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Neonatal septicaemia caused by diverse clones of *Klebsiella pneumoniae* & *Escherichia coli* harbouring $bla_{\text{CTX-M-15}}$

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***Background & objectives*: Information about the genetic diversity of the extended-spectrum β-lactamases (ESBLs) and the clonal relationship of the organisms causing neonatal infections is limited, particularly from India where neonatal mortality is high. This study was undertaken to investigate the molecular epidemiology and risk factors associated with neonatal septicaemia caused by ESBL-producing *Klebsiella pneumoniae* and *Escherichia coli*.**

***Methods*: Bloodstream isolates (n=26) of *K. pneumoniae* (n=10) and *E. coli* (n=16) from the neonates admitted in a tertiary care hospital in New Delhi during January to May 2008 were characterized. Antimicrobial susceptibility tests were carried out and ESBL production was assessed phenotypically. PCR was carried out for ESBL and *ampC* genes. Genotyping was performed by pulsed-field gel electrophoresis (PFGE). Conjugation experiments were done to determine the mobility of ESBL genes. Risk factors associated with ESBL-producing *K. pneumoniae* and *E. coli* infections were analysed.**

***Results*: Resistance rates to most of the antibiotics tested were high, except for imipenem. Among the isolates tested, 60 per cent of *K. pneumoniae* and 75 per cent of *E. coli* were ESBL producers. PFGE of the isolates demonstrated a vast diversity of genotypes with no epidemic clones. Despite the clonal diversity, $bla_{\text{CTX-M-15}}$ was detected in 100 per cent of ESBL-positive isolates. The other genes present in ESBL-positive isolates were $bla_{\text{TEM-1}}$, $bla_{\text{SHV-1}}$, $bla_{\text{SHV-28}}$, $bla_{\text{SHV-11}}$, and $bla_{\text{SHV-12}}$. Class 1 integrons were detected in 7 of 18 ESBL-positive isolates. Moreover, the plasmid carrying $bla_{\text{CTX-M-15}}$, in *E. coli* and *K. pneumoniae* were self transferable. Feeding through an enteral tube was identified as the only risk factor for sepsis by ESBL-producing organisms.**

***Interpretation & conclusions*: The study emphasises the presence of $bla_{\text{CTX-M-15}}$ in clonally diverse isolates indicating probable horizontal transfer of this gene. The widespread dissemination of CTX-M-15 is of great concern as it further confines the limited therapeutic interventions available for neonates.**

Key words CTX-M-15 - diverse clones - ESBLs - *Escherichia coli* - *Klebsiella pneumoniae* - neonatal sepsis - risk factor

Neonatal mortality in developing countries like India is very high[1,2]. Microbial infections leading to sepsis is a major contributor to neonatal deaths in the developing world[3]. The overall fatality rate due to neonatal sepsis and pneumonia in developing countries is estimated to be about 25 per cent, based largely on data for infants treated in hospitals[3]. Developing countries like India, Nigeria, Democratic Republic of the Congo, Pakistan, and China were responsible for large proportions of the global total for neonatal death due to sepsis[4].

An important compounding factor in the treatment of these infections associated with sepsis is the emergence of extended-spectrum beta lactamase (ESBL)-producing *Enterobacteriaceae*[5]. ESBLs are derived from genes by mutation in the narrower-spectrum TEM-1, TEM-2 or SHV-1 ß-lactamases[6,7]. Additionally, other types of ESBLs (CTX-M) that are plasmid-mediated, exhibit an overall preference for cefotaxime and ceftriaxone[8]. At present, more than 100 genetically distinct TEM, SHV, OXA and CTX-M-type ESBLs have been characterized (*http://www.lahey.org/studies/webt.asp*).

Among Gram-negative bacilli the two most important bacterial pathogens causing neonatal sepsis in developing countries are *Klebsiella pneumoniae* and *Escherichia coli*[9,10]. Studies on ESBL-mediated resistance mechanisms in adult patients from developing countries have been carried out[11-18]. However, studies on the types of ESBLs and the clonal relationship of the organisms producing them, especially in neonatal infections from India are limited[19-21].

