Research Article

Occurrence of various β-lactamase enzyme producing Enterobacteriaceae in the hospital effluent: a wake-up call

Anusuya Devi Devaraju, Rajesh Ramachander

Department of Microbiology, Sri Siddhartha Medical College, Tumkur, Karnataka, India.
Correspondence to: Anusuya Devi Devaraju, E-mail: annu151983@gmail.com

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Abstract

Background: Enterobacteriaceae producing extended-spectrum β-lactamase (ESBL), AmpC, and metallo-β-lactamases (MBL) have been increasingly reported worldwide. These organisms usually exhibit multidrug resistance that is not always detected in routine susceptibility tests. This leads to uncontrolled spread of ESBL- and AmpC-producing organisms and related treatment failures. Hence, detection of ESBL, AmpC, and MBL is important in the routine clinical laboratory.

Objective: To investigate the presence of different classes of β-lactamase enzymes in clinical isolates of Enterobacteriaceae.

Materials and Methods: A total of 100 consecutive Enterobacteriaceae, that is, *Escherichia coli*, *Klebsiella* spp., *Citrobacter* spp., and *Proteus* spp., isolates from various clinical samples were included in this study. Detection of ESBL production was carried out by phenotypic confirmatory test as per Clinical and Laboratory Standards Institute guidelines. AmpC production was detected by AmpC disk test and MBL by EDTA disk potentiation test.

Result: Among the 100 clinical isolates tested, ESBL production was seen in 34 (34%), AmpC in 16 (16%), ESBL and AmpC coproduction in 24 (24%), and MBL in 8 (8%) isolates.

Conclusion: The study emphasizes the high prevalence of multidrug-resistant Enterobacteriaceae producing β-lactamase enzymes of diverse mechanisms. Thus proper antibiotic policy and measures to restrict the indiscriminative use of cephalosporins and carbapenems should be taken to minimize the emergence of this multiple β-lactamase-producing pathogens.

KEY WORDS: AmpC β-lactamases, extended-spectrum β-lactamases, coexistence, prevalence, gram-negative bacteria

Introduction

The members of the Enterobacteriaceae are gram-negative, fermentative bacilli and have an important role in nosocomial and acquired infections. The predominant mechanism for resistance to β-lactam antibiotics in gram-negative bacteria is by the synthesis of β-lactamases. β-Lactamases are enzymes produced by some bacteria and are responsible for their resistance to β-lactam antibiotics such as penicillins, cephamycin, and carbapenems.[1,2] β-Lactamase deactivates the molecular antibacterial properties of β-lactam antibiotics, thereby breaking and opening the common element in their molecular structure. Some of these enzymes include extended-spectrum β-lactamase (ESBL), AmpC, and carbapenemase.[2,3]

ESBLs are plasmid-mediated β-lactamase that are capable of efficiently hydrolyzing penicillin, narrow- and broad-spectrum cephalosporins, and monobactams (Aztreonam), but they do not hydrolyze cephamycin or carbapenems (imipenem or meropenem). β-Lactamase inhibitors such as clavulanic acid, sulbactam, and tazobactam are generally inhibit ESBL-producing strains.[3,6] ESBL-producing isolates are most commonly found in *Klebsiella pneumoniae* and in *E. coli*.[4,5]

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AmpC β-lactamase is primarily chromosomal and plasmid-mediated and are resistant to β-lactamase inhibitors, such as clavulanic acid, but can hydrolyze cephamycin. Carbapenems are one of the antibiotics of last resort for many bacterial infections such as *E. coli* and *K. pneumoniae* producing AmpC and ESBL, but the emergence of carbapenamase with versatile hydrolytic capacities has the ability to hydrolyze pencillins, cephalosporins, monobactams, and carbapenems.[6,7]

Infection caused by organisms producing such enzymes has resulted in poor outcomes, reduced rate of clinical and microbiological responses, longer hospital stays, and greater hospital expenses.[8] Physical contact is the most likely mode of transmission and the gastrointestinal tract of colonized or infected patients is the most frequent reservoir while transient carriage of bacteria on the hands of health-care workers may lead to transmission to patients.[8,9]

The spread of these resistant bacteria in hospitals all over the world, conferring multiple antibiotic resistances in the treatment and management of life-threatening infections necessitate this study. With the increase in occurrence and types of these multiple β-lactamase enzymes, early detection is crucial, the benefits of which include implementation of proper antibiotic therapy and infection control policy. Hence, this study was designed to investigate the presence of different classes of β-lactamase enzymes in the clinical isolates of Enterobacteriaceae.

