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Extended Spectrum Beta-lactamase (ESBL)-mediated resistance to third generation cephalosporins and conjugative transfer of resistance in Gram-negative bacteria isolated from hospitals in Tamil Nadu, India

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ABSTRACT

Clinical pathogens especially Gram-negative bacteria developing resistance to third-generation cephalosporins are making the clinical outcome more complicated and serious. This study was undertaken to evaluate the distribution of extended-spectrum beta-lactamases in Tamil Nadu regions in India. For this study, clinical samples were collected from five different hospitals located in Tamil Nadu and ESBL producing Gram-negative isolates were characterized. Minimal inhibitory concentration (MIC) was performed using cefotaxime and ceftazidime. The bla_{ESBL} producing genes were screened using multiplex PCR for the genes, CTX-M group-1,-2,-8,-9,-26. Conjugation studies were performed using *E. coli* AB1157 as a recipient for the isolates harboring plasmid-borne resistance following broth-mating experiment. In total, 1500 samples were collected and 599 Gram-negative bacteria were isolated that included *Escherichia coli* (n=233), *Klebsiella pneumoniae* (n=182), *Pseudomonas aeruginosa* (n=79), *Citrobacter* spp. (n=30), *Proteus mirabilis* (n=28), *Salmonella* spp. (n=21), *Acinetobacter baumannii* (n=12), *Serratia* spp. (n=6), *Shigella* spp. (n=4), *Morganella morganii* (n=3) and *Providencia* spp. (n=1). MIC results showed that 358 isolates were resistant to cefotaxime and ceftazidime. Further, ESBL gene amplification results showed that 19 isolates had CTX-M group-1 gene including *E. coli* (n=16), *K. pneumoniae* (n=2) and *P. aeruginosa* (n=1) whereas one *M. morganii* isolate had CTX-M group-9 gene in their plasmid. Through conjugation studies, 12/20 isolates were found to be involved in the transformation of its plasmid-borne resistance gene. Our study highlighted the role of horizontal gene transfer in the dissemination of plasmid-borne bla_{CTX-M} resistance genes among ESBL producing isolates.

Keywords: Beta-lactamase; cephalosporin; cefotaxime; transconjugation; plasmid-borne resistance.

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INTRODUCTION

The arousal of β-lactamase production in bacteria provoked the emergence of beta-lactam resistance. Beta-lactamase is an enzyme which actively participates in the hydrolysis of β-lactam antibiotics; as a consequence, it loses its antibacterial activity against the pathogen [**Paterson *et al.*, 2005**]. ESBL producing Gram-negative bacteria are rapidly evolving and are the up-most clinical vexation by considering the potential risk of disseminating the infection [**Rawat and Nair, 2006**]. The infections caused by such resistant bacteria are increasing over the past decade and has become a worldwide epidemic. Cephalosporin-resistant bacteria were also found to have co-resistance to other antibiotics such as tetracyclines, sulfonamides and aminoglycosides posing serious therapeutic challenge [**Cantón and Coque, 2006**]. Among the β-lactam antibiotics, the third generation cephalosporin plays a vital role in treating serious infections caused by Gram-negative bacteria [**Paterson, 2006**]. The antibacterial activity of cephalosporin group of antibiotics was inhibited greatly by the production of ESBLs. In *Enterobacteriaceae* family, *E. coli* and *K. pneumoniae* were confessed as major ESBL producing organism [**Livermore *et al.*, 2007**]. This enzyme was also effectively produced by other members of the *Enterobacteriaceae* and by certain non-fermenting Gram-negative bacteria [**Livermore *et al.*, 2007**]. The concern towards ESBL producing *Klebsiella* has increased by the report from Germany revealed that there is an emergence of plasmid-mediated resistance to ESBL and was later reported in India and France [**Jain *et al.*, 2003; Upadhyay *et al.*, 2015**]. Urinary tract infections caused by ESBL producing *E. coli* and *K. pneumoniae* are of great concern because of the lesser availability of last-line treatment options [**Salvatore and Resman-Targoff, 2015**]. CTX-M emerge as the prevalent family of ESBL, which can actively be involving in imparting resistance to advanced generations in cephalosporin group of antibiotics [**Livermore, 2012**]. Among the various CTX-M variants, CTX-M-15 was considered to be the more prominent and it was first reported from the Indian subcontinent [**Pitout and Laupland, 2008**]. The emanation of plasmid- mediated resistance and other mobile genetic elements made chances for the spread of resistance genes rapidly by horizontal gene transfer [**Karim *et al.*, 2001**]. The mobilization of resistance genes was greatly encouraged by the various genetic elements like insertion sequences, a segment which provides switch around mechanism of resistance to the bacteria for their survival [**Karim *et al.*, 2001**]. The evolution of bacteria was highly supported by horizontal gene transfer (HGT) by taking up the genes or operons from the pool of mobile genetic elements [**Karim *et al.*, 2001**]. More studies on the prevalence of ESBL producers among nosocomial pathogens and

