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Research Article

PREVALENCE OF EXTENDED SPECTRUM BETA-LACTAMASES AMONG ENTEROBACTERIACEAE AND THEIR ANTIBIOGRAM PATTERN FROM VARIOUS CLINICAL SAMPLES

LAVANYA SEGAR[1]*, SHAILESH KUMAR[2], NOYAL MARIA JOSEPH[3], UMADEVI SIVARAMAN[1]

[1]Department of Microbiology, Mahatma Gandhi Medical College and Research Institute, Puducherry, India. [2]Department of Microbiology, Indira Gandhi Institute of Medical Sciences, Patna, Bihar, India. [3]Department of Microbiology, JIPMER, Puducherry, India. Email: lavanyavijay21@gmail.com

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ABSTRACT

Objective: This study was attempted to evaluate the prevalence of extended spectrum beta-lactamase (ESBL) among Enterobacteriaceae from different clinical samples from a tertiary care hospital in Puducherry.

Methods: A total of 204 Gram-negative isolates from different clinical samples were studied. All isolates were identified, and antimicrobial susceptibility testing was done by standard microbiological procedures. ESBL production was detected by phenotypic confirmatory disc diffusion test. The test was carried according to Clinical Laboratory Standards Institute guidelines.

Results: Out of 204 isolates, 78 (38.2%) tested positive for ESBL production. *Klebsiella pneumoniae* (50.9%) showed the maximum ESBL positivity. Amikacin, piperacillin-tazobactam and imipenem are the most effective drugs for the treatment of infections caused by ESBL producing organisms.

Conclusion: High prevalence of ESBL producing Enterobacteriaceae in hospitals, with a tendency for multidrug-resistance, suggests that routine detection is mandatory as this may help in regulating hospital antibiotic policy.

Keywords: Extended spectrum beta-lactamase, Phenotypic confirmatory disc diffusion test, antimicrobial resistance, Enterobacteriaceae.

INTRODUCTION

In early 1980s, third-generation cephalosporins were introduced into clinical practice, and this marked a major breakthrough as they were effective against most beta (β)-lactamase producing organisms [1]. In addition, they had the advantage of less nephrotoxic effects compared to that of aminoglycosides and polymyxins [1]. In 1983, the first report was published on plasmid-encoded β-lactamases capable of hydrolyzing the extended-spectrum cephalosporins [1,2]. β-lactamases are bacterial enzymes that inactivate β-lactam ring containing antibiotics. Extended spectrum β-lactamases (ESBLs) are β-lactamases containing serine in their active site and capable of hydrolyzing penicillins, all cephalosporins, monobactam, and aztreonam, thereby causing resistance to them. However, they do not hydrolyze cephamycin antibiotics such as cefoxitin and cefotetan. They are generally inactive against the carbapenem antibiotics such as imipenem, meropenem, and ertapenem. ESBL's are inhibited by β-lactamase inhibitors such as clavulanic acid, sulbactum, and tazobactam. This property is used to detect and confirm ESBLs.

Approximately, 500 different ESBLs are described, which are due to mutations in the broad-spectrum β-lactamase enzymes that were initially named TEM and SHV (TEM-1, TEM-2, SHV-1) [1].

Intensive care units are often the main source of ESBL production in hospitals [1]. Other units found to be affected by ESBL's are hematology, oncology, burns, neurosurgical, renal, obstetrics and gynecology, and geriatric units [1].

Various risk factors for developing infection with ESBL-producing organisms include seriously ill patients with prolonged hospital stays, prolonged usage of invasive medical devices such as urinary catheters, endotracheal tubes, central venous lines, and increased antibiotic use [1].

At present even, patients without any recognized risk factor for multidrug-resistant organisms are found to have ESBL-producing Enterobacteriaceae [3,4]. Hence, diagnosis of ESBL-producing organisms has become important for both general hospitals and private laboratories.

