Annals of R.S.C.B., Vol. 24, Issue 1, 2020, pp. 861- 872
Received 18April2020; accepted 23June2020

Molecular detection of CTX-M gene in extended spectrum β-lactamases producing multidrug-resistant Gram-negative bacterial isolates

M. R. Ramesh Kumar[1], N. Arunagirinathan[1,2]*, Mohamed Adil. A.A[2], Revathi[2]. K, V. Pandiruba[1], P. Indu[1], B. Senthamilselvan[1], Syed Madani Z[1]

[1]Department of Microbiology and Biotechnology, Presidency College (Autonomous), Affiliated to University of Madras, Chennai, Tamil Nadu, India

[2]Central Research Laboratory, Meenakshi Academy of Higher Education and Research(Deemed to be University), Chennai, Tamil Nadu, India

Abstract

Production of β-lactamases is the most important mechanism for bacterial resistance to penicillin, cephalosporins, and carbapenems.Antibiotic resistant bacterial isolates pose a higher burden to health care settings.In this study, extended spectrum β-lactamase (ESBL), metallo β-lactamase (MBL) and AmpC β-lactamase (AmpC) production were screened among Gram-negative bacterial isolates from the clinical specimens and the positivity of CTX-M gene among ESBL producers was analyzed. ESBLs, MBL, and AmpC producers were identified using Combination Disc Method(CDM). Antibiotic susceptibility of bacterial isolates was screened using Kirby-Bauer disc diffusion method. Positivity of CTX-M genein ESBL producing bacterial isolates wasanalyzed by Polymerase chain reaction and DNA sequencing. Out of 73 bacterial isolates, 38 were *Escherichia coli,* 21*Klebsiella pneumoniae,* 10*Pseudomonas aeruginosa,*3 *Proteus vulgaris, and* 1*Enterobacter aerogenes*A total of45 isolates were identified as ESBL producers followed by 14were AmpC, 11 wereboth ESBL and AmpC,and only 2 wereMBL producers. Five ESBL producing isolates showed positive for CTX-M andsequencing analysis showed all five were belonging to CTX-M-15 type.This study finding revealed thatCTX-M-15 type variant was found among ESBL producers. CTX-M-15 gene positive isolates showed positive for multiple β-lactamase production and possess high level of drug resistance profile compared to non-CTX-M positive ESBL producers.

Key words:*Klebsiella pneumoniae*, ESBL, β- lactamases, MBL, AmpC, CTX-M-15.

1. INTRODUCTION

Production of β-lactamases is the most important mechanism for bacterial resistance to β-lactam antibiotics. Especially extended spectrum β-lactamases (ESBLs) producing bacteria are showing resistance to penicillin and cephalosporin antibiotics. ESBLs production is mostly encountered in *Escherichia coli* and *Klebsiellasp.* and other Enterobacteriaceae species[1]. The metallo β-lactamases (MBLs)are requiring divalent cations as a cofactor forits activity and efficiently hydrolyze all β-lactam antibiotics except aztreonam[2].In Gram-negative bacteria, AmpC β-lactamase production is either chromosome or plasmid mediated. AmpC beta-lactamasesare conferring resistance to amino-penicillins, cephalosporins, oxyimino-cephalosporins, cephamycins, and monobactams. Cloxacillin and 3-aminophenylboronic acid inhibit AmpC beta-lactamases, while AmpC beta-lactamase activity is not affected by the ESBL inhibitor clavulanic acid[3,4,5].

Annals of R.S.C.B., Vol. 24, Issue 1, 2020, pp. 861- 872
Received 18April2020; accepted 23June2020

In ESBLs producing bacteria, CTX-M is one of the plasmid-mediated enzymes with significant clinical impact and more than 109 CTX-M variants have been identified. CTX-M producers display a high level of resistance to cefotaxime than to ceftazidime[6].CTX-M variants were classified into the 5 major phylogenetic groups like CTX-M-1, -2, -8, -9, and -25[7,8].The prevalence of CTX-M variants is geographically different, but CTX-M-15 and CTX-M-14 are the most common variants identified inclinically important bacterial etiologies. Most of the ESBL producers are having multidrug-resistant profile and carry other genes that responsible for aminoglycosides and trimethoprim-sulfamethoxazole (TMP-SMX) resistance[9]. Therefore,the spread of ESBLs producing bacterial pathogens in the hospital and community settings causes a significant threat by limiting the therapeutic options[10,11]. In this study, the gram-negative bacterial strains isolated from clinical specimens were studied for ESBL, MBL, and AmpC enzymes production and ESBL producers were analyzed for positivity of CTX-M gene.

