

Phenotypic detection of extended spectrum, AmpC, Metallo beta-lactamases and their coexistence in clinical isolates of commonly isolated gram negative bacteria in GKGH hospital, Bhuj

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Abstract

Broad-spectrum beta lactam antibiotics are the most commonly used antibiotics in the clinical setting. Resistance to these beta lactam antibiotics mediated by extended spectrum beta lactamases (ESBLs), AmpC beta lactamases and Metallo beta lactamases (MBLs) enzymes is an increasing common public health problem. Determination of their prevalence is essential for epidemiological purpose and to formulate an effective antibiotic policy to avoid treatment failure.

A total of 934 clinical isolates comprising of *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus spp.* And *A cinetobacter baumannii*, which were recovered from various clinical specimens over a period of a year i.e., from January 2017 to December 2017, were studied. Antibiogram profile was determined by Kirby-Bauer disc diffusion method and was interpreted by using CLSI guidelines. Detection of ESBL was done using ceftazidime and ceftazidime-clavulanic acid combination discs method, AmpC producers were detected by AmpC disc test and MBL production was done using Imipenem-EDTA combined disc test.

Among 934 isolates 246(26.3%) strains were ESBL producers, 91(9.7%) AmpC producers and 143(15.3%) were MBL producers. The co-production of ESBL/AmpC/MBL beta lactamases was observed in 160(17.1%).

The study emphasizes the need for the early detection of isolates that produce these enzymes to avoid therapeutic failures and nosocomial outbreaks.

Keywords: Extended spectrum beta-lactamases, AmpC beta-lactamases, Metallo beta-lactamases, Prevalence, Co-production.

Introduction

There are many mechanisms for beta lactam antibiotics becoming resistance in treating the bacterial infections one of the most important mechanism involved is the production of beta lactamases enzymes, such as extended-spectrum beta-lactamases (ESBL), AmpC beta-lactamases and carbapenemases.¹

ESBLs are enzymes capable of cleaving third-generation cephalosporins and aztreonam.² ESBLs are inhibited by clavulanic acid and have been closely associated with resistance to other classes of antibacterial. Such organisms producing these enzymes become multi-drug resistant leading to limited therapeutic options for infections.³

AmpC β -lactamases are cephalosporinases and are poorly inhibited by clavulanic acid, they can be differentiated from ESBLs by their ability to hydrolyze and inactive cephalosporins, cephamycins, aminopenicillins and monobactams. The enzyme exhibit both chromosomal and plasmid mediated mechanism of resistance.⁴

Metallo beta-lactamases (MBL) are considered as broad spectrum enzymes that has the capability to hydrolyse all the beta lactam antibiotics except monobactams and are not inhibited by beta lactamases inhibitors such as clavulanic acid and sulbactam.⁵ MBL producers are multiple drug resistant isolates which are also capable to become resistance to aminoglycosides and fluoroquinolones which create a difficult scenario with no option of therapeutic treatment.⁶

Widespread and indiscriminate use of antibiotics create an environment for pathogenic bacteria to become more notorious and to mutate themselves and also created a selective pressure to become a mutated forms which carry multiple drug resistance genes and these isolates are now an ever increasing concern since they are resistant to many available antibiotics posing a challenge in proper treatment of patients in the health care settings.⁷

Isolates carrying a multiple beta lactamases can be associated with the false susceptibility reports if not detected in routine culture and sensitivity. This carry a chance to provide the prescription of the inappropriate therapy for the infected patients, hence, it is necessary to know the accurate prevalence of the β -lactamases producing isolates and its antibiotic susceptibility. The present study was done to detect the prevalence of ESBL, AmpC β -lactamases, MBL and their coexistence in commonly isolated gram negative bacilli.

Materials and Methods

The study was carried out in Microbiology Department, Gujarat Adani Institute of Medical Sciences, Bhuj. It was a cross sectional study done for a year i.e., from January 2017 to December 2017. All the specimens sent for routine culture and sensitivity test to the dept. of Microbiology from different departments and ICUs were included in this study.

Identification of isolates

Microscopy was done for each specimen by gram staining method and was inoculated to the appropriate culture media and kept for 18-24 hrs in incubator at 37°C. The culture was

identified further by studying morphology, staining and standard biochemical tests.⁸

Antimicrobial susceptibility testing

Isolates which were recovered from the clinical specimen were tested for its susceptibility to different antibiotics by the disc diffusion method of Kirby-Bauer following the guidelines given by Clinical Laboratory Standard Institute (CLSI).⁹

ESBL phenotypic detection test

All Gram negative bacteria isolates were screened for ESBL production by disk diffusion method. Isolates with zones of inhibition of ≤ 22 mm for ceftazidime and ≤ 27 mm for cefotaxime indicated ESBL production. The less susceptible or resistant isolates were subjected to confirmatory test.⁹

