

PREVALENCE AND MOLECULAR CHARACTERIZATION OF *bla*_{CTX-M-15}-PRODUCING PATHOGENIC GRAM-NEGATIVE BACTERIA FROM VARIOUS CLINICAL SAMPLES

SINDHUJA S, SURESHKUMAR BT, JANAKI S, THENMOZHI S*

Department of Microbiology, Vivekanandha College of Arts and Sciences for Women (A), Elayampalayam - 637 205, Tiruchengode, Namakkal District, Tamil Nadu, India. Email: stmmicro@gmail.com

Received: 25 June 2018, Revised and Accepted: 25 June 2018

ABSTRACT

Objective: The objective of this study was to describe the prevalence and molecular characterization of *bla*_{CTX-M-15}-producing pathogenic Gram-negative bacteria from various clinical samples isolated from clinically suspected patients.

Methods: In this study, clinical samples of urine, stool, sputum, and pus were collected from 244 patients with nosocomial infections. The phenotypic identification of extended-spectrum β -lactamases (ESBL) was confirmed by double-disk synergy test and combined disk diffusion test. *In vitro*, the susceptibility pattern of antimicrobial agents against pathogenic isolates was performed by Kirby-Bauer disk diffusion method. The identification of *bla*_{CTX-M-15}-producing *Escherichia coli* was assessed by polymerase chain reaction method.

Results: The frequency of ESBL-producing pathogenic bacteria from screened was 6 (46.15%). *In vitro*, susceptibility to pathogenic bacteria showed that the majority of isolates were highly susceptible to amoxicillin-clavulanic acid (97.87%), ofloxacin (93.33%), and *Pseudomonas aeruginosa* showed 100% sensitive to ceftazidime, cefotaxime, cefixime, cefoperazone, and meropenem (92.30%). The rates of resistance to other antibiotics varied from <26.66%. Among six tested isolates, only one *E. coli* isolates showed *bla*_{CTX-M-15} gene.

Conclusion: Due to the increase of *E. coli* with multiple ESBL genes, continuous surveillance should be needed in clinical field to use of appropriate antibiotics and the control of infections.

Keywords: CTX-M-15 gene, Clinical samples, Extended-spectrum β -lactamases, *Escherichia coli*, Multidrug resistance.

© 2018 The Authors. Published by Innovare Academic Sciences Pvt Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>) DOI: <http://dx.doi.org/10.22159/ajpcr.2018.v11i10.25111>

INTRODUCTION

Extended-spectrum β -lactamases (ESBLs) are enzymes produced by pathogens belonging to *Enterobacteriaceae*, most commonly *Escherichia coli* and *Klebsiella pneumoniae* [1]. The most common bacterial infections in humans are the urinary tract infections (UTIs), both in the community and the hospitals. In India, the incidence of UTI is about 50,000/million persons per year. Beta-lactam (β -lactam) antimicrobials are characterized by having a four-membered cyclic amide (β -lactam ring) as part of their chemical structure. A β -lactam ring is a four-membered lactam (a lactam is a cyclic amide). It is named as such because the nitrogen atom is attached to the β -carbon atom relative to the carbonyl [2].

ESBLs are known for their ability to hydrolyze β -lactam antibiotics such as penicillins, cephalosporins, and monobactams, resulting in antimicrobial therapy failure [3]. The CTX-M enzymes are the predominant type of ESBL found in many regions of the world, including Asia, South America, Europe, and Africa [4]. In the past 5–10 years, the incidence of infections caused by *Enterobacteriaceae* producing ESBL has increased rapidly mainly attributed to the successful distribution of CTX-M enzymes between *E. coli* causing urinary tract and bacteremic infections [5]. CTX-M-type β -lactamases (CTX-Ms) are broad-spectrum β -lactamases derived from the chromosomally encoded β -lactamases of *Kluyvera* sp. So far, >70 CTX-M types have been isolated and have been divided into five clusters because of amino acid sequence: CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, and CTX-M-25. Native CTX-Ms are cefotaximases that usually hydrolyze cefotaxime rather than ceftazidime. However, point mutations can extend their target spectrum to ceftazidime. Thus, CTX-M-15 and CTX-M-27 are derived by a single Asp240Gly substitution from CTX-M-3 and CTX-M-14, respectively [6].

Infections by ESBL-producing organisms are a worldwide problem. Extensive use of broad-spectrum antibiotics in hospitals has contributed to an increased carriage of *Klebsiella*, and subsequently, the development of multidrug-resistant strains that produce ESBL [7]. Critical care units provide a favorable environment for the antimicrobial-resistant organisms to disseminate. There is recent increase in number of ESBL producers because of the emergence of CTX-M beta-lactamases produced by *Enterobacteriaceae*. They colonize the intestinal flora and spread with greater intensity in the community and hospital. This study was designed to assess the prevalence and molecular characterization of CTX-M-15 (ESBL)-producing pathogenic Gram-negative bacteria (GNB) from various clinical samples.