2. MATERIALS AND METHODS

Isolation and identification of bacterial isolates

Clinical specimens were streaked on MacConkey agar, 5% sheep blood agar, and Eosine Methylene Blue agar andincubated at 37°C for 24hours to check the growth pattern.Gram-staining, catalase, oxidase, and biochemical tests such as sugar fermentation, indole, methyl-red, Voges-Proskauer, citrate, and nitrate tests were performed for bacterial identification[12].

Combination Disc Methodfor ESBLs, AmpC and MBL production

For identification of ESBL producerscefotaxime and ceftazidime, alone and in combination with clavulanic acid were used.In this method, bacterial culture suspension adjusted to 0.5 McFarland's standard was used tomake lawn culture on Mueller Hinton Agar (MHA) plate. The cefoxitin (30 µg) and cefoxitin- cloxacillin (30 µg/ 200 µg) discs were used for screening of AmpC productionand imipenem (10µg) alone and in combination with EDTA (750µg) were were used for screening of MBL production. After incubating overnight at 37^{o}C, a more than 5mm increase in the zone diameter was interpreted as positive for respective β-lactamase production[13].

Antibiotic Sensitivity Test

The antibiotic susceptibility of the bacteriawasanalyzed using the following antibiotics such as amikacin(30mcg), aztreonam (30mcg), tetracycline (30mcg), piperacillin-tazobactam(100/10mcg), ciprofloxacin (5mcg), co-trimoxazole (25mcg), gentamicin (10mcg), nalidixic acid(30mcg), cefoperazone (75mcg), doxycycline (30mcg), cefotaxime (30mcg), ceftazidime (30mcg), cefoxitin(30mcg) and imipenem (10mcg) (Himedia, Mumbai) were used to determine the resistance profile of the isolates. This test was done on MHA plates using Kirby-Bauer disc diffusion method according to CLSI guidelines[13].

Polymerase Chain Reaction (PCR)

Annals of R.S.C.B., Vol. 24, Issue 1, 2020, pp. 861- 872
Received 18April2020; accepted 23June2020

PCR analysis for CTX-M gene from ESBL producers was carried out usingCTX-MU1 forward primer 5'ATGTGCAGYACCAGTAARGT 3' and CTX-MU2 reverse primer 5' TGGGTRAARTARGTSACCAGA 3'. Cycling parameters include initial denaturation at 94°C for 7min, followed by 35 cycles of denaturation at 94°C for 50 sec, annealing at 50°C for40 sec, amplification at 72°C for 1min and final extension at72°C for5min[14]. 1% agarose gel with ethidium bromide (50 μg/ml) was made in 0.5X TAE buffer was used. 10μL of the amplified PCR product was mixed with 2μL of sample loading dye and electrophoresis was performed at 100V for 20min.

Phylogenetic analysis

The CTX-M gene sequences from this study were aligned with 22 sequences of CTX-M belonging to variousCTX-M groups that wereretrieved from GenBank using the program Muscle software. The aligned sequence was edited and the phylogenetic tree was constructed using MEGA6. The phylogenetic relationships among strains were reconstructed by the Maximum Likelihood method with a boot-strap of 500 using Kimura-2 parameter.

Statistical analysis

Chi-square test was used for determining the significance of association. The p value<0.05 was considered as significant. Statistical analysis was done by SPSS software version 10.0.

3. RESULTS

A total of 73 bacterial strains were isolated from various clinical samples (62 from Urine, 2 from Sputum, 6 from Pus, 2 from Throat swab, and 1 from Wound samples) (Table 1). *E.coli*was the predominantly isolated bacterium in this study. The percentages of isolates were *E.coli*(52.03%),*K.pneumoniae*(28.76%), *P. aeruginosa*(13.69%), *Proteus vulgaris*(4.10%)and *Enterobacter aerogenes*(1.36%). Among the total isolates, 30 (41.09%) were isolated from males and 43 (58.90%) were from females. Sex and age-wise distribution of various clinical isolates was shown in Table 2. A total of 45 isolates were identified as ESBL producers by Combination Disc Method and among them, 44.44% were *K. pneumoniae*, 42.22%were *E.coli*, 6.66%were *P.aeruginosa*, 4.44%were *P. vulgaris* and 2.22%were *E.aerogenes*. Twenty six isolates showed resistance to cefoxitin andamong them, 19.17% were tested positive forAmpC production.Among the AmpC producers, 71.42% were *K.pneumoniae, P. aeruginosa* (14.28%), *P. vulgaris* (7.14%),and *E.coli*(7.14%).Out of 11isolates producing both ESBL and AmpC, 9 were *K. pneumoniae*and 2were *P. aeruginosa*. Ten isolates showed resistance to imipenem and 2.73% of *P. aeruginosa* isolates showed MBL production (Table 4).