An important mechanism of antibiotic resistance in Gram-negative bacteria is the production of ESBL's [5]. *Klebsiella pneumoniae* and *Escherichia coli* are the main pathogens producing ESBLs [5]. However, recent reports from various parts of the world, suggest other Enterobacteriaceae, and Pseudomonadaceae [5], also as ESBLs producers. Other ESBL-producing Gram-negative pathogens are *Proteus mirabilis*, *Enterobacter* spp., *Salmonella, Acinetobacter baumannii,* and *Pseudomonas aeruginosa*.

Clinical Laboratory Standards Institute (CLSI) recommends, routine antimicrobial susceptibility testing to include screening isolates for ESBL production using ceftazidime (CAZ), aztreonam, cefotaxime, ceftriaxone or cefpodoxime, followed by phenotypic confirmation of the positive cases [6-8]. Screening tests are based on the ability of the isolates to show decreased susceptibility to extended-spectrum cephalosporins and confirmatory tests are based on the effectiveness of the screening antibiotic in the presence of a β-lactamase inhibitor [5]. This study was attempted to evaluate the prevalence of ESBL among Enterobacteriaceae from different clinical samples from a tertiary care hospital in Puducherry.

METHODS

A total of 204 Gram-negative organisms isolated from various clinical specimens, as part of routine diagnostic activities in the Clinical Microbiology Laboratory of Mahatma Gandhi Medical College and Research Institute, Puducherry, were studied. The study included both outpatients and inpatients, all age groups and both sexes. The samples were processed, and isolates were identified to species level according to standard microbiological laboratory procedures.

Antimicrobial susceptibility testing

All the Gram-negative isolates were subjected to antimicrobial susceptibility testing by the Kirby-Bauer disc diffusion method

according to CLSI guidelines [6]. The antimicrobial panel included ampicillin (10 µg), amoxycillin/clavulanic (CAC) acid (20/10 µg), piperacillin/tazobactam (100/10 µg), imipenem (10 µg), ceftriaxone (30 µg), cefotaxime (30 µg), amikacin (30 µg), gentamicin (10 µg), co-trimoxazole (1.25/23.75 µg), cefaperazone (75 µg), and ciprofloxacin (5 µg). Those isolates that showed resistance to at least three different classes of antimicrobial agents were termed as multidrug-resistant.

Screening test for ESBL

Disk diffusion method for ESBL screening was performed using ceftriaxone (30 µg) and those isolates that displayed resistance were considered positive for a screening test.

The phenotypic confirmatory disc diffusion test (PCDDT)

All strains which gave a positive result in ESBL screening test were subjected to confirmation by PCDDT. The test strain was inoculated onto a plate of Mueller-Hinton agar, and CAZ (30 µg) discs alone and in combination with CAC acid (CAZ + CAC acid, 30/10 µg) were applied [6]. An increase in the zone of inhibition by ≥5 mm around the combination disks as that of cephalosporin alone was considered to be positive [6].

Quality control

E. coli ATCC 25922 was used for the quality control of the Kirby-Bauer disk diffusion method. *K. pneumoniae* ATCC 700603 and *E. coli* ATCC 25922 were used for the quality control of the ESBL testing methods [6].

RESULTS

The present study was conducted in the Department of Microbiology, at a tertiary health care hospital to determine the prevalence of ESBL producing Gram-negative bacilli from various clinical samples. About 204 Gram-negative bacilli were isolated from various clinical samples, out of which 112 were males and 92 females. Antibiotic sensitivity testing done with a panel of antibiotics showed, a maximum sensitivity for imipenem (64.71%) followed by amikacin (59.8%), piperacillin/tazobactam (54.9%), ciprofloxacin (13.23%), and gentamicin (11.27%) (Table 1). A high resistance rate was seen for amoxycillin-clavulanic (99.51%), cefatoxime (99.51%), ceftriaxone (97.06%), and co-trimoxazole (94.61%) (Table 1).