Materials and Methods

A total of 100 consecutive, nonrepetitive clinical isolates of Enterobacteriaceae isolated from various clinical samples, such as pus (34), urine (28), sputum (25), ear swab (6), body fluid (4), and blood (3), were included in this study. All the isolates were identified biochemically by the standard methods (14) and were stored at 4°C in 0.2% semisolid agar until used.

Antimicrobial Susceptibility Testing

The antibiogram of the isolates was determined by the standard Kirby–Bauer disk diffusion method (3). The following antibiotics disks (Himedia, Mumbai) were used, such as ampicillin (10 μg), amikacin (30 μg), gentamicin (10 μg), co-trimoxazole (25 μg), ciprofloxacin (5 μg), cefotaxime (30μg), ceftazidime (30 μg), ceftriaxone (30 μg), cefoxitin (30 μg), and imipenem (10 μg). The zone diameters were interpreted as per Clinical Laboratory Standards Institute recommendations (9). *E. coli* ATCC 25922 strain was used for quality control.

Detection of ESBL Production

Isolates that were resistant to third-generation cephalosporins were tested for ESBL production by combination disk method using cefotaxime (30 μg), cefotaxime/clavulanic acid (10 μg), ceftazidime (30 μg), and ceftazidime/clavulanic acid (10 μg). An increase in diameter of cephalosporin + clavulanate disk inhibition zone ³5 mm when compared to cephalosporin disk alone was interpreted as evidence of ESBL production.[4]

Detection of AmpC Production

Isolates that yielded a cefoxitin zone diameter less than 18 mm and resistant to 3GC (screen positive) were tested for AmpC enzyme production by AmpC disk test.[7] Briefly, 0.5 McFarland suspension of ATCC *E. coli* 25922 was inoculated on the surface of Mueller–Hinton agar plate. A 30 μg cefoxitin disk was placed on the inoculated surface of the agar. A sterile plain disk inoculated with several colonies of the test organism was placed beside the cefoxitin disk almost touching it, with the inoculated disk face in contact with the agar surface.

After overnight incubation at 37°C, the plates were examined for either an indentation or a flattening of the zone of inhibition, indicating enzymatic inactivation of cefoxitin (positive result), or the absence of a distortion, indicating no significant inactivation of cefoxitin (negative result).[7]

Detection of MBL production

Metallo-β-lactamase (MBL) production was detected by meropenem–EDTA disk test. Two 10 μg meropenem disks were placed on the plate, and appropriate amounts of 10 μL of 0.5 M EDTA solution were added to one of them to obtain the described concentration (750 μg). The inhibition zones of meropenem and meropenem–EDTA disks were compared after 16–18 hours of incubation in air at 35°C. If the increase in inhibition zone with meropenem and EDTA disk was ≥5 mm, then the meropenem disk alone was considered to be the MBL producer. Carbapenemase production was further confirmed by modified Hodge test (MHT).[4,8]

Results

Antimicrobial Susceptibility Testing

Out of the 100 total isolates tested, 57 (57%) strains were resistant to 3GC (cefotaxime, ceftazidime, ceftriaxone) while 43 (43%) were susceptible. Majority of the *Klebsiella*, *E. coli*, and *Enterobacter* isolates showed multidrug resistance. They were resistant to at least one non-lactam antibiotic (amikacin, gentamicin, co-trimoxazole, and tetracycline.

ESBL-, AmpC-, and Carbapenemase-Producing Isolates

Out of the 100 isolates screened for ESBL production, 34 were confirmed to produce ESBL giving an overall prevalence of 34%. The highest prevalence of ESBLs was found in *E. coli* (17%), followed by *K. pneumoniae* (12%), *Enterobacter* spp. (2%), *Proteus mirabilis* (1%), and *Proteus vulgaris* (1%), and the least ESBL prevalence was 34% [Table 1, Figure 1].