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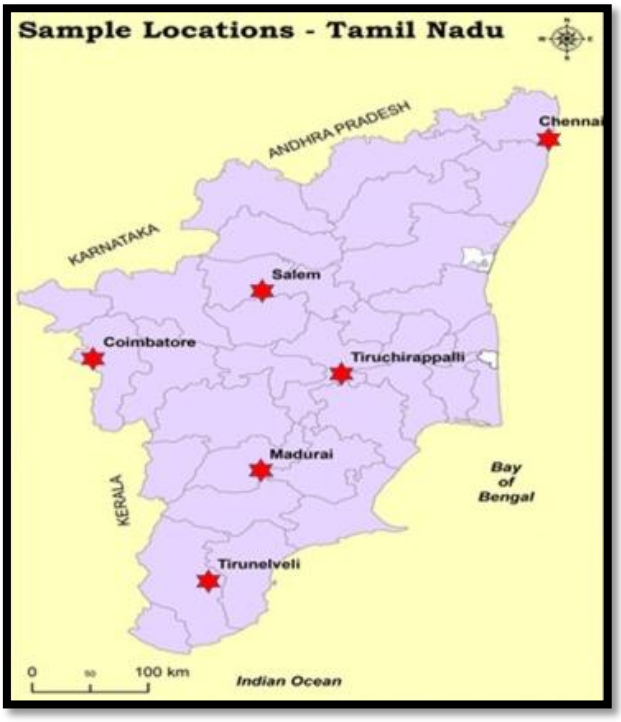
plasmid-mediated resistance transmissibility will provide more insight into the clinical problems. Our study aimed to find out the prevalence of ESBL producers among Gram-negative bacteria isolated from clinical samples within Tamil Nadu, South India and also to reveal the role of bacterial conjugation in disseminating plasmid-borne $bla_{\text{CTX-M}}$ gene.

MATERIAL AND METHODS

Clinical Samples and Study Area

A total of 1500 samples (urine, blood, pus, stool, ET secretion, sputum, semen, vaginal swab, cerebrospinal fluid, throat, swab, wound swab, tracheoscopy, bronchial wash, catheter tip and pleural fluid) were collected from patients during the period of 2010 to 2012. The clinical samples were obtained from various locations in Tamil Nadu from both male and female patients between the age group of 3 to 60 years. The inpatient and outpatient samples from the microbiology laboratories of Billroth Hospital- Chennai, Viva lab- Salem, Miritasanjivini Laboratory - Coimbatore, Bose Laboratory - Madurai, KAPV Medical College - Tiruchirappalli and Annai Vellankanni Hospital– Tirunelveli were collected (fig.1). This study was approved by the Institutional Ethics Committee for Human Research (IEC) No: DM/2010/101/23 project number-4.