In this study, isolates of *K. pneumoniae* and *E. coli* causing bloodstream infection from neonates admitted to a tertiary care centre in New Delhi, India were analysed. The aim of the study was to carry out *(i)* molecular characterization of the ESBL genes in *E. coli* and *K. pneumoniae* isolates involved in neonatal sepsis, *(ii)* assessment of any significant differences in the clinical or demographical variables in the neonates that predispose them to acquisition of ESBL-producing organisms, *(iii)* determination of the molecular typing of the ESBL-producing isolates to ascertain the clonal carriage of the ESBL genes.

Material & Methods

Clinical setting and bacterial isolates: Isolates included in the study were collected from septicaemic neonates during January to May 2008 from Department of Microbiology at Vardhman Mahavir Medical College and Safdarjang Hospital, tertiary care centre in New Delhi. This centre has two nurseries, one for intramural admissions (born at the hospital) and one for extramural admissions (born outside the hospital). During the study period, a total of 1443 neonates were admitted to the nurseries, among whom, 405 neonates were suspected to have sepsis. Of these 405, 177 (43.7%) neonates were culture positive. Among these 177 neonates, 63 (35.59%) had aerobic Gram-negative bacilli (GNB) in their blood. Of the 63 GNBs, *K. pneumoniae* (n=16) and *E. coli* (n=10) were isolated. All *K. pneumoniae* and *E. coli* isolates obtained during this period were included in the study. Details of the neonates from whom *K. pneumoniae* and *E. coli* have been isolated are included in Table I.

Blood specimen collection and identification of the isolates: One ml blood for culture was drawn with aseptic precautions from a peripheral vein. Cultures were processed in BacT/ALERT 3D system (bioMe´rieux, Marcy l'Etoile, France). For any culture which flagged positive, Gram stain was performed and subculture was done on appropriate medium based on the Gram stain: MacConkey agar and 5 per cent sheep blood agar for Gram-negative and Gram-positive organisms, respectively. Bottles were incubated in the system for up to seven days, at the end of which all negative bottles were subcultured once on blood agar before discarding. The isolates were identified by different biochemical tests and further verified by using an ID32E or ID32GN system (bioMe´rieux).

Antibiotic susceptibility and MIC determination: Antibiotic susceptibility was determined as per Clinical and Laboratory Standards Institute (CLSI) criteria[22]. *E. coli* ATCC 25922 was used as quality control strain. The minimum inhibitory concentration (MIC) values (µg/ml) for cefotaxime, ceftazidime and cefepime were determined using E-tests (AB Biodisk, Solna, Sweden) and interpreted according to manufacturer's instructions.

ESBL confirmatory test: ESBL production was confirmed using the cephalosporin/clavulanic acid (cefotaxime/clavulanic acid and ceftazidime/clavulanic acid) combination disk test[22]. Fifty per cent of the isolates that were positive in the combination disk test were again confirmed by the double disk synergy test[5].

PCR detection and sequencing of β-lactamases & integrons: Genotypic analysis for bla_{SHV}, bla_{TEM}, bla_{OXA-1} and bla_{CTX-M} was performed for isolates found to be positive in ESBL confirmatory test and subsequently

Table I. Demographic data and clinical features of the septicaemic neonates with *E. coli* or *K. pneumoniae* blood infection