Allisolates were screened for MDR profile by Kirby- Bauer disc diffusion method with standard antibiotics. All the clinical isolates showed the highest degree of resistance to cefotaxime (94.52%) followed by nalidixic acid (82.19%), ciprofloxacin (78.08%), and doxycycline (72.60%). Imipenem resistance was noted among 82.19% of the isolates followed

Annals of R.S.C.B., Vol. 24, Issue 1, 2020, pp. 861- 872
Received 18April2020; accepted 23June2020

byamikacin(61.64%), and cefoxitin(56.16%) (Table 3). *E.coli* showed highest resistance to cefotaxime (94.73%) followed by nalidixic acid(84.21%), cefoperazone (81.57%), ciprofloxacin (76.31%), co–trimoxazole (73.68%), doxycycline(73.68%), and tetracycline (71.05%) and highly susceptible to imipenem (97.36%) followed by cefoxitin (73.68%), and amikacin (65.78%). *K. pneumoniae* showed high level of resistance to cefotaxime (95.23%) followed by ceftazidime (90.47%), aztreonam (85.71%), ciprofloxacin (80.95%) and cefoperazone (80.95%) and showed high susceptibility to imipenem (61.90%).*P.aeruginosa* showed 100% resistance to cefotaxime and doxycycline, 80% to nalidixic acid, and 100%sensitive toimipenem, ceftazidime, amikacin and piperacillin-tazobactam. *Proteus vulgaris* showed 100% resistance to doxycyline, tetracycline, ciprofloxacin and 100% sensitive to amikacin, tetracycline and cefoxitin. *Enterobacter aerogenes*. showed 100 % resistance to cefotaxime, ceftazidime, aztreonam, piperacillin-tazobactam, ciprofloxacin, co– trimoxazole and gentamicin (Table 3).

A total of 10 isolates showed resistance to cefotaxime and more than 5mm zone of inhibition was increased when cefotaxime combined with clavulanic acid, hence the above 10 isolates were subjected to PCR for identification of CTX-Mgene. It was found that five ESBL positive bacterial isolates showed positive for CTX-Mgenewith a gene size of 593bp. Molecular characterization of clinical isolates showed 50% positive of CTX-M gene among ESBL producers(Fig. 1). All CTX-M positive isolates showed positive for AmpC β-lactamase production and 2 showed positive for both MBL and AmpC β-lactamase production. CTX-M gene positive isolates showed a high level of drug resistance profile compared to non-CTX-M positive ESBL producing isolates.

All five CTX-M genes were subjected to DNA sequencing and identified that all these five CTX-M genes were belonging to CTX-M-15 type. The accession numbers of gene sequences determined in the present study and deposited in GenBank(KF640078-KF640080 and KF378591-KF378592).A total of 22 CTX-M gene sequencesbelonging to five different groups, namely, CTX-M group1, 2, 9, 8, and 25 were used as the reference sequencesand 5 sequences obtained from our study were used for the construction of the Phylogenetic tree. The Maximum likelihood algorithm with a boot strapping of 500 was employed using MEGA6 software. All CTX-M sequences obtained in thisstudy fell in to the CTX-M group 1, and the sequences were closely related to AY080894, EU082208, FJ973572, AY267213 and FJ668755 whereas HQ734697 and HQ734702 belonging to same group fell into a separate sub lineage (Fig. 2).

Table 1: Bacterial isolates from various clinical samples

S. No	Organism	Clinical Samples				
		Urine	Sputum	Pus	Wound	Throat swab

Annals of R.S.C.B., Vol. 24, Issue 1, 2020, pp. 861- 872
Received 18April2020; accepted 23June2020

1	*Escherichia coli*	37(50.68%)	-	1(1.36%)	-	-
2	*Klebsiella pneumoniae*	15(20.54%)	1(1.36%)	3(4.10%)	-	2(2.73%)
3	*Pseudomonas aeruginosa*	6(8.21%)	1(1.36%)	2(2.73%)	1(1.36%)	-
4	*Proteus vulgaris*	3(4.10%)	-	-	-	-
5	*Enterobacter aerogenes*	1(1.36%)	-	-	-	-