Figure 1: Sampling locations: Five different regions were selected in Tamil Nadu for sample collection.



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Isolation and Identification of Clinical Isolates

The samples were collected in sterile containers and were cultured immediately on blood agar and characterized on MacConkey agar plates. Using standard microbiological procedures the clinically important bacteria were isolated from the respective samples. Bacterial isolation mainly focused on *Enterobacteriaceae* and all the isolated strains were identified at species level using automated methods, API 20E identification system (BioMerieux, Marcy, Etoile, France) and further identified using the standardized microbial identification by ID 32GN strips.

Antibiotic Susceptibility Testing

A. Disc diffusion method

Antibiotic susceptibility testing was performed for all the isolates by disc diffusion method and the results were elucidated according to CLSI guidelines. The following antibiotics were used, ampicillin (10 µg), amikacin (30 µg), gentamicin (10 µg), ciprofloxacin (30 µg), ofloxacin (5 µg), cefotaxime (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg),cefpodoxime (10 µg), cefepime (30 µg), aztreonam (30 µg), imipenem (10 µg), meropenem (10 µg), piperacillin-tazobactam (100/10 µg), amoxicillin-clavulanic acid (20/10 µg) and nitrofurantoin (30 µg) (Hi-Media, India). The reference strains used for this study were *E. coli* ATCC - 25988, *K. pneumoniae* ATCC -700603 and *P. aeruginosa* ATCC – 27853. ESBL enzyme producing isolates were determined by the combination disk technique using the test inoculum of 10^5 CFU/mL. The antibiotic disks containing ceftazidime (30 µg), cefotaxime (30 µg) and cefpodoxime (10 µg) were tested alone or in combination with clavulanic acid (10 µg). That isolates that showed an increase in their inhibitory zones, when tested against ceftazidime-clavulanate, cefotaxime-clavulanate or cefpodoxime-clavulanate of at least >5mm when compared to ceftazidime, cefotaxime or cefpodoxime alone respectively, were determined to be ESBL producers.

B. Minimum Inhibitory Concentration

MIC was performed with micro-broth dilution method for ceftazidime and cefotaxime following the CLSI guidelines. In brief, two-fold serial dilutions were done by adding 100 µL of MH broth in 96 well plate and the respective antibiotics were amended with the concentration ranging from 0.25 to 256 mg/L respectively. Later, an inoculum of 10^5 CFU/ml was inoculated on the respective wells and incubated for 16 hours. For this study, *K.*

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pneumoniae ATCC700603 and *E. coli* ATCC25988 were used as positive and negative controls, respectively.

Molecular studies

A. DNA Isolation

DNA was obtained using a modified alkaline lysis method. Briefly, a single colony of each bacterium was inoculated from MacConkey agar into 5 mL of LB broth and incubated at 37°C for 18 h. Cells were pelleted and resuspended in 100 µL of glucose 50 mM, 25 mM tris-Cl and 10 mM EDTA (pH 8.0), and RNase was added to a final concentration of 20 µg/mL. To the solution, 200 µl of 0.2N NaOH, 1% SDS was added. In the tubes, 150 µL of 5M potassium acetate, glacial acetic acid and water was added and stored at 4°C for 5 min and centrifuged. The supernatant was extracted with two volumes of phenol: chloroform. The nucleic acid was precipitated by adding 100% ethanol. The pellet was washed with 70% ethanol and nucleic acid was recovered by centrifugation; the DNA pellet was resuspended in sterile distilled water and stored at 4°C. The plasmid profile of each strain was observed in 0.8% of agarose gel.

B. Polymerase Chain Reaction

The amplification of genes coding for $bla_{\text{CTX-M}}$ was performed by PCR using the primers reported in our previous study [**Ramesh *et al.*, 2015**]. Polymerase chain reaction was accomplished in 50 µL reactions, approximately 10ng of plasmid DNA as a template, reaction volume containing: 10 pmol of each primer, 200µm dNTP, 1.5mM $MgCl_2$, 1X Taq buffer and 2U of Taq polymerase (Genei, Bangalore, India). All the PCR products were sequenced.