Patient name/ Isolate no.	Isolate name	Sex	Birth weight (g)	Gestational age (wk)	Intramural / Extramural birth	Mode of delivery	EOS / LOS	Ventilation	Enteral feeding tube	Maternal risk factors	Outcome
P1 / E1	EC	M	2500	37	Extramural birth	SVD	EOS	Yes	Yes	Leaking per vaginum	NK
P2 / E4	KP	F	3800	40	Intramural birth	LUCS	EOS	No	Yes	Meconium stained liquour	Discharge
P3 / E5	EC	M	1900	37	Extramural birth	LUCS	LOS	No	Yes	None	Discharge
P4 / E10	EC	F	1400	32	Intramural birth	SVD	EOS	No	Yes	Leaking per vaginum	LAMA
P5 / E14	EC	M	3000	39	Intramural birth	SVD	EOS	Yes	Yes	Leaking per vaginum, Meconium stained liquour, Hepatitis B positive	Death
P6 / E16	KP	M	1750	33	Intramural birth	SVD	EOS	Yes	Yes	Leaking per vaginum	Discharge
P7 / E27	EC	M	2000	32	Intramural birth	SVD	EOS	Yes	Yes	Meconium stained liquour	Death
P8 / E34	EC	M	2200	37	Intramural birth	SVD	EOS	No	Yes	Leaking per vaginum, Meconium stained liquour,	Death
P9 / E47	EC	M	3300	40	Intramural birth	LUCS	LOS	No	No	None	Discharge
P10 / E55	KP	F	2500	37	Intramural birth	SVD	EOS	No	Yes	Anaemia and preeclampsia and no sepsis setting in mother	NK
P11 / E57	KP	M	2300	40	Intramural birth	SVD	LOS	No	No	Bleeding per vaginum	Discharge
P12 / E65	EC	M	2340	37	Extramural birth	SVD	LOS	No	Yes	None	NK
P13 / E67	KP	M	1500	33	Intramural birth	SVD	EOS	No	Yes	Leaking per vaginum oligo-hydramnios	Discharge
P14 / E68	EC	M	2200	40	Intramural birth	SVD	EOS	No	No	Leaking per vaginum	Discharge
P15 / E73	EC	M	3000	37	Intramural birth	SVD	LOS	No	No	None	NK
P16 / E75	EC	M	2000	34	Intramural birth	SVD	LOS	No	No	None	Discharge

Contd...

Patient name/ Isolate no.	Isolate name	Sex	Birth weight (g)	Gestational age (wk)	Intramural / Extramural birth	Mode of delivery	EOS / LOS	Ventilation	Enteral feeding tube	Maternal risk factors	Outcome
P17 / E78	KP	F	3500	37	Extramural birth	SVD	LOS	No	No	None	Discharge
P18 / E91	EC	M	2700	37	Intramural birth	LUCS	EOS	Yes	Yes	delivery by traditional birth attendant	Death
P19 / E98	EC	M	3200	37	Extramural birth	SVD	LOS	No	No	None	LAMA
P20 / E99	KP	F	1200	38	Intramural birth	SVD	EOS	No	Yes	None	Discharge
P21 / E104	EC	F	1200	32	Intramural birth	SVD	LOS	No	Yes	None	Death
P22 / E108	KP	F	700	30	Intramural birth	SVD	LOS	Yes	Yes	Leaking per vaginum	Death
P23 / E113	KP	F	1200	28	Intramural birth	SVD	LOS	Yes	Yes	Leaking per vaginum, Meconium stained liquour	NK

Among the 26 neonates with septicaemia due to *E. coli* or *K. pneumoniae* clinical details were available for 23 neonates. EC, *Escherichia coli*; KP, *K. pneumoniae*; M, male; F, female; SVD, spontaneous vaginal delivery; LUCS, low uterine caesarean delivery; EOS, early onset sepsis (onset of sepsis within 48 h of delivery); LOS, late onset sepsis (onset of sepsis at >48 h of delivery); LAMA, left against medical advice; NK, not known