The susceptibility of the isolates to cefoxitin disk showed that 21 isolates equivalent to 46.3% were either found to be resistant or showed reduced susceptibility to cefoxitin. The overall prevalence of AmpC β-lactamases was 16%. Similar to ESBL, *E. coli* had the highest prevalence of 8% followed by *K. pneumoniae* (5%), *Proteus* spp. (2%), and *Enterobacter* (1%).

Table 1: Prevalence of ESBL, AmpC, and carbapenemase producers among Enterobacteriaceae

Bacterial species	Number of isolates screened	ESBL positive (%)	AmpC positive (%)	MBL positive (%)
Escherichia coli	48	17	8	2
Klebsiella pneumoniae	30	12	5	6
Enterobacter	6	2	1	—
Citrobacter	9	1	—	—
Proteus mirabilis	5	1	1	—
Proteus vulgaris	2	1	1	—
Total	100	34	16	8

ESBL, extended-spectrum β-lactamase; MBL, metallo-β-lactamases.

Table 2: Different b-lactamase-mediated resistance mechanism in AmpC-producing Enterobacteriaceae (*n* = 100).

ESBL + MBL (%)	AmpC + ESBL (%)	AmpC + MBL (%)
16	24	5

ESBL, extended-spectrum β-lactamase; MBL, metallo-β-lactamases.

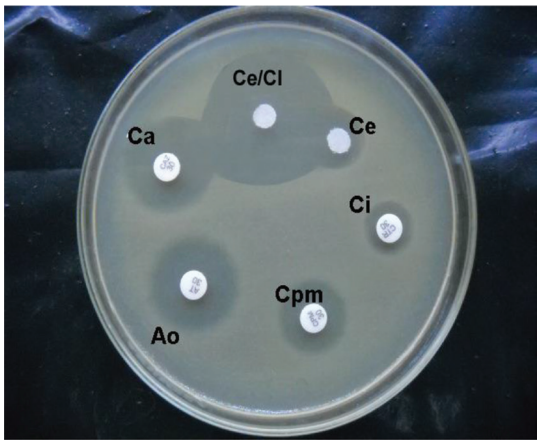

Figure 1: Extended-spectrum β-lactamase detection.

Figure 3: Metallo-β-lactamases detection.

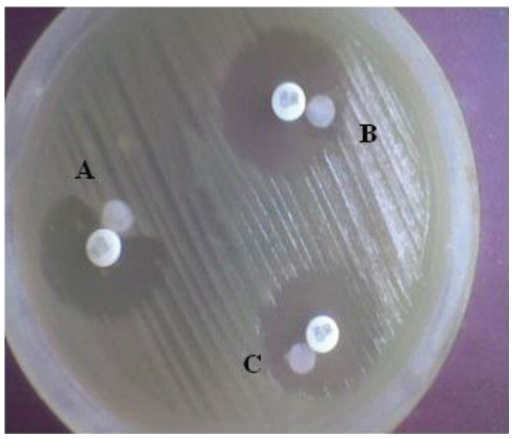

Figure 2: AmpC disk test: presence of blunting toward cefoxitin disk indicates test positive (A) absence of blunting indicates test negative (B and C).

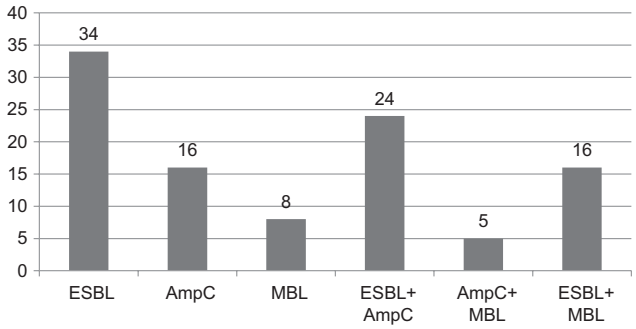

Figure 4: Distribution of β-lactamases. ESBL, extended-spectrum β-lactamase; MBL, metallo-β-lactamases.

Among the AmpC producers, 10% showed indentation (high production of AmpC enzyme) while 6% showed flattening (low production of AmpC enzyme) [Figure 2].