Transferability assay

To study the transferability of resistance, mating experiments were performed using plasmid-free *E. coli* AB1157 (Str^r) as recipient strain and all the ESBL producing *E. coli* as donors [**Vaidya, 2011**]. Overnight cultures of both donor and recipient strains grown in MH broth at 37°C were mixed together at 1:10 (v/v) proportion and incubated at 37°C for at least 4 h without shaking. Then, 0.1 mL of the mixture was spread onto the surface of MH agar plates containing streptomycin (100 mg/L) and cefotaxime (2 mg/L). The transconjugants growing on the selection plates were subjected to an ESBL screening, antibiotic susceptibility testing and PCR to confirm the possible acquisition of resistance.

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Statistical analysis

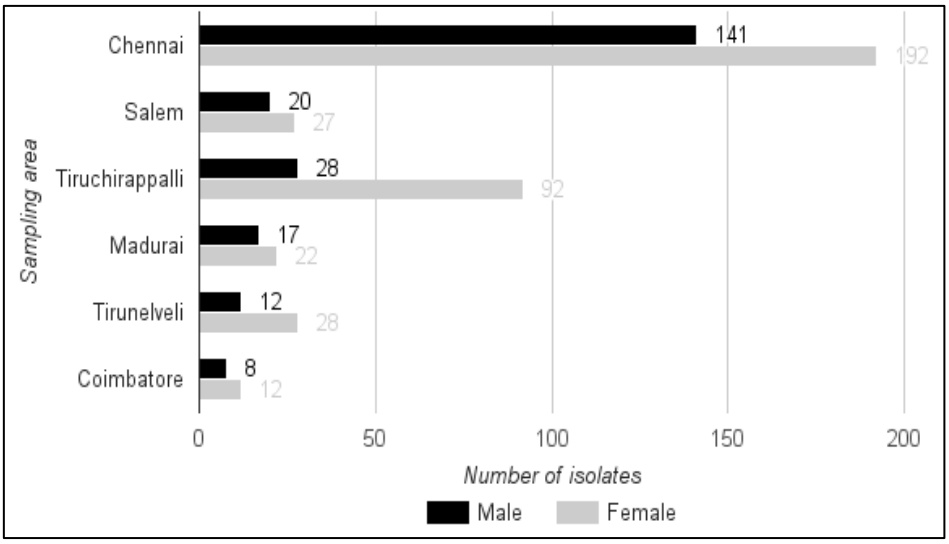
Data were recorded and entered into a Graph Pad software (online) database. Analyses were performed using Chi-square test, or Fisher exact test when appropriate to compare proportions. All statistical analyses were two-sided, and significance was set at $p < 0.05$.

RESULTS

Isolation of Gram-negative bacteria from clinical samples

Out of 1500 samples collected during the study period, 599 (39.9%) Gram-negative bacteria were isolated (Table 1). The clinical distribution of isolates includes, 346 (57.7%) from urine, 80 (13.3%) from stool, 55 (9.1%) from pus, 44 (7.3%) from blood, 31 (5.1%) from wound swab, 17 (2.8%) from sputum, 10 (1.6%) from endotracheal secretion, 4 (0.6%) from throat swab, 3 (0.5%) from bronchial wash, 2 (0.3%) from semen, 2 (0.3%) from vaginal swab, 2 (0.3%) from cerebrospinal fluid, 2 (0.3%) from tracheoscopy, 1 (0.1%) each from catheter tip and pleural fluids. From the samples, a total of 226 isolates from male and 373 from female patients were isolated (fig.2)

Figure 2: Distribution of Gram-negative bacteria among male and female patients in the five sampling locations.