whole open reading frame (ORF) of PCR products were amplified for sequencing[23-25]. PCR controls for bla_{SHV} and bla_{TEM} were *K. pneumoniae* ATCC 700603 and *E. coli* ATCC 35218, respectively. Control DNAs were used for detection of three groups of bla_{CTX-M} genes (provided by Dr Guillaume Arlet, Service de Bacteriology, Hospital Tenon, France). PCR was carried out to detect class 1, 2 and 3 integrons in all isolates and presence of plasmid-mediated *amp*C genes was also checked in cefoxitin-resistant isolates using PCR[26,27]. PCR controls were used for *amp*C genes (MIR-1, DHA-1, ACT-1 and FOX-5) (kind gift of George A. Jacoby, Lahey clinic, Massachusetts). PCR products were purified using QIAquick PCR Purification Kit (Qiagen, USA), sequenced directly on both strands using the BigDye Terminator v$^{3.1}$ Cycle Sequencing Kit and analysed with an automated sequencer ABI3100 genetic analyser (Applied Biosystems**,** Foster City, CA). Sequence analysis was performed using the Lasergene DNASTAR sequence analysis software (DNAStar, Madison, USA). The BLASTN program was used for database searching (*http://www.ncbi.nlm.nih.gov/BLAST/*).

Pulsed-field gel electrophoresis (PFGE): PFGE was performed following the Pulse Net protocol (*www.cdc.gov/pulsenet/protocols.htm*). Overnight cultures of the isolates on Tryptic Soy Agar with 5 per cent sheep blood were suspended in the cell suspension buffer (100mM Tris, *p*H8.0, 100mM EDTA, *p*H 8.0). The optical density (600 nm) was adjusted to 1.3-1.4. Plugs were prepared by mixing 200 µl of the bacterial suspension [with 10 µl of Proteinase K (Takara, Japan)] with 200 µl of 1 per cent plug agarose (Seakem Gold Agarose, Cambrex, USA). Cell lysis was carried out in lysis buffer (50mM Tris: 50mM EDTA, *p*H 8.0, 1% Sarcosyl, 100 µg/ml Proteinase K) at 50ºC. Digestion of DNA was performed by overnight incubation of the plug in the presence of 50 U/plug of the restriction endonuclease *Xba* I (New England Biolabs, USA) at 37ºC. Electrophoresis was performed in a 1 per cent agarose (Pulse certified agarose, BioRad, Hercules, CA, USA) on a CHEF DRIII apparatus (Bio-Rad Laboratories) with 6V/cm for 19 h at 14ºC with an initial switch time of 2.2 to 52.0 sec. *Salmonella* Braenderup H9812 was included as a molecular weight marker. *Xba*I macrorestriction patterns were compared visually

and interpreted according to the criteria of Tenover *et al*[28].

Conjugation: Conjugal transfer of bla_{CTX-M} to sodium azide-resistant *E. coli* J53 recipient (provided by George A. Jacoby, Lahey clinic, Burlington, Massachusetts, USA) was attempted by a broth mating assay[29]. Transconjugants were selected on Luria-Bertani agar plates containing cefotaxime (4 µg/ml) and sodium azide (100 µg/ml). The approximate sizes of the plasmids in the test isolates and transconjugants were estimated using logarithmic plots, generated with plasmids of known molecular mass[30]. *E. coli* K12 V517 (sizes of the plasmids are 54.2, 7.2, 5.6, 5.1, 2.7 & 2 kb) and *Shigella flexneri* YSH 6000 (sizes of the plasmids are 212, 3.9 & 2.7 kb) were used as reference standards. The presence of bla_{CTX-M} in the transconjugants was confirmed by PCR.

Statistical analysis: Fisher's Exact test was used for the assessment of risk factor for sepsis by comparing variables. This included sex, gestational age, birth weight, place of delivery (intramural/extramural), mode of delivery, use of a mechanical ventilator, type of feeding (feeding of expressed breast milk by enteral feeding tubes or breast feeding) and maternal risk factors (maternal pyrexia, meconium stained liquor, urinary tract infection, leaking per vaginum for more than 24 h duration, Hepatitis B positive, anaemia, preeclampsia and delivery of baby by traditional birth attendant). All comparisons were unpaired and all tests of significance were two-tailed.