Table 2:Age and sex-wise distribution of bacterial isolates

S.No	**Age**	***Escherichia coli* (38)**		***Klebsiella pneumoniae* (21)**		***Pseudomonas aeruginosa* (10)**		***Proteus vulgaris* (3)**		***Enterobacter aerogenes* (1)**	
		Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
1	1 - 20	3	5	3	2	2	3	2	-	-	-
2	21 – 40	4	8	1	6	-	1	-	1	-	1
3	41 - 60	3	6	3	2	-	1	-	-	-	-
4	Above 60	4	5	4	-	1	2	-	-	-	-
Total		14	24	11	10	3	7	2	1	-	1

Table 3:Antibiotic susceptibility of bacterial isolates from clinical samples

S. No	**Organism**	**Interpretation**	**Antibiotics**													
			AMK	**ATM**	**TET**	**TZP**	**CIP**	**SXT**	**GEN**	**NAL**	**CFP**	**DOX**	**IPM**	**FOX**	**CTX**	**CAZ**
1	*E.coli*	Sensitive (S)	25	5	10	5	7	9	7	5	5	9	37	28	1	21
		Intermediate (I)	9	9	1	22	2	1	12	1	2	1	1	2	1	3

Annals of R.S.C.B., Vol. 24, Issue 1, 2020, pp. 861- 872
Received 18April2020; accepted 23June2020

		Resistant (R)	4	24	27	11	29	28	19	32	31	28	-	8	36	14
2	*K. Pneumonaie*	Sensitive (S)	10	-	9	3	3	10	2	4	2	9	13	7	-	-
		Intermediate (I)	3	3	2	8	1	1	4	1	2	-	1	3	1	2
		Resistant (R)	8	18	10	10	17	10	15	16	17	12	7	11	20	19
3	*P. aeruginosa*	Sensitive (S)	6	1	3	6	-	1	5	1	-	-	8	2	-	6
		Intermediate (I)	1	3	3	1	3	4	1	1	7	-	1	1	-	2
		Resistant (R)	3	6	4	3	7	5	4	8	3	10	1	7	10	2
4	*P. vulgaris*	Sensitive (S)	3	-	-	3	-	-	1	-	-	-	1	3	-	1
		Intermediate (I)	-	2	-	-	-	1	-	-	2	-	-	-	1	-
		Resistant (R)	-	1	3	-	3	2	2	3	1	3	2	-	2	2
5	*E. aerogenes*	Sensitive (S)	1	-	1	-	-	-	-	-	-	1	1	1	-	-
		Intermediate (I)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Resistant (R)	-	1	-	1	1	1	1	1	1	-	-	-	1	1

AMK= amikacin, ATM=aztreonam, TET=tetracycline, TZP= piperacillin-tazobactam, CIP=ciprofloxacin, SXT-trimethoprim-sulfamethoxazole,GEN=gentamicin, NAL=nalidixic acid, CFP=cefoperazone, DOX= doxycycline, IPM= imipenem, FOX=cefoxitin, CTX= cefotaxime, CAZ=ceftazidime

Table 4:Positivity of ESBL, MBL, and AmpC production among bacterial isolates

S. No	**Organisms**	**ESBL**		***p* value**	**AmpC**		***p* value**	**MBL**		***p* value**
		Total	**%**		**Total**	**%**		**Total**	**%**	
1	*E. coli*(38)	19	26.02	1.000	1	01.36	<0.001*	-	-	
2	*K. pneumonia*(21)	20	27.39	<0.001*	10	13.69	0.827	-	-	
3	*P. aeruginosa*(10)	3	04.10	0.206	2	02.73	0.058	2	02.73	0.058
4	*P.vulgaris*(3)	2	02.73	0.564	1	01.36	0.564	-	-	
5	*E. aerogenes.*(1)	1	01.36	1.000	-	-		-	-	

Note: * Statistically significant

Annals of R.S.C.B., Vol. 24, Issue 1, 2020, pp. 861- 872
Received 18April2020; accepted 23June2020