Identification of Clinical Isolates

The isolates belonged to 11 different genera of Gram-negative bacteria including *E. coli* (n=233, 38%), *K. pneumoniae* (n=182, 30%), *P. aeruginosa* (n=79, 13%), *Citrobacter* spp. (n=30, 5%), *Proteus mirabilis* (n=28, 4%), *Salmonella* spp. (n=21, 3%), *A. baumannii* (n=12,

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2%), *Serratia* spp. (n=6, 1%), *Shigella* spp. (n=4, 0.6%), *Morganella morganii* (n=3, 0.5%) and *Providencia* spp. (n=1, 0.1%). Among the isolates, *E. coli* and *K. pneumoniae* were the most predominant species in all the six sampling locations and the majority of the strains were obtained from urine samples followed by stool and pus. The maximum number of the aforementioned Gram-negative bacteria was isolated from the patients between the age group of 15 to 45; in addition, samples collected from females were found to encounter pathogenic Gram-negative bacteria more frequently than from the male patients.

Antibiotic Susceptibility Testing

Among the bacterial pathogens isolated from the clinical samples from various locations in Tamil Nadu, 498 (83.1%) out of 599 strains were found to be cephalosporin-resistant by disk diffusion studies. The antimicrobial sensitivity patterns showed that the overall resistance pattern for ampicillin was 87.5% followed by cefepime (82.5%), cefotaxime (79.2%), ceftriaxone (78.2%), ceftazidime (77.5%), cefpodoxime (76.5%), ofloxacin (70.8%), gentamicin (67.7%), ciprofloxacin (64.5%), amikacin (57.3%), nitrofurantoin (26.5%), piperacillin-tazobactam (21.8%), amoxicillin-clavulanic acid (21.5%), imipenem (3.9%), and meropenem (3.6%). The overall sensitivity for meropenem was 96.4%, followed by imipenem (96.1%), amoxicillin-clavulanic acid (78.5%) and piperacillin-tazobactam (78.1%). The overall prevalence of isolates resistant to cephalosporins was 84.3%, 72.3%, 81.6%, 79.4%, 95% and 80% in Chennai, Salem, Tiruchirappalli, Madurai, Tirunelveli and Coimbatore respectively. In the case of combination disk method, out of 498 isolates screened, only 358 (64.7%) isolates were positive and 140 (35.2%) isolates were negative for ESBL production (Table 2).

MIC was performed for those isolates that were found to be cephalosporin-resistant by disk diffusion and combination-disk methods. Of the 358 isolates screened for the MIC analysis using ceftazidime and cefotaxime, 85% of the isolates showed MIC ≥64 µg/mL. However, cefotaxime and ceftazidime resistant isolates with MIC values >256 µg/mL were 36 and 40 respectively (Table 3). In total, 305 isolates were found to be cefotaxime and ceftazidime resistant through MIC results that included *E. coli* (n=127), *Klebsiella pneumoniae* (n=93), *Pseudomonas aeruginosa* (n=36), *Citrobacter* spp. (n=14), *Proteus mirabilis* (n=15), *Salmonella* spp. (n=9), *Acinetobacter baumannii* (n=5), *Serratia* spp. (n=3), *Shigella* spp. (n=2) and *Morganella morganii* (n=1).

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Table 1. Comparison of prevalence of Gram-negative bacteria among clinical samples of different age group and sex