Results

In comparison to *K. pneumoniae*, isolates of *E. coli* demonstrated higher resistance to the β-lactamase stable β-lactams, *e.g.* 3rd generation cephalosporins (cefotaxime, ceftazidime, cefpodoxime, and ceftriaxone), monobactams (aztreonam), aminoglycoside (gentamicin), and fluoroquinolone (ciprofloxacin). On the other hand, *K. pneumoniae* isolates showed higher resistance than *E. coli* against ampicillin, piperacillin, amikacin and netilmicin. Only imipenem exhibited 100 per cent activity in both isolates. Rates of resistance to β-lactam and non-β-lactam antibiotics were as follows (in *K. pneumoniae* versus in *E. coli*, respectively): ampicillin, 100 vs 88 per cent; piperacillin, 100 vs 88 per cent; cefotaxime, 60 vs 75 per cent; ceftazidime, 30 vs 50 per cent; cefpodoxime, 60 vs 75 per cent; ceftriaxone, 60 vs 75 per cent; azteonam, 60 vs 75 per cent; amikacin, 40 vs 25 per cent; gentamicin, 60 per cent vs 75 per cent; netilmicin, 50 vs 19 per cent; and ciprofloxacin, 60 vs 91 per cent. Cefoxitin resistance was low in both *K. pneumoniae* [30% (3/10)] and *E. coli* [6% (1/16)] isolates. All ESBL-negative isolates showed MIC values for cefotaxime, ceftazidime and cefepime in the range of 0.094 - 0.5 µg/ml and values for ESBL-positive isolates are presented in Table II.

The overall percentage of ESBL-positive isolates in cephalosporin/clavulanic acid disk test was 60 per cent (6/10) for *K. pneumoniae* and 75 per cent (12/16) for *E. coli*. Isolates of *K. pneumoniae* and *E. coli* which were positive for ESBL by phenotypic tests showed presence of bla_{CTX-M} group 1. Among ESBL-positive *E. coli* (n=12), bla_{OXA-1} was present in 100 per cent of isolates but none showed the presence of bla_{SHV} and bla_{TEM}. ESBL-positive *K. pneumoniae* (n=6) isolates possessed bla_{OXA-1} [83% (5/6)], bla_{TEM} [100% (6/6)] and bla_{SHV} [67% (4/6)]. Sequencing revealed the presence of $bla_{TEM-1,}$ bla_{SHV-1}, bla_{SHV-28}, bla_{SHV-11}, bla_{SHV-12} and $bla_{CTX-M-15}$ in ESBL-positive isolates (Table II). Plasmid-mediated AmpC β-lactamases could not be detected in any of the cefoxitin-resistant isolates (n=4).

Only seven ESBL-positive isolates (E5, E31, E55, E78, E98, E104 & E113) were found to possess class 1 integron which was confirmed by sequencing. However other classes of integrons could not be identified in any of these strains. Molecular typing of *K. pneumoniae* (n=10) revealed diversity (Fig. 1). Among *E. coli* (n=16) isolates, one clonal cluster was identified which consisted of three isolates (E5, E10 & E31). Another cluster of *E. coli* consisted of two (E27 & E34) closely related isolates (Fig. 2). CTX-M-15 was detected in all isolates that were ESBL-positive irrespective of their genotypes.

Different sizes of megaplasmids (~54.2 Kb upto >212 Kb, data not shown) were isolated from ESBL-positive isolates (12 *E. coli* & 6 *K. pneumoniae*) hourbouring $bla_{CTX-M-15}$ gene. Conjugal transfer of $bla_{CTX-M-15}$ gene carried out for one isolate each of *E. coli* and *K. pneumoniae* was successful. The presence of $bla_{CTX-M-15}$ was confirmed by PCR in the transconjugants and plasmid analysis indicated that these had acquired megaplasmids present in the donor isolates (Fig. 3).