Out of the 204 Gram-negative bacilli, 78 (38.2%) were confirmed as ESBL producers by PCDDT. ESBL positivity was seen more in females (40.2%) as compared to males (36.6%) (Table 2). The age group more commonly affected was between 21 and 30 years (n=27) (Table 2).

K. pneumoniae (50.9%, n=29) showed the maximum ESBL prevalence followed by *Proteus vulgaris* (50%, n=5) and others, as shown by PCDDT.

The maximum ESBL production was seen in sputum (75%, n=6) followed by catheter tips (66.7%, n=2) and others.

Most of the ESBL producers were from intensive care unit (ICU) (n=15, 51.7%) followed by obstetrics and gynecology (n=22, 47.8%) unit and general surgery (n=27, 37.5%).

ESBL producers showed maximum resistance to cefatoxime (77%), cefaperazone (77%), and amoxycillin-CAC acid (77%) while amikacin showed maximum sensitivity (55%) (Table 6).

The ESBL producers had more sensitive isolates for amikacin (70.5%), imipenem (66.7%), and piperacillin/tazobactam (61.5%) (Table 6) as compared to their non-ESBL producing counterparts ($p>0.05$) (Table 7).

Similarly, ESBL producers showed more resistance to amoxycillin-CAC acid (98.7%), cefaperazone (98.7%), and cefotaxime (98.7%) (Table 6) as compared to ESBL non-producers (Table 7).

Table 1: Antibiotic susceptibility pattern of Gram-negative bacilli from various clinical samples

Antibiotics	Sensitive (%)	Resistance (%)
Ampicillin	0.98	98.53
Amoxycillin-CAC acid	-	99.51
Cefaperazone	0.98	99.02
Cefotaxime	-	99.51
Ceftriaxone	0.98	97.06
Co-trimoxazole	4.9	94.61
Amikacin	59.8	27.5
Ciprofloxacin	13.23	84.8
Gentamicin	11.27	86.27
Piperacillin-Tazobactam	54.9	42.65
Imipenem	64.71	34.8

CAC: Clavulanic

Table 2: Age and gender-wise distribution of ESBL producing Enterobacteriaceae in different age groups.

Age interval	Males	Females
0-10	0	0
11-20	1	0
21-30	10	17
31-40	9	9
41-50	6	6
51-60	8	2
61-70	6	2
71-80	1	1

ESBL: Extended spectrum beta-lactamase

Table 3: ESBL producers and their prevalence

Isolates	n (%)
Klebsiella pneumoniae (57)	29 (50.9)
Proteus vulgaris (10)	5 (50)
Pseudomonas aeruginosa (8)	3 (37.5)
Citrobacter (3)	1 (33.3)
Escherichia coli (88)	29 (32.9)
Acinetobacter (35)	11 (31.4)

ESBL: Extended spectrum beta-lactamase

Table 4: ESBL producers and their prevalence

Samples	*n* (%)
BAL (1)	1 (100)
Indwelling devices (1)	1 (100)
Sputum (8)	6 (75)
Catheter tip (3)	2 (66.7)
HVS (2)	1 (50)
Blood (2)	1 (50)
Pus (92)	35 (38.04)
ET TUBE (23)	8 (34.8)
Urine (72)	23 (31.9)

BAL: Bronchoalveolar lavage, HVS: High vaginal swab, ET TUBE: EndoTracheal tube, ESBL: Extended spectrum beta-lactamase

Table 5: Department wise distribution of ESBL producers

Wards	n (%)
General surgery (72)	27 (37.5)
General medicine (22)	6 (27.3)
ICU (29)	15 (51.7)
OG (46)	22 (47.8)
Ophthalmology (1)	1 (100)
Orthopedic (8)	2 (25)
Pulmonary medicine (1)	1 (100)
Urology (20)	4 (20)