MATERIALS AND METHODS

Study design

The study was designed to investigate the prevalence and molecular characterization of CTX-M-15-producing pathogenic GNB in various clinical samples. The collection of clinical sample and identification of isolates were carried out in Vivekanandha Medical Care Hospital (VMCH), Elayampalayam, Tiruchengode, Tamil Nadu (India), to purpose of microbiological culture and sensitivity test. In our study, the clinical isolates and its secondary data were obtained from VMCH, and farther process was carried out at the Department of Microbiology in Vivekanandha College of Arts and Science for Women, Elayampalayam, Tiruchengode, Tamil Nadu (India), during the period of March 2016–July 2016.

Inclusion and exclusion criteria

While collecting clinical samples, those clinically suspected subjects who have not taken antibiotic therapy had been included in the study and if the suspected cases that have undergone therapy were excluded from the study.

Identification of pathogenic GNB

The obtained isolates were inoculated into Nutrient agar and MacConkey agar and incubated at 37°C for 24 h. Then, isolated organism was identified according to the Bergey's manual of determinative bacteriology. The confirmed isolates of *E. coli*, *K. pneumoniae*, and *Pseudomonas aeruginosa* were maintained at 4°C on nutrient agar slants for further use.

Antibiotic susceptibility test

Susceptibility testing of bacterial isolates was performed using the disc diffusion method as described by the Clinical and Laboratory Standards Institute (CLSI) [8]. Antimicrobial discs used imipenem (10 mcg), meropenem (10 mcg), ceftazidime (30 mcg), cefotaxime (30 mcg), cefixime (5 mcg), cefoperazone (75 mcg), amoxiclav (amoxicillin/clavulanic acid) (30/10 mcg), aztreonam (30 mcg), amikacin (30 mcg), norfloxacin (5 mcg), ofloxacin (30 mcg), and nitrofurantoin (300 mcg). The diameter of the zone of inhibition for each antibiotic was measured and interpreted as resistant, sensitive. The antibiotic disks used were obtained from Hi-Media Pvt. Ltd., Mumbai, India.

Screening of multidrug-resistant (MDR) isolates

The antibiogram patterns of the GNB were analyzed. The isolates which were showed resistant to more than three classes of antibiotics are considered as a multidrug resistance isolates [9].

Screening test for ESBLs

The isolates were tested for their susceptibility to the third-generation cephalosporins (3 GCs), for example, ceftazidime (30 µg), cefotaxime (30 µg), and ceftriaxone (30 µg) using the standard disc diffusion method as recommended by the CLSI. If a zone diameter of <22 mm for ceftazidime, <27 mm for cefotaxime, and <25 mm for ceftriaxone was recorded, the strain was considered to be "suspicious for ESBL production" [10]. Only those isolates which were resistant to one of the 3 GCs were selected for the study and were processed for ESBL production.

Phenotypic confirmatory tests for ESBLs

The presence of ESBL between selected isolates positive on screening was confirmed using following two methods on *in vitro* condition.

Combination disc method (CDM)

This test requires the use of a third-generation cephalosporin antibiotic disc alone and in combination with tazobactam. In this study, a disk of ceftazidime (30 µg) alone and a disk of ceftazidime+tazobactam (30/10 µg) were used. Both the disks were placed at least 25 mm apart, center-to-center, on a lawn culture of the test isolate on Mueller-Hinton agar (MHA) plate and incubated overnight at 37°C. Difference in zone diameters with and without tazobactam was measured [10].

Interpretation

When there is an increase of ≥5 mm in inhibition zone diameter around combination disk of ceftazidime + tazobactam versus the inhibition zone diameter around ceftazidime disk alone, it confirms ESBL production.

Double-disc synergy test (DDST)

In DDST, synergy was determined among a disc Augmentin (20 mg amoxicillin + 10 µg clavulanic acid) and 30 mg of disc of cefotaxime and ceftazidime antibiotics placed at a distance of 15 mm apart from the center disc on the surface of culture of the resistant isolate under test on MHA. The test organisms were considered to produce ESBL if the zone size around the test antibiotic disc was more than 5 mm and above toward the augmentin disc. This increase occurs because the clavulanic acid present in the Augmentin disc inactivates ESBL enzymes produced by the test organism [11].

Detection of CTX-M

Aztreonam, ceftazidime, cefotaxime, and cefotaxime-clavulanic acid, and ceftriaxone are used to detect CTX-M type ESBL. CTX-M strains

are resistant to cefotaxime and sensitive to cefotaxime plus clavulanic acid. The antibiotics were placed on Muller-Hinton agar (MHA) plate. After incubating overnight at 37°C, more than 5 mm increase in the zone diameter for combination with clavulanic acid was interpreted as positive for CTX-M production.