Risk factors for sepsis due to ESBL-producing organisms were examined for neonates (Table III). Among all the factors analysed for acquisition of ESBL-producing organisms, use of enteral feeding tubes was found to be the only significant risk factor (P=0.02). No significant association was found between

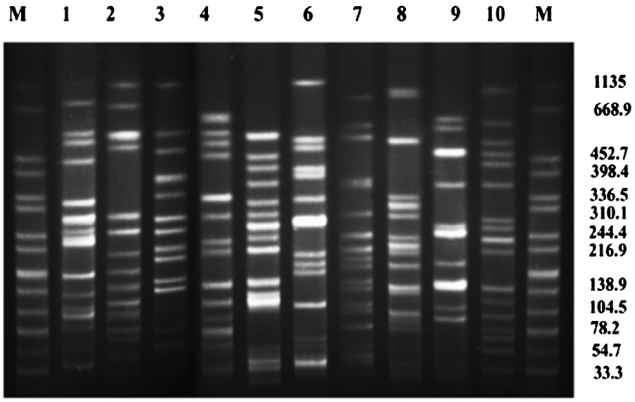

Fig. 1. Pulsed-field gel electrophoresis (PFGE) of *Xba*I-digested genomic DNA of CTX-M-15 producing *K. pneumoniae* (Lane 1-10) isolated from blood of neonates. Lane M: *Salmonella* serotype Braenderup H9812 as reference standard (band sizes in kilobases).

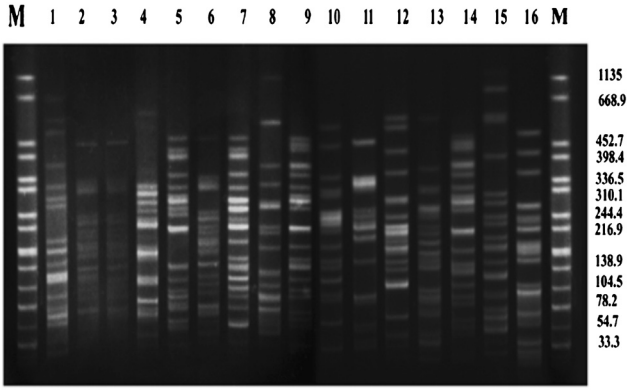

Fig. 2. Pulsed-field gel electrophoresis (PFGE) of *Xba*I-digested genomic DNA of CTX-M-15 producing *E. coli* (Lane 1-16) isolated from blood of neonates. Lane M: *Salmonella* serotype Braenderup H9812 as reference standard (band sizes in kilobases).

Table II. Determination of MIC values and sequence analysis of ESBL genes in ESBL-positive *K. pneumoniae* and *E. coli* isolates

Organisms	MIC of cefotaxime (µg/ml)	MIC of ceftazidime (µg/ml)	MIC of cefepime (µg/ml)	SHV	TEM	OXA	CTX-M
K. pneumoniae (n=6)							
E16	≥32	≥256	12	-	TEM-1	OXA-1	CTX-M 15
E55	≥32	16	4	-	TEM-1	OXA-1	CTX-M 15
E65	≥32	≥256	≥256	SHV-1	TEM-1	OXA-1	CTX-M 15
E98	≥32	≥256	16	SHV-12	TEM-1	-	CTX-M 15
E104	≥32	16	6	SHV-11	TEM-1	OXA-1	CTX-M 15
E113	≥32	16	16	SHV-28	TEM-1	OXA-1	CTX-M 15
E. coli (n=12)							
E1	≥32	32	16	-	-	OXA-1	CTX-M 15
E5	≥32	32	16	-	-	OXA-1	CTX-M 15
E10	≥32	32	4	-	-	OXA-1	CTX-M 15
E27	≥32	32	8	-	-	OXA-1	CTX-M 15
E31	≥32	32	16	-	-	OXA-1	CTX-M 15
E34	≥32	32	64	-	-	OXA-1	CTX-M 15
E63	≥32	16	≥256	-	-	OXA-1	CTX-M 15
E67	≥32	12	6	-	-	OXA-1	CTX-M 15
E78	≥32	8	4	-	-	OXA-1	CTX-M 15
E82	≥32	96	≥256	-	-	OXA-1	CTX-M 15
E91	≥32	128	≥256	-	-	OXA-1	CTX-M 15
E99	≥32	16	3	-	-	OXA-1	CTX-M 15