Clinical sample	Age group	Distribution of clinical samples and isolates						Total number of samples	Total number of isolates
		Samples			Isolates				
		Male	Female	P value	Male	Female	P value		
Urine	Below 15	80	90	0.087	38	49	0.0001	815	346
	b/w15-45	172	163		91	96			
	Above 45	138	172		32	40			
Blood	Below 15	17	11	0.193	2	7	0.076	126	44
	b/w15-45	41	22		6	18			
	Above 45	12	23		3	8			
Pus	Below 15	8	13	0.441	6	9	0.190	152	55
	b/w15-45	44	36		8	15			
	Above 45	19	32		2	15			
Stool	Below 15	21	21	0.399	5	18	0.025	195	80
	b/w15-45	53	42		10	31			
	Above 45	29	29		6	10			
Endotracheal secretion	Below 15	3	2	0.843	1	2	0.333	25	10
	b/w15-45	6	7		1	6			
	Above 45	4	3		0	0			
Sputum	Below 15	9	6	0.885	2	3	0.433	49	16
	b/w15-45	11	13		1	5			
	Above 45	5	5		1	4			
Semen	Below 15	1	2	0.185	0	2	0.529	20	2
	b/w15-45	7	4		0	0			
	Above 45	5	1		0	0			
Vaginal swab	Below 15	0	1	0.725	0	0	1.000	8	2
	b/w15-45	1	3		1	0			
	Above 45	2	1		0	1			
Cerebrospinal fluid	Below 15	1	0	0.499	0	0	0.529	1	2
	b/w15-45	0	0		0	1			
	Above 45	0	0		0	1			
Throat swab	Below 15	2	2	0.813	0	1	1.000	17	4
	b/w15-45	3	4		0	1			
	Above 45	4	2		1	1			
Wound swab	Below 15	11	9	0.409	2	7	0.185	75	31
	b/w15-45	16	17		2	12			
	Above 45	14	8		4	4			

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Tracheoscopy	Below 15	1	0	0.373	0	1	0.529	4	2
	b/w15-45	2	1		0	1			
	Above 45	0	0		0	0			
Bronchial Wash	Below 15	3	2	0.506	1	2	1.000	8	3
	b/w15-45	1	1		0	0			
	Above 45	1	0		0	0			
Catheter Tip	Below 15	0	1	1.000	0	1	0.529	5	2
	b/w15-45	2	0		0	0			
	Above 45	0	2		0	0			

p value = male vs female. b/w- between. $p<0.05$ was considered as statistically significant.

Table 2: Comparison of two different phenotypic test results used to identify the ESBL producer.

Sampling location	Distribution of ESBL producer-phenotypic analysis			
	By disk-diffusion test		By combination-disk method*	
	Total isolates	**ESBL producer**	**Total isolates**	**ESBL producer**
Chennai	333	281 (84.3)	281	188 (66.9)
Salem	47	34 (72.3)	34	34 (100)
Tiruchirappalli	120	98 (81.6)	98	79 (88.7)
Madurai	39	31 (79.4)	31	28 (90.3)
Tirunelveli	40	38 (95)	38	22 (57.8)
Coimbatore	20	16 (80)	16	07 (43.7)
Total (%)	599	498 (83.1)	498	358 (71.8)

*Bacterial isolates that were found to be ESBL producer using disk-diffusion method was further studied for combination-disk method.

Plasmid profiling and multiplex PCR

From the antibiotic susceptibility studies, 40 isolates that had MIC values >256 µg/mL were selected for plasmid profiling. Out of 40 selected isolates, 28 (70%) yielded plasmids of the size ranging from 1 kb to 100 kb while the remaining were found to be plasmid-free. Multiple plasmids were seen in *E. coli, K. pneumoniae* and *P. aeruginosa*. There were some isolates that harbored six or seven plasmids of varying size, for instance, an *E. coli* isolated from urine sample carried seven plasmids and other *E. coli* had six plasmids, and *K. pneumoniae* with five plasmids were isolated from stool samples. Therefore, *E. coli* and *K. pneumoniae* were the most predominant that had more than two plasmids of varying size. All the isolates that had one or more plasmids were subjected to conjugation experiments.