Isolation of genomic DNA by phenol, chloroform method

The positive isolates were used for the isolation of genomic DNA to detect the CTX-M-15 (ESBL) gene by molecular method. Single colony of the culture of GNB was inoculated into a conical flask containing 50 ml of sterile LB broth (without antibiotic). The culture is incubated overnight at 37°C. After incubation, transfer 2 ml of culture into Eppendorf tube and centrifuged at 10,000 rpm for 5 min at 4°C. Discard the supernatants gently by aspiration. The pellet obtained must be suspected in 1 ml of sodium chloride buffer. Add 250 µl of 10% sodium dodecyl sulfate and mix well. The mixture must be incubated in water bath at 60°C for 15 min. Cool at room temperature and to this mixture adds 500 µl of phenol, chloroform in the ratio 1:2 centrifuge at 10,000 rpm for 5 min at 4°C. Transfer the supernatant in to a fresh eppendorf tube. Equal volume of ice-cold 95% ethanol must add to the supernatant. The reaction mixture must be incubated in ice for 15–30 min (if precipitation is delayed). The DNA can be spooled with the help of a tip and transferred into fresh tube and centrifuged at maximum speed for 5 min and the supernatant can be discarded. The DNA pellet must be washed with 90% ethanol. This step must be repeated once again, final wash was done with 75% ethanol and air dried for 15 min. Dissolve the DNA pellet in 20 µl TE buffer. Add 10 µl of tracking dye in to 20 µl of DNA sample, mix it well, and load the wells (0.7% agarose). Connect electrophoresis apparatus to power pack and perform electrophoresis at 50 V using 1X TBE as the running buffer. Visualize the DNA bands under UV-transilluminator.

Molecular identification of *bla*_{CTX-M-15} (ESBL) gene by polymerase chain reaction (PCR) technique

The isolated DNA was subjected to detect the CTX-M-15 (ESBL) gene using specific primers (Table 1).

PCR technique has been used to amplify genes encoding the CTX-M-15 β-lactamases from genomic DNA of greatest resistant pattern of *E. coli* strain. DNA amplification was performed in an Eppendorf thermal cycler (Roche Co., Germany) in a final volume of 25 µL containing 2.5 µL of ×10 PCR buffer, 2.5 µM of dNTP mix, 10 µM of each primer, 0.2 U of Taq DNA polymerase, and 0.5 µL of the template DNA. PCR conditions for the CTX-M gene composed an initial denaturation step for 5 min at 94°C, followed by 34 cycles of 94°C for 45 s, 55°C for 45 seconds, and 72°C for 1 min, with extension at 72°C for 5 min. The primers used in the above-mentioned reactions can amplify the DNA fragments correspond with 451 base pair (bp) of CTX-M-15 gene. The PCR products were loaded on a 1% (W/V) agarose gel with 0.5 µg/ml ethidium bromide and were analyzed by gel electrophoresis method.

RESULTS

The data regarding this study and other background variables such as age, sex, antibiogram resistance pattern, and enzyme production were elicited from the secondary data obtained from the hospital. The collected samples and information were subjected to statistical analyses to explicate extensively the prevalence of extended β-lactamase products among the isolates in the study area. The study is based on the sample of 244 subjects (Fig. 1).

Table 1: Sequence of the primers used to detect the CTX-M-15 gene

Primers	Sequence	Amplicon size
Forward	5'- AAA AAT CAC TGC GCC AGT TC-3'	415bp
Reverse	5'- AGC TTA TTC ATC GCC ACG TT- 3'	

Isolation and identification of pathogenic GNB

A total of 244 various clinical samples were streaked on nutrient agar, HiCrome UTI agar, and MacConkey agar. The *E. coli* produced pink to purple color and *K. pneumoniae* produced blue color mucoid colonies on HiCrome UTI agar (Fig. 2). *P. aeruginosa* produced green color pigmentation on nutrient agar (Fig. 3). Of the 244 samples, 114 samples showed positive for growth and 130 for no growth. From positive growth, 88 samples showed positive for GNB and 26 samples showed other organisms such as Gram-positive. The isolated colonies

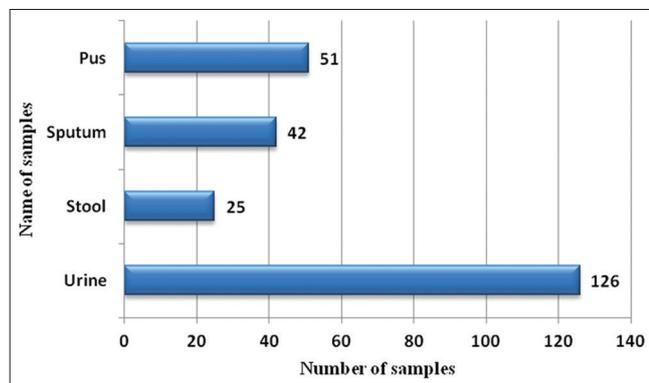


Fig. 1: Collection of clinical samples



Fig. 2: *Escherichia coli* and *Klebsiella pneumoniae* on urinary tract infections agar

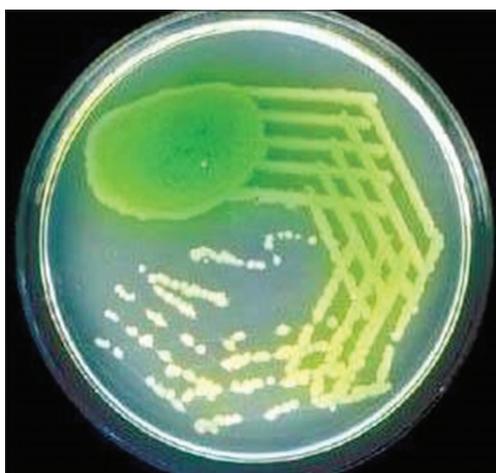


Fig. 3: *Pseudomonas aeruginosa* on nutrient agar

were identified according to the Bergey's manual of determinative bacteriology (Tables 2 and 3).