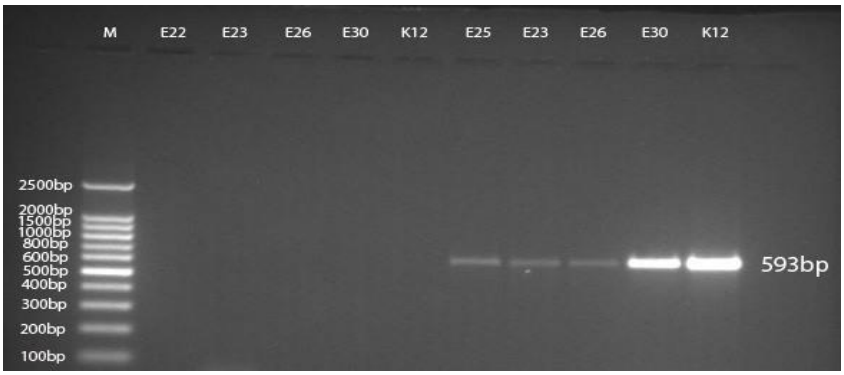

Fig. 1: Positivity of CTX-M gene among ESBL producing bacterial isolates

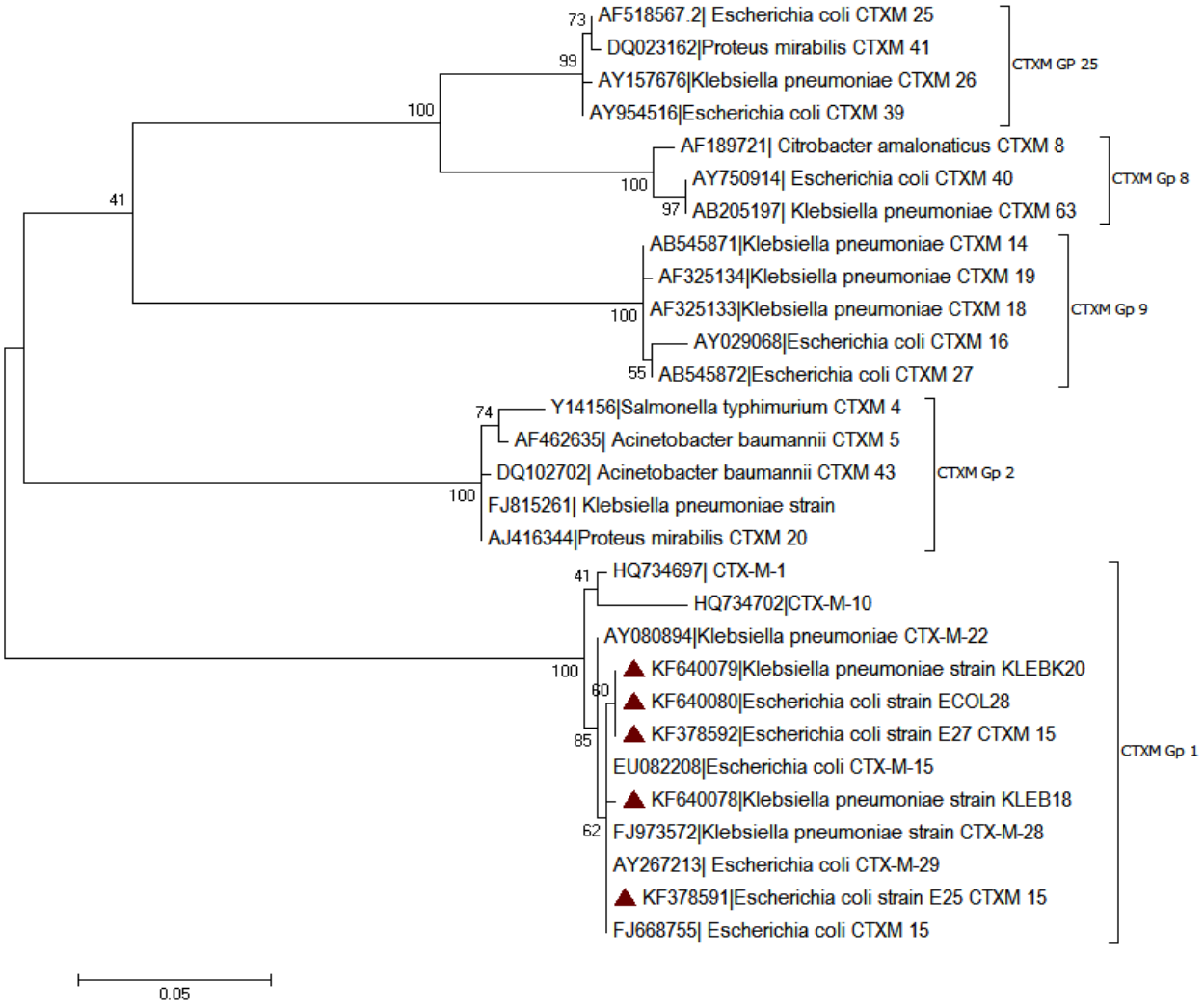

Fig. 2: Phylogenetic tree for comparison of CTX-M gene sequences with CTX-M reference sequences