Furthermore, 10 out of the 100 isolates (10%) produce carbapenemase. The highest prevalence of carbapenemase producers was in *K. pneumoniae* (6%) and *E. coli* (2%) [Table 1, Figure3].

Coproduction of ESBL, AmpC, MBL, and Carbapenemase

The coproduction of ESBL, AmpC, MBL, and carbapenemase was also observed among the isolates. Various combinations of different types of enzymes were found particularly in *E. coli* and *K. pneumoniae* [Table 2, Figure 4].

Discussion

The infections that are caused by multidrug-resistant gram-negative bacilli that produce various β-lactamase enzymes have been reported with an increasing frequency and they are associated with a significant morbidity and mortality.[8] The numerous β-lactamases are encoded either by the chromosomal genes or by the transferable genes, which are located on the plasmids or the transposons.[9] Initially, these enzymes were commonly found in the *Klebsiella* spp. and in the *E. coli*, but now, these are produced by all members of Enterobacteriaceae and other gram-negative bacilli.[11] The growing increase in the rate of antibiotic resistance of these isolates is a major cause of concern. β-Lactam has been the mainstay of treatment for serious infections, the most active of these being carbapenems, which are advocated for use in treatment of infections caused by ESBL-producing Enterobacteriaceae,[10,12] particularly, *E. coli* and *K. pneumoniae*. Pathogens that produce ESBL or AmpC β-lactamases along with carbapenemases are particularly challenging for clinicians and are a major threat worldwide.[13,15]

In our study, the prevalence of various β-lactamases in the gram-negative bacteria, which included the Enterobacteriaceae, was 69%, which was alarmingly high. The ESBL production was (34%) found to be maximum as compared to the other β-lactamases. According to the mentioned studies, it seems that the prevalence of β-lactamases-producing Enterobacteriaceae in different parts of the world can be varied from 0% to more than 70%. This difference could be due to the factors such as differences in the type and mode of antibiotic consumption that cause genetic mutations in bacteria and producing the mentioned enzymes.[16] In addition, cultural, nutritional, and ethnic differences in various populations caused variations in the normal flora.[17] Different phenotypic methods in various studies could also be another reason.

Out of the 21 (21%) of the isolates showing resistance to cefoxitin in this study, only 16 (16%) were AmpC producers. Cefoxitin resistance in this type of AmpC-negative isolates could be due to a decreased permeability of porins. It was 17.3% in Kolkata[15] and 22.9% in a study that was done by Bandekar et al.,[11] in burn patients, whereas a study that was conducted by Bhattacharjee et al.[12] showed 22% AmpC-producing *Pseudomonas aeruginosa*.

In our study, 8% of the isolates were MBL producers. Several studies from India have shown a prevalence rate of 8%–10% of Enterobacteriaceae isolates being carbapenemase producers.[19]

The coexistence of ESBL and MBL was reported in 16% isolates, whereas the AmpC and MBL coproduction was shown by 5% isolates and the AmpC and ESBL coproduction was shown by 24% isolates. A study that was conducted by Arora et al. reported the AmpC and MBL coproduction in 46.6% isolates and the ESBL and AmpC coproduction in 3.3% isolates.[15]

The increase in the prevalence of the AmpC-, MBL-, and ESBL-producing isolates may be indicative of the ominous trend of more and more isolates acquiring the resistance mechanisms, thus rendering the antimicrobial armamarium ineffective. In our study, the multidrug-resistant strains showed co-resistance to the fluoroquinolones and aminoglycosides, but they were moderately susceptible to imipenam and the ampicillin-sulbactam combination, which was in concordance with the findings of other studies.[17,18]

Conclusion

Microbiology laboratories must be able to detect resistant pathogens in a timely manner, especially those that are falsely susceptible in vitro to drugs that may be considered for therapy of infected patients. Hence routine Antibiotic Sensitivity Testing must include disks of ceftazidime and cefotaxime for detection of ESBLs, and imipenam and imipenam + EDTA for detection of MBLs. We found that all cefoxitin-resistant strains showing a zone £14 mm were found to be AmpC producers. So this could be used for presumptive identification of AmpC producers. Inclusion of MHT in routine may not be very useful as per today, as many MBL producers were not detected by this test. It also cannot be carried out easily on routine basis. So MHT is also known to produce false positives in ESBL-producing organisms.

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