Antibiotic susceptibility test

A total of 12 antibiotics of seven classes were used against *E. coli*, *K. pneumoniae*, and *P. aeruginosa*. More than 80% of the isolates were sensitive for imipenem, cefixime, amoxycylav, aztreonam, and ofloxacin. Specifically, *P. aeruginosa* exhibited <10% resistance to all antibiotics and 100% sensitive to ceftazidime, cefotaxime, cefixime, cefoperazone, amoxycylav, aztreonam, and ofloxacin (Table 4).

Screening of MDR and ESBL-producing pathogenic GNB

Of the 88 isolates, 18 (66.66%) isolates of *E. coli* and 6 (22.22%) isolates of *K. pneumoniae* were screened to be MDR strains in urine samples. 1 (3.70%) *K. pneumoniae* isolate in pus and 2 (7.40 %) isolates of *K. pneumoniae* in sputum were screened as MDR. In ESBL screening test, 8 (61.53%) *E. coli*, 4 (30.76%) *K. pneumoniae* from urine, and 1 (7.69%) *K. pneumoniae* from pus sample were positive for ESBL production. All the isolates in stool showed negative for both MDR and ESBL (Table 5).

Multidrug resistance pattern of GNB clinical isolates

Of the 88 GNB isolates, the drug resistance pattern shows that among the 37 MDR isolates, 13 (35.1%) show resistant to 1 drug, 7 (18.9%) to 2 drugs, 4 (10.8%) to 3 drugs, 1 (2.70%) to 4 drugs, 2 (5.4%) to 5 drugs, 1 (2.70%) to 6 drugs, 1 (2.70%) to 7 drugs, 3 (8.10%) to 8 drugs, 4 (10.8%) to 9 drugs, and 1 (2.70%) to 10 drugs. Of the resistant organisms, 3 (8.10%) show resistant to 8 drugs, 4 (10.8%) show resistant to 9 drugs, and 1 (2.70%) shows resistant to 10 drugs (Fig. 4).

Phenotypic confirmation of ESBL producers

A total of 13 ESBL isolates screened were subjected to the phenotypic confirmatory methods. In this, 5 (83.33%) isolates of *E. coli* and 1 (16.6%) isolate of *K. pneumoniae* showed strongly positive for ESBL production in both the methods and seven isolates were identified as non-ESBL producers (Table 6 and Figs. 5 and 6).

Age- and sex-wise prevalence of ESBL-producing GNB

The study subjects were classified into three age groups, one isolate of *K. pneumoniae* was found in age group among 20–40 followed by 41–60 for one isolates of *E. coli* and four *E. coli* in the age group of 61–80 years. In sex, males are the more prevalent than female that is distributed in three isolates of *E. coli* in male and two in female and one isolates *K. pneumoniae* were from male (Table 7).

Table 2: Distribution of pathogenic GNB growth in clinical samples

Clinical samples (n=244)	Growth	GNB	Others
Urine (n=126)	72	61	11
Stool (n=25)	4	3	1
Sputum (n=42)	17	10	7
Pus (n=51)	21	14	7
Total	114	88	26

GNB: Gram-negative bacteria

Table 3: Frequency of pathogenic GNB isolates

Clinical samples	n (%)		
	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>P. aeruginosa</i>
Urine (n=126)	44 (93.61)	9 (60)	8 (30.76)
Stool (n=25)	3 (6.38)	0 (0)	0 (0)
Sputum (n=42)	0 (0)	4 (26.66)	6 (23.07)
Pus (n=51)	0 (0)	2 (13.33)	12 (46.15)
Total (n=244)	47 (99.99)	15 (99.99)	26 (99.98)

E. coli: *Escherichia coli*, *K. pneumoniae*: *Klebsiella pneumoniae*,

P. aeruginosa: *Pseudomonas aeruginosa*, GNB: Gram-negative bacteria

Table 4: Antibiotic resistance and sensitivity profile of *E. coli*, *K. pneumoniae*, and *P. aeruginosa*