Annals of R.S.C.B., Vol. 24, Issue 1, 2020, pp. 861- 872
Received 18April2020; accepted 23June2020

4. DISCUSSION

In recent years, the emergence and spread of ESBL producing MDR bacteria has increased worldwide. The spread of CTX-M genes harboring *E. coli*isolates are dramatically increased and they are highly associated with community-acquired infections[15]. Obtaining current epidemiological data of ESBL producing drug-resistant bacteriamight be helpful in the planning of effective empirical therapy and infection control measures.In this study, a total of 73 bacterial isolates were isolated from various clinical samples. Five different Gram-negative bacterial strains were isolated in the study and among them *E.coli* (52.1%) was predominantly isolated followed by *K. pneumoniae* (28.76%), *Pseudomonas aeruginosa* (13.69%), *Proteus vulgaris* (4.1%) *and E. aerogenes*(1.36%). Similar to this study, Abdulrahman *et al.*(2005) [16]also reported that *E.coli*, *Klebsiella sp.*,and *Pseudomonas sp.*are the common isolates obtained in their study.Urinary Tract Infection (UTI) is the most common infectious disease diagnosed in both hospitalized and community patients. The increase in nosocomial UTI is a serious concern because of the longer hospitalization and higher health care costs. The availability of new antimicrobial agents and improved pharmaceutical management of urinary tract disorders has been recently improved. Francesco *et al.* reported that among the pathogens which cause UTI, *E.coli* is the major bacterial pathogen isolated from urine sample[17].This study also showed that out of 62 urine samples processed, 37 showed culture positive for *E.coli*.

In our previous study[18],a high rate of ESBL production (71.01%) was reported in *E.coli*isolatesfollowed by *K.pneumoniae* (36.6%) and *P.aeruginosa* (25%) and the emergence of a multidrug resistance profile in these isolates from patients in Chennai. In this study, it was found that among the urinary isolates,*E.coli* and *K. pneumoniae* showed a high level of ESBL production.According to a study by *Quinn et al*.ESBL producing *E. coli* isolatesshowed resistance to TMP-SMX, nalidixic acid, gentamicin, and ciprofloxacin[19]. This study also revealed that ESBL producers showed resistance to ciprofloxacin, nalidixic acid, gentamicin, andSXT.AmpC beta-lactamase was detected in 14 (19.17%) isolates. Out of 14 isolates, 4 (5.47%) showed resistance only to AmpC beta-lactamases and 10 isolates showed both AmpC and ESBL. The positivity of AmpC production is higher in this study compared to other studies which reported AmpC positive rate as8 and 43%[20-23].

The emergence of MBL producing gram-negative bacterial pathogens poses not only a therapeutic problem and it is also a serious concern for infection control in clinical setting. Hemlatha *et al*. (2005)[24]reported that 16% of *P. aeruginosa* isolates were imipenem resistant and 14% were MBL producers. Behera *et al*.[25] found that 14.8% of *P. aeruginosa*isolates were resistant to imipenem and 10.53% were tested positive for MBL production.

CTX-M type ESBLs is preferentially hydrolysis cefotaxime and in some regions, it has been the most widely disseminated ESBL enzyme in the Enterobacteriaceae[26]. The CTX-M is an

important beta-lactamase enzyme responsible for the high drug resistance profile and it presents either alone or in combination with other ESBL genes[27]. Pournaras *et al.*[28]reported 87% positivity of CTX-M gene in ESBL producers isolated from a tertiary care hospital in Greece. In a multi-centric study from Russia, 35.9% of *E.coli* isolated harbored CTX-M gene and 34.9% of*K. pneumoniae*[29]. In Italy, a national survey study reported that CTX-M producing strains were reported with remarkably variable rates (1.2–49.5% were ESBL producers) among the centres[30].In our study, the CTX-M gene positivity was 50%. This rate is higher when compared to the study of Kumar *et al.*(2015)[5]who reported 21.42% of ESBL producing bacteria harbored CTX-M gene.