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Plasmid DNA was used to screen CTX-M group genes (1, 2, 8, 9, and 26) and our data revealed that group-1 CTX-M was predominantly found in all the sampling locations included in the study. CTX-M-group-1 which was the most prevalent ESBL gene was detected in 19 isolates (95% of ESBLs), that includes, 16 (80%) of *E. coli*, 2 (10%) of *K. pneumoniae*, 1 (5%) of *P. aeruginosa* and followed by group-9 in one isolate (5%), in *M. morganii*. More than one CTX-M beta-lactamases (Group-1, -9) were found in one *E. coli* strain. None of the isolates from any of the locations had shown the presence of all the five CTX-M group genes. All the amplified CTX-M group (1 and 9) genes were sequenced and GenBank accession numbers were obtained.

Table 3. Comparison of Minimal Inhibitory Concentration of isolates as determined using cefotaxime and ceftazidime.

Sampling location	*MIC results as obtained by micro-broth dilution method			
	Cefotaxime (≤ 256µg/ml)	**Cefotaxime** (≥ 256µg/ml)	**Ceftazidime** (≤256µg/ml)	**Ceftazidime** (≥256µg/ml)
Chennai (n=188)	170	18	175	9
Salem (n=34)	28	4	25	7
Tiruchirappalli (n=79)	70	8	68	13
Madurai (n=28)	28	2	27	1
Tirunelveli (n=22)	21	2	19	6
Coimbatore (n=7)	5	2	4	4
Total (%)	322 (89.9)	36 (10.4)	318 (88.8)	40 (11.4)
	358		358	

*Bacterial isolates that were found to be ESBL producers by earlier phenotypic studies were taken for MIC determination.

Transferability assay

From the molecular studies, 20 isolates that were found to be carrying plasmid-borne CTX-M genes were selected for broth-mating experiments. Among the 20 isolates, 12 were found to be involved in conjugating plasmid-borne $bla_{\text{CTX-M}}$ resistance to *E. coli* AB1157 at the frequency of 10^5. Further, the conjugated plasmids were amplified by multiplex PCR for the presence of CTX-M gene in each transconjugant. As expected, among the 12 positive isolates, 11 isolates were found to carry CTX-M group 1 gene and one isolate was found to carry group 9. The twelve isolates that were involved in transferability includes, eight *E. coli*, two *K. pneumoniae*, one each of *P. aeruginosa* and *M. morganii*.

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DISCUSSION

The prevalence of CTX-M type ESBL producers has progressively increased throughout the health care settings though it may vary between geographical locations [**Manohar *et al.*, 2017a,b; Nachimuthu *et al.*, 2016**]. Infections due to ESBL producing strains are infamous for unfavorable outcomes or high mortality rates. Our study showed that *K. pneumoniae* was the most common (56%, n=75) ESBL producer from the sampling locations, followed by *P. aeruginosa* with 29% (n=39), and *E. coli* with 15% (n=20). Basavaraj *et al.*, have reported that, out of 218 *Enterobacteriaceae* isolates, *E. coli* (57.8%) was most prevalent, followed by *K. pneumoniae* (25.6%), *Citrobacter* sp. (6.5%), *Proteus* sp. (6.5%), *Salmonella* sp. (1.8%) and *Enterobacter* sp. (1.8%) [**Metri *et al.*, 2011**]. In this study, the patients between the age groups of 15 to 45 were most predominantly infected with Gram-negative ESBL producing isolates. The study also recorded that the female patients were most frequently infected than the male patients in all six sampling locations of Tamil Nadu. Similarly, in another study, female patients were found to be infected with ESBL-producing *E. coli* [**Kiratisin *et al.*, 2008**]. The majority of the patients isolated with ESBL producing isolates were over 60 years old and this may be due to the increased hospitalization of the patients in the medical and surgical units. This shows that the antimicrobial resistance in Gram-negative bacteria has built up progressively during the last few decades, leading to increased incidence of outbreaks of infections due to the existence of multi-drug resistant bacteria [**Shahid *et al.*, 2011**].