Antimicrobial agent (mcg)	Symbol	<i>E. coli</i> (47)		<i>K. pneumoniae</i> (15)		<i>P. aeruginosa</i> (26)	
		Sensitive (%)	Resistant (%)	Sensitive (%)	Resistant (%)	Sensitive (%)	Resistant (%)
Beta-lactam drugs							
Carbapenem							
Imipenem (10 mcg)	IPM	38 (80.85)	9 (19.14)	13 (86.66)	2 (13.33)	25 (96.15)	1 (3.84)
Meropenem (10 mcg)	MRP	41 (87.2)	6 (12.7)	11 (73.33)	4 (26.66)	24 (92.30)	2 (7.69)
Cephems							
Ceftazidime (30 mcg)	CAZ	43 (91.48)	4 (8.51)	11 (73.3)	4 (26.66)	26 (100)	-
Cefotaxime (30 mcg)	CTX	44 (93.61)	3 (6.38)	10 (66.66)	5 (33.33)	26 (100)	-
Cefixime (5 mcg)	CFM	41 (87.23)	6 (12.76)	13 (86.66)	2 (13.33)	26 (100)	-
Cefoperazone (75 mcg)	CPZ	40 (85.10)	7 (14.89)	11 (73.3)	4 (26.66)	26 (100)	-
β-lactam/β-lactamase inhibitors combination							
Amoxyclav (amoxicillin/clavulanic acid) (30/10 mcg)	AMC	46 (97.87)	1 (2.12)	15 (100)	-	26 (100)	-
Monobactam							
Aztreonam (30 mcg)	AT	40 (85.10)	7 (14.89)	13 (86.66)	2 (13.33)	24 (92.30)	2 (7.69)
Aminoglycosides							
Amikacin (30 mcg)	AK	35 (74.46)	12 (25.53)	12 (80)	3 (20)	23 (88.46)	3 (11.53)
Fluoroquinolones							
Norfloxacin (5 mcg)	NX	37 (78.72)	10 (21.27)	13 (86.66)	2 (13.33)	24 (92.30)	2 (7.69)
Ofloxacin (5 mcg)	OF	40 (85.10)	7 (14.89)	14 (93.33)	1 (6.66)	23 (88.46)	3 (11.53)
Nitrofurantoin							
Nitrofurantoin (300 mcg)	NIT	36 (76.59)	11 (23.40)	13 (86.66)	2 (13.33)	26 (100)	-

E. coli: *Escherichia coli*, *K. pneumoniae*: *Klebsiella pneumoniae*, *P. aeruginosa*: *Pseudomonas aeruginosa*

Table 5: Screening of MDR and ESBL-producing pathogenic GNB

Clinical samples	Total isolates	GNB	Screened MDR (%)	Screened ESBL (%)
Urine (n=126)	GNB=61	<i>E. coli</i> (44)	18 (66.66)	8 (61.53)
	MDR=24	<i>K. pneumoniae</i> (9)	6 (22.22)	4 (30.76)
	ESBL=12	<i>P. aeruginosa</i> (8)	-	-
Stool (n=25)	GNB=3	<i>E. coli</i> (3)	-	-
	MDR=0	<i>K. pneumoniae</i> (0)	-	-
	ESBL=0	<i>P. aeruginosa</i> (0)	-	-
Pus (n=51)	GNB=14	<i>E. coli</i> (0)	-	-
	MDR=1	<i>K. pneumoniae</i> (2)	1 (3.70)	1 (7.69)
	ESBL=1	<i>P. aeruginosa</i> (12)	-	-
Sputum (n=42)	GNB=10	<i>E. coli</i> (0)	-	-
	MDR=2	<i>K. pneumoniae</i> (4)	2 (7.40)	-
	ESBL=0	<i>P. aeruginosa</i> (6)	-	-
Total			27 (99.98)	13 (99.98)

GNB: Gram-negative bacteria, MDR: Multidrug resistance, ESBL: Extended-spectrum beta lactamase, *E. coli*: *Escherichia coli*, *K. pneumoniae*: *Klebsiella pneumoniae*, *P. aeruginosa*: *Pseudomonas aeruginosa*

Table 6: Phenotypic confirmation of ESBL producers

GNB isolates	Phenotypic confirmatory methods		
	ESBL screened by disc diffusion method (%)	CDM and DDST	
		ESBL (%)	Non-ESBL (%)
<i>E. coli</i> (n=47)	8 (61.53)	5 (83.33)	3 (42.85)
<i>K. pneumoniae</i> (n=15)	5 (38.46)	1 (16.6)	4 (57.14)
<i>P. aeruginosa</i> (n=26)	-	0 (0)	0 (0)
Total (n=88)	13 (14.77%)	6 (46.15%)	7 (53.84%)

E. coli: *Escherichia coli*, *K. pneumoniae*: *Klebsiella pneumoniae*, *P. aeruginosa*: *Pseudomonas aeruginosa*, DDST: Double-disc synergy test, CDM: Combination disc method, ESBL: Extended-spectrum β -lactamases

Age- and sex-wise prevalence of pathogenic GNB

The age-wise distribution of pathogenic GNB is shown in Fig. 7. The age group was classified into five. The age among 61–80 was mostly infected with *E. coli* 30 (63.82%) followed by *K. pneumoniae* 8 (53.33%) and *P. aeruginosa* 12 (46.15%), respectively. The prevalence of pathogenic GNB was more between the male as compared to female. Between the total of 88 isolates, 49 (55.68%) were from male and 39 (44.31%) from female (Fig. 8).

Molecular characterization of *bla*_{CTX-M-15}-producing pathogenic GNB

In PCR experiment, totally six isolates were subjected to detect the *bla*_{CTX-M-15} gene. Among that, only one isolate of *E. coli* gave positive PCR result for CTX-M-15 gene (Fig. 9).