In conclusion, ESBLs producers were high compared to MBL and AmpC β-lactamase producers and all β-lactamase producing isolates exhibited a multidrug resistance profile. In this study, all CTX-M gene positive isolates were identified as CTX-M-15 variant type and the findings provided that evidence of CTX-M15 type of ESBLs producing bacteria is majorly present in the community. Multiple β-lactamase production might be the reason for genotypic and phenotypic variations. Strict adherence to the hospital antibiotic policy and good infection control practices could play a significant role in reducing the emerging of antibiotic resistance in bacterial pathogens.

Conflict of Interest: None to declare.

Acknowledgement: Authors would like to acknowledge the Bioinformatics Infrastructure Facility Centre, Presidency College (Autonomous), Chennai for assisting the phylogenetic analysis study.

REFERENCES

1. Teklu DS, Negeri AA, Legese MH, Bedada TL, Woldemariam HK, Tullu KD. Extended-spectrum beta-lactamase production and multi-drug resistance among Enterobacteriaceae isolated in Addis Ababa, Ethiopia. Antimicrobial Resistance & Infection Control. 2019;8(1):39.
2. Bush K, Jacoby GA, Medeiros AA. A functional classification scheme for β-lactamases and its correlation with molecular structure. Antimicrobial Agents and Chemotherapy. 1995; 39(6):1211-1233.
3. Beesley T, Gascoyne N, Knott-Hunziker V, Petursson S, Waley SG, Jaurin B, Grundström, T. The inhibition of class C beta-lactamases by boronic acids. Biochemical Journal. 1983; 209(1):229–233.
4. Jacoby GA.AmpC β-lactamases. Clinical Microbiology Reviews. 2009;22(1):161–182.
5. Tan TY, Ng LS, He J, Koh TH, Hsu LY. Evaluation of screening methods to detect plasmid mediated AmpC in *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus mirabilis*. Antimicrobial Agents and Chemotherapy. 2009; 53(1):146–149.

6. Baraniak A, Fiett J, Hryniewicz W, Nordmann P, Gniadkowski M. Ceftazidime hydrolyzing CTX-M-15 extended-spectrum β-lactamase (ESBL) in Poland. Antimicrobial Agents and Chemotherapy. 2002; 50(3):393–396.
7. Pallecchi L, Bartoloni A, Florelli C, Mantella A, Di Maggio T, Gamboa H, *et al*.Rapid dissemination and diversity of CTX-M extended-spectrum β-lactamase genes in commensal *Escherichia coli* isolates from healthy children from low-resource settings in Latin America. Antimicrobial Agents and Chemotherapy. 2007; 51(8):2720–2725.
8. Mirzaee M, Pourmand MR, Chitsaz M, Mansouri S. Antibiotic resistance to third generation cephalosporins due to CTX-M-type extended-spectrum β-lactamases in clinical isolates of *Escherichia coli*. Iranian Journal of Public Health. 2009; 38(1):10–17.
9. Pitout JD, Nordmann P, Laupland KB, Poirel L. Emergence of *Enterobacteriaceae* producing extended-spectrum beta-lactamases (ESBLs) in the community. Antimicrobial Agents and Chemotherapy. 2005; 56(1), 52–59.
10. Padmini N, Ajilda AA, Sivakumar N, Sureka I, Kumar RS, Selvakumar G. Genetic determination and characterization of extended spectrum β-lactamase producing Escherichia coli and Klebsiella pneumoniae in a tertiary care Hospital, India. 2019; 18 (2): 145-150.
11. Nachimuthu R, Kannan VR, Bozdogan B, Krishnakumar V, Pandiyan S K, Manohar P. CTX-M-type ESBL-mediated resistance to third-generation cephalosporins and conjugative transfer of resistance in Gram-negative bacteria isolated from hospitals in Tamil Nadu, India. Access Microbiology. 2020; 1-8.
12. Cheesbrough M. District Laboratory Practice in Tropical Countries, *part* 2, 2nd ed. Cambridge University Press: London; 2000.
13. CLSI. Performance Standards for antimicrobial disc susceptibility tests. 11th ed. Approved Standards M02–A11, Clinical and Laboratory Standards Institute, Wayne; 2012.
14. Pagani L, Dell'Amico E, Migliavacca R. Multiple CTX-M-type extended-spectrum β-lactamases in nosocomial isolates of *Enterobacteriaceae* from a hospital in Northern Italy. Journal of Clinical Microbiology. 2003; 41(9):4264–4269.
15. Leylabadlo H E, Kafil HS, Yousefi M, Aghazadeh M, Asgharzadeh M. Persistent infection with metallo-beta-lactamase and extended spectrum β-lactamase producer *Morganella morganii* in a patient with urinary tract infection after kidney transplantation. Journal of Natural Science, Biology, and Medicine. 2016;7(2):179.
16. Abdulrahaman AK, Kumar A. Prevalence and antimicrobial susceptibility of extended spectrum beta lactamase producing *Escherichiacoli* and *Klebsiella pneumoniae* in a general hospital.Annals of Saudi Medicine.2005;25(3):239-242.
17. Francesco MA, Ravizzola G, Peroni L, Negrini R, Manca N. Urinary tract infections in Brescia, Italy: etiology of uropathogens and antimicrobial resistance of common uropathogens. Medical Science Monitor. 2007; 13(6):136-144.