(-), genes are absent in the isolates; ESBL, Extended-spectrum β-lactamse; MIC, Minimum inhibitory concentration; clinical breakpoints to term the isolates sensitive (S), intermediate (I) and resistant (R) for cefotaxime: ≤8, 16-32 & >64 µg/ml, respectively; for ceftazidime: ≤8, 16 & >64 µg/ml, respectively; for cefepime: ≤8, 16 & >64 µg/ml, respectively

ESBL production in this study group and mortality of neonates.

Discussion

There is limited information about the diversity of the ESBL-producing genes and the clonality of the organisms involved in neonatal infections in India. It is necessary to know whether the same bacterial clones are responsible for the infections or whether dissemination of a particular ESBL-gene via genetically unrelated bacterial clones has been taking place. This study carried out in neonates highlights the presence of ESBL-producing *K. pneumoniae* and *E. coli* of diverse

clonality in neonatal infections. The rate of resistance to most antibiotics was alarming, suggesting that the WHO recommended ampicillin and gentamicin combination as first line treatment of neonatal sepsis may no longer be effective[31]. Resistance to 3rd generation cephalosporins and monobactam was seen in these isolates, being higher amongst *E. coli* isolates. Ciprofloxacin resistance was more frequent in *E. coli* isolates, as reported previously[32]. Carbapenems which are reserve drugs for treatment of neonatal sepsis, showed 100 per cent sensitivity.

bla$_{SHV}$ genes were identified only in *K. pneumoniae* isolates. All different *bla*$_{SHV}$ genes identified were non-ESBL except for SHV-12 which is an ESBL type. In addition, TEM-1 was found only in *K. pneumoniae* and the occurrence of *bla*$_{TEM-1}$ genes were more than *bla*$_{SHV}$ genes in *K. pneumoniae* isolates which is similar to a previous study on neonatal sepsis[20]. The *E. coli* isolates thus harboured a different set of β-lactamase genes in comparison to the *K. pneumoniae* isolates.