DISCUSSION

The increase of resistance to antibiotics and the existence of multidrug resistant in ESBL producers have become an emerging public health

Table 7: Age- and sex-wise prevalence of ESBL producers

Age group	ESBL producer		Gender	ESBL producer	
	<i>E. coli</i>	<i>K. pneumoniae</i>		<i>E. coli</i>	<i>K. pneumoniae</i>
20-40	0 (0)	1	Male	3	1
41-60	1	0 (0)	Female	2	0
61-80	4	0 (0)			

E. coli: *Escherichia coli*, *K. pneumoniae*: *Klebsiella pneumoniae*, ESBL: Extended-spectrum β -lactamases

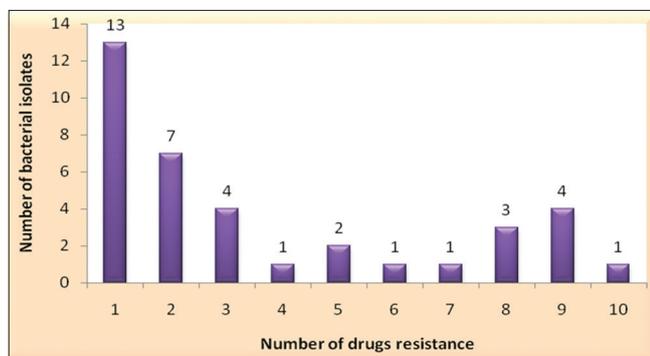


Fig. 4: Multidrug resistance pattern of Gram-negative pathogenic bacteria

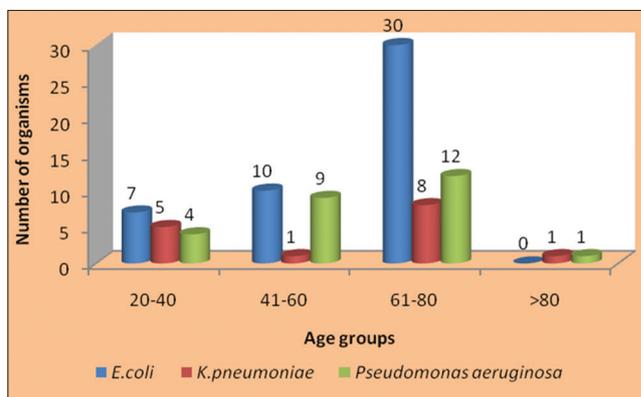


Fig. 7: Age-wise prevalence of pathogenic Gram-negative bacteria

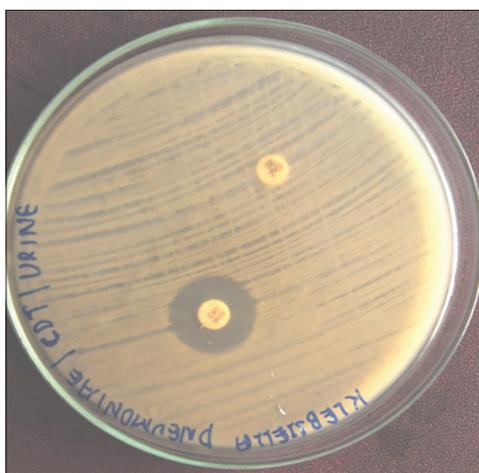


Fig. 5: Combination disc method

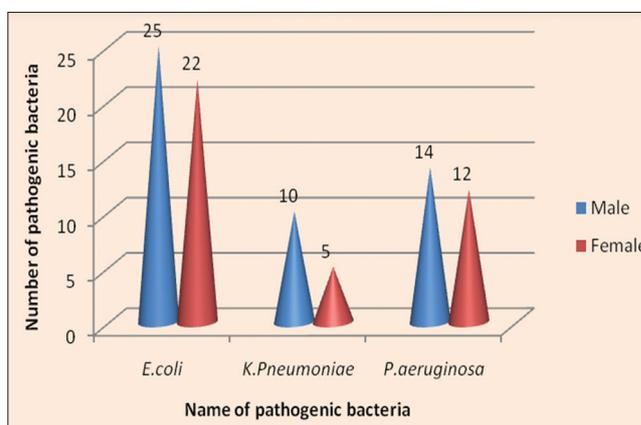


Fig. 8: Sex-wise prevalence of pathogenic Gram-negative bacteria



Fig. 6: Double-disc synergy test

problem due to the clinical failure of empirical treatment protocols [12]. Although the mechanism of resistance between ESBL-producing *E. coli* may be varied, it has been documented that the most common mechanism of resistance to β -lactam antibiotics in *E. coli* isolates is beta-lactamase production.