ICU: Intensive care unit, ESBL: Extended spectrum beta-lactamase

Table 6: Sensitivity pattern of ESBL positive organisms

Drug	Resistance (%)	Intermediate (%)	Sensitive (%)
Ampicillin	75 (96.1)	1 (1.3)	2 (2.6)
Amoxycillin-CAC acid	77 (98.7)	1 (1.3)	0
Cefaperazone	77 (98.7)	0	1 (1.3)
Cefotaxime	77 (98.7)	1 (1.3)	0
Ceftriaxone	74 (94.9)	3 (3.8)	1 (1.3)
Co-trimoxazole	70 (89.7)	1 (1.3)	7 (8.9)
Amikacin	15 (19.2)	8 (10.3)	55 (70.5)
Ciprofloxacin	60 (76.9)	2 (2.6)	16 (20.5)
Gentamicin	65 (83.3)	3 (3.8)	10 (12.8)
Piperacillin-tazobactam	29 (37.1)	1 (1.3)	48 (61.5)
Imipenem	26 (33.3%)	0	52 (66.7)

ESBL: Extended spectrum beta-lactamase, CAC: Clavulanic

Table 7: Sensitivity pattern of ESBL negative organisms

Drug	Resistance (%)	Intermediate (%)	Sensitive (%)
Ampicillin	126 (100)	0	0
Amoxycillin-CAC acid	126 (100)	0	0
Cefaperazone	125 (99.2)	0	1 (0.8)
Cefotaxime	126 (100)	0	0
Ceftriaxone	124 (98.4)	2 (1.6)	0
Co-trimoxazole	121 (96)	0	5 (3.9)
Amikacin	39 (30.9)	19 (15.1)	68 (53.9)
Ciprofloxacin	112 (88.9)	2 (1.6)	12 (9.5)
Gentamicin	111 (88.1)	2 (1.6)	13 (10.3)
Piperacillin-tazobactam	57 (45.2)	4 (3.2)	65 (51.6)
Imipenem	44 (34.9)	1 (0.8)	81 (64.3)

ESBL: Extended spectrum beta-lactamase, CAC: Clavulanic

Table 8: Prevalence rates of ESBL positive organisms from various studies

Study	Prevalence rate (%)
Subha and Ananthan [13]	6.6
Babypadmini and Appalaraju [14]	40.3
Mathur *et al.* [15]	68
Singhal *et al.* [16]	64
Rodrigues *et al.* [17]	53
Dalela [11]	61.6

DISCUSSION

ESBLs constitute an important antibiotic resistance mechanism by Gram-negative bacteria [1]. Worldwide the incidence of ESBL-producing Enterobacteriaceae has increased and spread significantly [1,5]. Prevention of the emergence and spread of ESBL-producing Enterobacteriaceae, pose a major challenge to the infection control teams. They are associated with high morbidity and mortality, in addition to having very limited therapeutic options [5]. Co-resistance to co-trimethoxazole, aminoglycosides and fluoroquinolones have also been reported in addition to decreased extended spectrum cephalosporins efficacy [5,9]. The major ESBLs producers are *K. pneumoniae* and *E. coli*, but more recently other Enterobacteriaceae, and Pseudomonadaceae have been reported, from various parts of the world [1,10].

Various studies have reported the prevalence rate of ESBL producers to be 6-68% (Table 8) [11-17]. This was in accordance with the findings of our study, which showed a prevalence rate of 38.2%.

Once an ESBL producing strain is detected, the laboratory should report it as "resistant" to all penicillins, cephalosporins, and aztreonam.

CLSI also recommended the use of PCDDT for the phenotypic confirmation of ESBL producers. PCDDT is an inexpensive and technically simple method for detection of ESBL producers. As the laboratory routinely tests, CAZ sensitivity by disc diffusion method, PCDDT requires only one disc to be added to the sensitivity plate. This would screen all Gram-negative bacteria for ESBL production [11].

Even though many laboratories detect and report ESBLs only in *E. coli* and *Klebsiella* species; ESBLs are known to occur in other species of Enterobacteriaceae.