18. Ramesh Kumar MR, Archana M, Vijaykanth N, Manikandan N, Arunagirinathan N. Detection of Extended Spectrum β-lactamase Producing Bacteria from Urinary Tract Infections from Chennai. In Proceedings of the Current Scenario in Biotechnology 2012; 252-257.
19. Quinn JP, Bradford PA, Goering RV, Nathan C, Bush K, Weinstein RA. Multiple antibiotic resistant *Klebsiella* and *Escherichia coli* in nursing homes. JAMA. 1999; 281(6):517-523.
20. Liu PYF, Gur D, Hall LMC, Livermore DM. Survey of the prevalence of β-lactamases amongst 1000 Gram-negative bacilli isolated consecutively at the Royal London Hospital. Journal of Antimicrobial Chemotherapy. 1992; 30(4):429-47.
21. Coudron PE. Inhibitor-based methods for detection of plasmid mediated AmpC beta-lactamases in *Klebsiella* spp., *Escherichia coli* and *Proteus mirabilis*. Journal of Clinical Microbiology. 2005; 43(8):4163-7.
22. Singhal S, Mathur T, Khan S, Upadhyay DJ, Chugh S, Gaind R, Rattan A. Evaluation of methods for AmpC beta-lactamase in Gram negative clinical isolates from tertiary care hospitals. Indian Journal of Medical Microbiology. 2005; 23(2):120-124.
23. Manchanda V, Singh NP, Shamweel A, Eideh HK, Thukral SS. Molecular epidemiology of clinical isolates of AmpC producing *Klebsiella pneumoniae.* Indian Journal of Medical Microbiology. 2006; 24(3): 177-181.
24. Hemlatha V, Sekar U, Kamat V. Detection of metallo-betalactamase producing *Pseudomonas aeruginosa* in hospitalized patients. Indian Journal of Medical Research. 2005; 122(2):148-152.
25. Behera B, Mathur P, Das A, Kapil A, Sharma V. An evaluation of four different phenotypic techniques for detection of metallo-betalactamase producing *Pseudomonas aeruginosa*. IndianJournal of Med Microbiology. 2008; 26(3):233-237.
26. Rossolini GM, Andrea MM, Mugnaioli C. The spread of CTX-M-type extended-spectrum beta-lactamases. Clinical Microbiology and Infection. 2008; 14(S1):33–41.
27. Goyal A, Prasad KN, Prasad A, Gupta S, Ghoshal U, Ayyagari A. Extended spectrum β-lactamases in *Escherichia coli* &*Klebsiella pneumoniae* & associated risk factors. Indian Journal of Medical Research. 2009; 129(1):695–700.
28. Pournaras S, Ikonomidis A, Kristo I, Tsakris A, Maniatis AN. CTX-M enzymes are the most common extended-spectrum β-lactamases among *Escherichia coli* in a tertiary Greek hospital. Journal of Antimicrobial Chemotherapy. 2004; 54(2):574–575.
29. Edelstein M, Pimkin M, Palagin I, Edelstein I, Stratshounski L. Prevalence and molecular epidemiology of CTX-M extended-spectrum β-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in Russian hospitals. Antimicrobial Agents and Chemotherapy. 2003; 47(12):3724–3732.
30. Mugnaioli C, Luzzaro F, De Luca F, Brigante G, Perilli M, Amicosante G, Stefani S, Toniolo A, Rossolini GM. CTX-M type extended-spectrum β-lactamases in Italy:

Annals of R.S.C.B., Vol. 24, Issue 1, 2020, pp. 861- 872
Received 18April2020; accepted 23June2020

molecular epidemiology of an emerging countrywide problem. Antimicrobial Agents Chemotherapy. 2006; 50(8):2700–2706.