a significant increase in ESBL producing *Klebsiella* spp. has been reported in India mostly identified using phenotypic methods¹³⁻¹⁶. Further, according to our earlier report (January- July, 2006), 130 patients with *K. pneumoniae* blood stream infections were identified with a very high proportion of these, (126 or 97%), producing ESBLs¹⁷. From our current study, 44 per cent of *K. pneumoniae* isolates carried both *bla*_{TEM} and *bla*_{SHV} genes, 41 per cent a *bla*_{TEM} gene only, and only 5 per cent a *bla*_{SHV} gene. In the past 15 years, CTX-M-type ESBLs have become more prevalent worldwide^{18,19}. Among our blood culture isolates, a very high incidence of multiple ESBL-gene carriage was detected, with the most notable result being the presence of CTX-M-15 in 92 per cent of isolates, as well as the combination of CTX-M-15 resistance and OXA-1 resistance in 82 per cent and 36 per cent of isolates possessing TEM, SHV, CTX-M and OXA-1 resistance combined. Two isolates (5%) were also meropenem resistant, with carbapenems currently being considered the preferred antimicrobial agent for the treatment of serious infections caused by ESBL-

producing *K. pneumoniae* in our hospital. This finding seriously limits treatment options, and causes great concern with respect to the adequate treatment and spread of ESBL resistant *K. pneumoniae* isolates both within hospitals and from the hospital environment to the community.

The spread of antimicrobial resistance in *K. pneumoniae* isolates is complicating the treatment of serious nosocomial infection worldwide, not least because resistance in *K. pneumoniae* is typically caused by the acquisition of plasmids containing multiple antimicrobial resistances (including genes coding for ESBL resistance)²⁰. Molecular characterization of such ESBL-carrying isolates is essential in allowing hospitals to identify the source of these pathogenic bacteria, whilst providing useful information regarding the distribution of clonally related ('outbreak' strains) or non-related ESBL genotypes. Further, monitoring of the spread of individual β -lactamase genes and their associated genetic platforms (in particular plasmids), provides a means to monitor for the appearance of new ESBL enzymes and genotypes, as well as establishing the dominance of older established ESBL enzymes/genotype combinations.

In conclusion, this study emphasizes the major role that CTX-M-15 plays in facilitating ESBL-mediated antimicrobial resistance in Puducherry, India, and reiterates its association with multiple antibiotic resistance determinants, including carbapenem resistance.

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