Our study revealed a slight female preponderance for ESBL production, and this was similar to the findings of other studies [9,12].

More than 75% of the studies implicated *K. pneumoniae* as the most common ESBL-producing organism [1]. Similarly in our study, *K. pneumoniae* was the most frequent encountered ESBL-producing organism (Table 3). *K. pneumoniae* (50.9%, n=29) showed the maximum ESBL production, followed by *P. vulgaris* (50%, n=5) and others, as shown by PCDDT.

Intensive care units are the most common areas, affected by ESBL production in hospitals [1]. This may be due to increased use of invasive devices and inappropriate use of newer β-lactam antibiotics which are being routinely prescribed to them [5]. In our study, about 51.7% (n=15) of ESBL isolates were from the ICU, followed by obstetrics and gynecology (n=22, 47.8%) (Table 5). Similar results were reported by other investigators [1,5,12]. Although ophthalmology and pulmonary medicine showed 100%, ESBL production, the sample size was too low (n=1).

Maximum ESBL production was seen in sputum (75%, n=6) followed by catheter tips (66.7%, n=2) and others (Table 4). This was in accordance to the study done by Sharma *et al.* [18]. Although bronchoalveolar lavage and indwelling devices showed 100% ESBL production, their sample numbers were too low (1 each).

In our study, we observed that a majority of the ESBL producers were susceptible to amikacin (55%), followed by imipenem (66.7%) and piperacillin-tazobactam (61.5%). Similarly, few studies showed these antibiotics to have a good activity against Gram-negative bacteria as compared to others [18-23]. Clinically, this of great concern as this leads to a limitation in the prescription of available antibiotics thus emphasizing judicious antimicrobial usage.

Maximum resistance was seen against cefotaxime (77%), cefaperazone (77%), and amoxycillin-CAC acid (77%). Co-resistance to co-trimoxazole (89.7%), ciprofloxacin (76.9%) and gentamicin (83.3%) has also been observed in our study. The efficacy of extended spectrum cephalosporins is compromised while co-resistance to co-trimethoxazole, aminoglycosides and fluoroquinolones has been reported [1,5,9,12,20].

ESBL producing organisms are the most common nosocomial pathogens. They pose a major problem in the area of infectious diseases as they are increasing rapidly and produce multidrug-resistance. Failure to contain ESBL-producing organisms leads to excessive use of carbapenems and the potential emergence of carbapenem-resistant pathogens. At present, the infection control teams face a major challenge in preventing the emergence and spread of ESBL-producing Enterobacteriaceae. In addition to the high morbidity and mortality, they have very limited therapeutic options for treatment.

New technologies such as molecular techniques and modified mass spectrometry technique (matrix assisted light desorption ionization time-of-flight) [24] are being suggested as quicker alternatives for routine laboratory diagnosis. However, these are available only in research facilities and are still new in their development. Hence, routine detection of ESBLs by conventional methods should be done

in every laboratory where molecular methods cannot be performed, as genotyping is useful only for the detection and confirmation of ESBLs and does not help in selection of appropriate antimicrobials.

CONCLUSION

The development and spread of ESBLs have been most likely accounted to the inappropriate use of antibiotics. Infection with strains expressing ESBLs is a challenge for both microbiologists and clinicians as they are having less therapeutic options. High prevalence of ESBL producing Enterobacteriaceae in hospitals, with a tendency for multidrug resistance, suggests that routine detection is mandatory as this may help in regulating hospital antibiotic policy. Monitoring and judicious usage of extended spectrum cephalosporins, periodic surveillance of antibiotic resistance patterns and efforts to decrease empirical antibiotic therapy will pave the way in combating these ESBL producing pathogens. The prevalence rate of ESBL producing Enterobacteriaceae, in our study is 38.2%. Amikacin, piperacillin-tazobactam and imipenem are the most effective drugs for the treatment of infections caused by ESBL producing organisms.

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