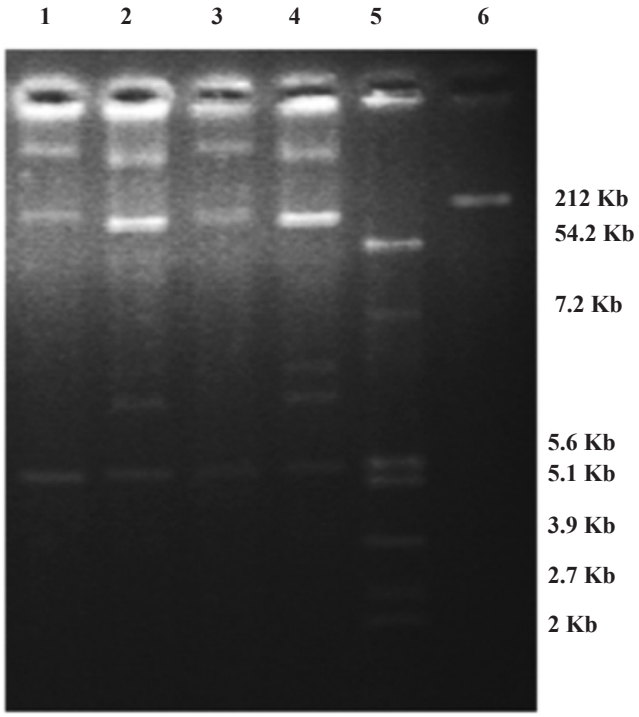

Fig. 3. Plasmid profiles of test isolates and transconjugants of *E. coli* and *K. pneumoniae* hourbouring *bla*$_{CTX-M-15}$. Lanes 1 & 2: transconjugant of *E. coli* and test isolate of *E. coli* from blood of neonates, respectively. Lanes 3 & 4: transconjugant of *K. pneumoniae* and test isolate of *K. pneumoniae* from blood of neonates, respectively. Lanes 5 & 6: *E. coli* K12 V517 and *Shigella flexneri* YSH 6000 were used as reference standards respectively.

Table III. Analysis of risk factors associated with sepsis due to ESBL-producing organisms in neonates (n=23)

Risk factors	Number of neonates	ESBL positive	*P* value
Sex			
Female	8	6	0.657
Male	15	9	
Place of delivery			
Intramural	18	10	0.122
Extramural	5	5	
Weight (g)			
Normal birth weight (≥2500)	9	5	0.657
Low birth weight (<2500)	14	10	
Gestational age (wk)			
Term ≥37	15	9	0.657
Pre-term <37	8	6	
Mode of delivery			
Caesarean	4	2	0.589
Normal	19	13	
Indication of maternal risk[#]			
Present	14	9	1.000
Absent	9	6	
Neonates on ventilation			
Yes	7	5	1.000
No	16	10	
Use of Enteral feeding tubes for feeding / Exclusive breast feeding			
Use of Enteral feeding[$] tubes Exclusive breast feeding	16 7	13 2	0.020[*]

[*]*P*<0.05
[#]Maternal risk factors, maternal pyrexia, meconium stained liquor, urinary tract infection, leaking per vaginum for more than 24 h duration, Hepatitis B positive, anaemia, preeclampsia and delivery with traditional birth attendant;
[$]Neonates fed with enteral feeding tubes were given expressed breast milk. Central venous cathedral was not used

The majority of the *bla*$_{TEM}$ and *bla*$_{SHV}$ genes present were the classical types and were not responsible for the ESBL phenotype. It was the *bla*$_{CTX-M-15}$ gene that was responsible for the ESBL phenotype. PFGE of the CTX-M-15-producing isolates revealed vast diversity and no epidemic clone could be seen. The clonal diversity and the possibility of dissemination of such plasmid mediated genes raise concern as experiments in the

laboratory show that transfer of such genes can occur by conjugation. In addition, the presence of integron-1 in some isolates probably allows dissemination of $bla_{\text{CTX-M-15}}$ gene.

Three *K. pneumoniae* and one *E. coli* isolates were found to be cefoxitin-resistant though plasmid-mediated AmpC β-lactamases were not detected. This could probably occur due to loss of porins in the isolates or the presence of chromosomally-encoded AmpC β-lactamases in the case of *E. coli*[33].

Infection with ESBL-producing organisms was significantly more among the neonates for whom expressed breast milk was fed through enteral feeding tubes in comparison to the neonates fed at the breast. Previous study showed that the use of the enteral feeding tube was an independent risk factor for colonization by multiple antibiotic-resistant *K. pneumoniae* and *E. coli* but the subjects were not neonates[34]. Further studies with a larger population need to be done to establish this association.

Though the study is limited by the small sample size, it highlights the presence of CTX-M-15 in clonally diverse *K. pneumoniae* and *E. coli* isolates indicating that $bla_{\text{CTX-M-15}}$ is probably disseminated horizontally. The high prevalence of ESBL organisms and a transmissible resistance gene ($bla_{\text{CTX-M}}$) is of great concern in a country with high population density and infant mortality rate. Though carbapenems remain the most active antibiotics, the high rate of ESBL-producing organisms may compel clinicians to increase the usage of carbapenems, with a probable increase in carbapenem resistance. The spread of ESBL-producing bacteria necessitates a change towards 'evidence-based' treatment practices, including prudent use of antibiotics.

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