In a study, there were 100 specimens of *E. coli* isolated from the urine samples and their resistance to antibiotics penicillin, amoxicillin, cephalexin, ceftriaxone, and imipenem was 100%, 100%, 43%, 46%, and 2%, respectively [13]. These results exhibited a high frequency of resistance between *E. coli* isolates to the common antibiotics, especially penicillin, amoxicillin, and cephalosporins such as cefotaxime and ceftazidime which are used routinely in the treatment of UTIs. According to another study that was conducted by the European Antibiotic Resistance Surveillance System in 2006, continual increase of resistant to the third-generation cephalosporins has been reported in over 800 laboratories from 31 countries [14]. Overall, due to indiscriminate use of antibiotics and low standard of personal and community hygiene, the percentage of *E. coli* resistance to chloramphenicol, ampicillin, tetracycline, aminoglycosides, and sulfonamides in developing



Fig. 9: Detection of gene encoding $bla_{\text{CTX-M-15}}$ in extended-spectrum β -lactamases-producing *Escherichia coli* by polymerase chain reaction. Lane 1 - 1Kb DNA ladder, Lane 2 - CTX-M-15 positive for *Escherichia coli*

countries is higher than industrialized countries [15]. Compare to above study, the contrast results were observed >80% of sensitive was observed for imipenem, cefixime, amoxycylav, aztreonam, and ofloxacin. Specifically, *P. aeruginosa* exhibited <10% of resistant to all antibiotics and 100% sensitive to ceftazidime, cefotaxime, cefixime, cefoperazone, amoxycylav, aztreonam, and ofloxacin.

In our study, the most age group infected with ESBL-producing *E. coli* and *K. pneumoniae* was between 41 and 60 and 61–80. Our findings, regarding the age of the patients, are in accordance with recent data that state aging is a risk factor for beta-lactamase-mediated resistance in patients infected with enterobacteria. In recent years, ESBL-producing *E. coli* isolates have increased frequently in different parts of the world, particularly Asia [16]. In our study, the ESBL-producing pathogenic GNB was 46.15% to *E. coli* and *K. pneumoniae* collected over a period of 6 months. The prevalence of ESBL-producing strains varies with geographic region, country studied, and institution. In this respect, several studies expressed that the ESBL production is much less frequent in Europe than in Latin America and Asia, and they are even less frequent in the Pacific than in North America [17]. This may be due to the differences in the type of samples, in the time of sample's collection, consumption of antibiotics, and differences.

The rate of resistance to multiple antibiotics between pathogenic isolates is usually common due to carrying multi-resistant genes and plasmids. In the present study, our isolates were resistant not only to beta-lactam but also to different antibiotic families including monobactam, nitrofurans, fluoroquinolones and aminoglycosides, and carbapenem. This obtained resistance pattern was similar to those commonly described in other studies [18]. As previous noted, the most prevalent types of ESBLs are SHV-, TEM-, and CTX-M-type that arise mainly due to mutations in beta-lactamase genes. We detected a beta-lactamase among the *E. coli* isolates of CTX-M-15 gene expresser. Although TEM and SHV variants were the most common ESBLs during the past decade, newly CTX-M beta-lactamases have emerged as prevalent ESBL worldwide type compared to the TEM and SHV genotypes [19].

The relation of pathogenic and ESBL-producing *Enterobacteriaceae* with respect to age and gender and reported more ESBL-positive isolates in males (65.33%) than females (34.67%) [20]. Similar findings were observed in the present study. Most of the medical laboratories in our country do not screen ESBL production. Appropriate antibiotic therapy and detection of ESBLs in laboratories

need to be carried out to prevent dissemination of these organisms and resistance genes. Our study reports showed that *E. coli* was a most ESBL producers which nearly correlate with a study conducted by Sandhiya et al. [21]. Between the wound/swab isolates, *Pseudomonas* was the predominant GNB followed by *Klebsiella* and *E. coli*. This result is in contrast to Divyashanthi et al. [22] where *Klebsiella* was the predominant GNB.

CONCLUSION

We analyzed the antimicrobial drug susceptibility and molecular detection of CTX-M-15 gene in clinical samples. The prevalence rates of the resistant strains have dramatically increased. These findings suggest that the cefotaxime-resistant isolates are genetically closely related and harbor a chromosome carrying the $bla_{\text{CTX-M-15}}$ gene and posing an urgent threat to public health. Therefore, more comprehensive surveillance is required to prevent farther spread of resistant strains.

ACKNOWLEDGMENT

The authors are thankful to Prof. Dr. M. Karunanithi, Chairman and Secretary, Vivekanandha Educational Institutions, Elayampalayam, and Dr. B. T. Sureshkumar, Principal, Vivekanandha College of Arts and Sciences for Women (Autonomous) Elayampalayam, Tiruchengode, Namakkal District (Tamil Nadu), for providing all the facilities for our research work.

AUTHOR'S CONTRIBUTIONS

Dr. B. T. Sureshkumar designed and supervised the research. S. Sindhuja performed all the experiments in the research. S. Thenmozhi and S. Janaki drafted the manuscript. The final version of the manuscript was revised by Dr. B. T. Sureshkumar.

CONFLICTS OF INTEREST

We have no conflicts of interest.