Our study showed the incidence of ampicillin resistant Gram-negative isolates, followed by cephalosporins such as cefepime, cefotaxime, ceftriaxone, ceftazidime, cefpodoxime, ofloxacin, gentamicin, ciprofloxacin. The overall sensitivity to meropenem was 96.4% followed by imipenem 96.1%, amoxicillin-clavulanic acid (78.5%) and piperacillin-tazobactam (78.1%) and these correlates very well with the findings of the earlier studies in the same geographical region. One prospective study had shown that *Enterobacteriaceae* isolates were resistant to cefotaxime (100%), ceftazidime (76%), cefepime (71%) and aztreonam (42%). Shahzad *et al.*, had found that the most prevalent isolates *Escherichia coli* 58.6%, *Klebsiella Sp.* 32.9% and *Pseudomonas* 8.6%, were resistant to most of the antimicrobials including cefazolin, ceftriaxone, cefuroxime, ampicillin and co-trimoxazole but sensitive to imipenem and meropenem [**Haque *et al.*, 2012**]. Our study found that out of 348 strains screened for the MICs of ceftazidime and cefotaxime, 36 (10%) isolates had MIC >256 µg/ml. A study by Bente-Olesen et al. (2013) showed that, out of 115 ESBL isolates, 92% produced CTX-M enzymes, most commonly CTX-M-15 (52%), but also CTX-M-14

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(19%), CTX-M-1 (11%), and CTX-M-27 (5%) [**Olesen *et al.*, 2013**]. Our present investigation also confirmed that the isolates from Chennai were positive for group-1 and group-9 CTX-M. The CTX-M-group-1 was the most predominant ESBL detected in 19 isolates (95% of ESBLs) followed by group-9 and this was in accordance with another study which detected *bla*$_{\text{CTX-M-1}}$ and *bla*$_{\text{CTX-M-9}}$ at 66.9% (87/130) and 54.6% (71/130) respectively, whereas none of the isolates was positive for *bla*$_{\text{CTX-M-2}}$, *bla*$_{\text{CTX-M-8}}$, and *bla*$_{\text{CTX-M-25}}$ groups [**Shi *et al.*, 2015**].

Further, the role of horizontal gene transfer in spreading antibiotic resistance is being studied. Our study proved that the Gram-negative bacteria with plasmid-borne CTX-M gene can transfer its resistance. The isolates of *E. coli, K. pneumoniae, E. cloacae* and *M. morganii* were found to involve in conjugating its resistance to recipient *E. coli* AB1157. Silva-Sanchez *et al.,* had assayed 104 isolates for mating, of which 68.4% of the isolates were successful, corresponding to 64% of Kp-ESBL 68% of Ecl-ESBL, and 81% of Ecl-ESBL [**Silva-Sanchez et al., 2011**]. In general, most transconjugants were acquired cefotaxime resistance by an ESBL gene encoded on the largest plasmid (70 kb) found in the respective clinical isolate. In addition, these transconjugants co-expressed other antibiotic resistance markers such as aminoglycosides (kanamycin and gentamicin), chloramphenicol and tetracycline.

CONCLUSION

In conclusion, we had found out the dissemination of ESBL producers from various regions within Tamil Nadu, India. The study also characterized the *bla*$_{\text{CTX- M}}$ group-1 followed by group-9 β-lactamases in Gram-negative bacteria isolated from Tamil Nadu, while the prevalence of CTX-M-15 has been reported previously. The study could highlight the efficiency of clinical isolates to conjugate its plasmid-borne resistance. This finding may well explain the reason for the dissemination of resistance determinants within bacteria isolated from nosocomial infections. This study puts forward the importance of using cephalosporins more effectively considering the global scenario of antibiotic resistance.

Acknowledgments: Authors would like to thank Bharathidasan University, Tiruchirappalli, for supporting the study. We would also like to thank Indian Initiative for Management of Antibiotic Resistance (IIMAR) for their partial funding.

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