REFERENCES

1. Kenneth S. Extended-spectrum- β -lactamase, AmpC, and Carbapenemase. *J Clin Microbiol* 2010; 48: 1019-102.
2. Essack SY. The development of β -lactam antibiotics in response to the evolution of β -lactamases. *Pharmaceut Res* 2001; 18: 1391-9.
3. Oberoi L, Singh N, Sharma P, Aggarwal A. ESBL, MBL and AmpC β Lactamases Prodical centering Superbugs - Havoc in the Intensive Care Units of Punjab India. *J Clin Diagn Res* 2013; 7: 70-73.
4. Bonnet R. Growing group of extended spectrum - Lactamases the CTX-M enzymes. *Antimicrob Agents Chemother* 2004; 48(1): 1-14.
5. Abhilash KP, Veeraraghavan B, Abraham OC. Epidemiology and outcome of bacteremia caused by extended spectrum beta-lactamase (ESBL)-producing *Escherichia coli* and *Klebsiella spp.* in a tertiary care teaching hospital in south India. *J Assoc Physicians India* 2010; 58(Suppl): 13-7.
6. Olson AB, Silverman M, Boyd DA, McGeer A, Willey BM, Pong-Porter V. Identification of a progenitor of the CTX-M-9 group of extended-spectrum beta-lactamases from *Kluyvera georgiana* isolated in Guyana. *Antimicrob Agents Chemother* 2005; 49: 2112-5.
7. Parja SC. *Text Book of Microbiology*. Elsevier 2009; 326-329.
8. Wayne PA. Clinical and Laboratory Standards Institute (CLSI). *Performance standards for antimicrobial susceptibility testing: Seventeenth informational supplement*. M100-S17. CLSI; 2007.
9. Fennel J, Vellinga A, Hanahoe B, Morris D, Boyle F, Higgins F, Lyins M, Connell K, Keudy D, Cormican, M. Increasing prevalence of ESBL production among Irish clinical *Enterobacteriaceae* from 2004 to 2008: An observational study. *BMC Infectious Diseases* 2012; 12: 1-8.
10. Wayne PA. Clinical and Laboratory Standard Institute (CLSI). *Performance standards for antimicrobial susceptibility testing, Twenty-First informational supplement M100-S20. Clinical and laboratory standards Institute* 2010; 30: 46.
11. Jarlier V, Nicolas MH, Fournier G, Philippon A. Extended spectrum beta lactamases conferring transferable resistance to newer beta lactam agents in *Enterobacteriaceae*: Hospital prevalence and susceptibility patterns. *Rev Infect Dis* 1988; 10: 867-878.

12. Ahmed OI, Hady SA, Ahmed TM, Ahmed IZ. Detection of bla SHV and bla CTX-M genes in ESBL producing *Klebsiella pneumoniae* isolated from Egyptian patients with suspected nosocomial infections. Egypt J Med Hum Genet 2013; 14(3): 277-83.
13. Abdi S, Ranjbar R, Vala MH, Jonaidi N, Bejestany OB, Bejestany FB. Frequency of bla_{TEM}, bla_{SHV}, bla_{CTX-M} and qnrA among *Escherichia coli* isolated from urinary tract infection. Arch Clin Infect Dis 2014; 9(1): 18690.
14. Gagliotti C, Balode A, Baquero F, Degener J, Grundmann HG, Ur D. *Escherichia coli* and *Staphylococcus aureus*: Bad news and good news from the European Antimicrobial Resistance Surveillance Network (EARS-Net, formerly EARSS), 2002 to 2009. Euro Surveill 2011; 16(11): 19819.
15. Sana T, Rami K, Racha B, Fouad D, Marcel A, Hassan M. Detection of genes TEM, OXA, SHV and CTX-M in 73 clinical isolates of *Escherichia coli* producers of extended spectrum beta-lactamases and determination of their susceptibility to antibiotics. Int Arab J Antimicrob Agents 2011; 1: 1-6.
16. Molton JS, Tambyah PA, Ang BS, Ling ML, Fisher DA. The global spread of healthcare-associated multidrug-resistant bacteria: A perspective from Asia. Clin Infect Dis 2013; 56(9): 1310-8.
17. Coque TM, Baquero F, Canton R. Increasing prevalence of ESBL producing *Enterobacteriaceae* in Europe. Euro Surveill 2008; 13(47): 19044.
18. Mshana SE, Imirzalioglu C, Hossain H, Hain T, Domann E, Chakraborty T. Conjugative IncFI plasmids carrying CTX-M-15 among *Escherichia coli* ESBL producing isolates at a university hospital in Germany. BMC Infect Dis 2009; 9: 97.
19. Barguigua A, Otmani F, Talmi M, Bourjilat F, Haouzane F, Zerouali K. Characterization of extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* isolates from the community in Morocco. J Med Microbiol 2011; 60(9): 1344-52.
20. Shah AA, Hasan F, Ahmed S, Hameed A. Extended spectrum beta-lactamase in *Enterobacteriaceae*: Related to age and gender. New Microbiol 2002; 25: 363-6.
21. Sandhiya R, Lakshmi Priya R, Esthermary S. Antibiotic susceptibility pattern and ESBL prevalence in *Escherichia coli* isolates from pus samples in a tertiary care hospital. Int J Pharm Biol Sci 2015; 7(3): 263-264.
22. Divyashanthi CM, Adithyakumar S, Bharathi N. Study Of Prevalence And Antimicrobial Susceptibility Pattern Of Bacterial Isolates In A Tertiary Care Hospital. Int J Pharm Pharm Sci 2015; 7(1): 185-190.