Those strains positive in ESBL screening test were brought for confirmation by performing CLSI double disc diffusion test. *E.coli* ATCC 25922 (ESBL negative) and *K.pneumoniae* ATCC 700603 (ESBL positive) were used as controls in the study. A lawn culture of the test bacteria was plated on Mueller Hinton agar (MHA). Ceftazidime (30 μ g) and the combination disc ceftazidime plus clavulanic acid (30 μ g + 10 μ g) was placed with a distance of 25mm apart similarly with Cefotaxime (30 μ g) and the combination disc cefotaxime plus clavulanic acid (30 μ g + 10 μ g). An increase of ≥ 5 mm in zone of inhibition for ceftazidime + clavulanic acid when compared to ceftazidime alone similarly ≥ 5 mm in zone of inhibition of cefotaxime + clavulanic acid compared to cefotaxime alone was identified as ESBL producers, as per recommendations of CLSI.⁹

AmpC phenotypic detection test

All the isolates were screened for AmpC production by testing their susceptibility to ceftoxitin disc (30 μ g). Isolates with an inhibition zone diameter of ≤ 14 mm were screened as AmpC positive.¹⁰

Detection of AmpC producers was done using AmpC disc test. On a MHA plate *E.coli* ATCC 25922 was inoculated with a lawn culture method and allowed it to dry and a moistened blank disc with sterile normal saline was inoculated with few colonies of test strain and then placed

next to a ceftoxitin disc (30 μ g) already placed on MHA plate. An indentation of ceftoxitin inhibition zone adjacent to disc containing the test strain, was considered as AmpC producers.¹¹

MBL phenotypic detection test

Screening of MBL production was done for all the isolates using imipenem (10 μ g) and meropenem (10 μ g) discs (Himedia) by Kirby Bauer disc diffusion method. Isolates with zone diameter of ≤ 19 mm were considered as potential MBL producers.⁹

All the isolates with positive screening test were subjected to confirmatory test by Imipenem-EDTA Combined Disc test. Briefly, the isolate to be tested was inoculated on a MHA plate by lawn culture method. A disc of Imipenem (10 μ g) was placed on MHA plate and in a distance of 20mm apart another disc of Imipenem in combination with EDTA, Imipenem-EDTA disc (10-750 μ g) is placed and incubated at 37 $^{\circ}$ c for 18-24 hrs. If there is increase in the zone of inhibition of Imipenem-EDTA combination disc by ≥ 7 mm when it is compared to Imipenem disc alone then the strains are confirmed to be MBL producers.¹²

Results

Total 934 isolates were recovered from different clinical specimens. Majority of the isolates were from wound infections followed by respiratory tract, urine, blood and other body fluids.

The commonly isolated gram negative bacteria in routine culture and sensitivity were: *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Proteus spp.*, and *Acinetobacter baumannii*.

The major beta lactamases production was observed in *Escherichia coli*, followed by *Klebsiella pneumonia* and *Pseudomonas aeruginosa*, Fig. 1 provides the isolate wise distribution of beta lactamase production.

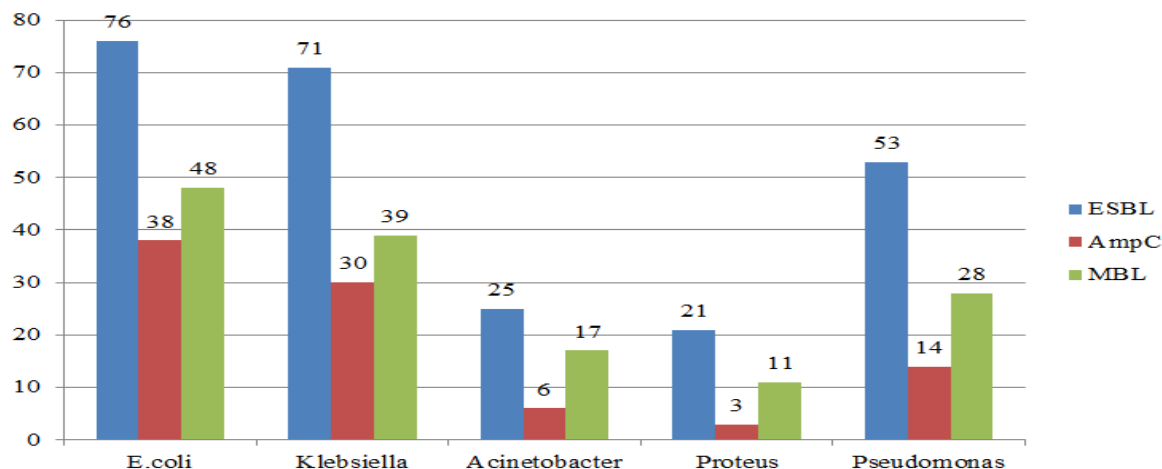


Fig. 1: Isolate wise distribution of ESBL, Amp C & MBL producers

Among 934 strains isolated, 640 strains were positive beta lactamases producers, out of which 246(26.3%) strains were ESBL producers, 91(9.7%) AmpC producers and 143(15.3%) were MBL producers. The co-production of

these beta lactamase enzymes was observed in total 160(17.1%) isolates, Fig. 2 gives the distribution of total beta-lactamases producing isolates.

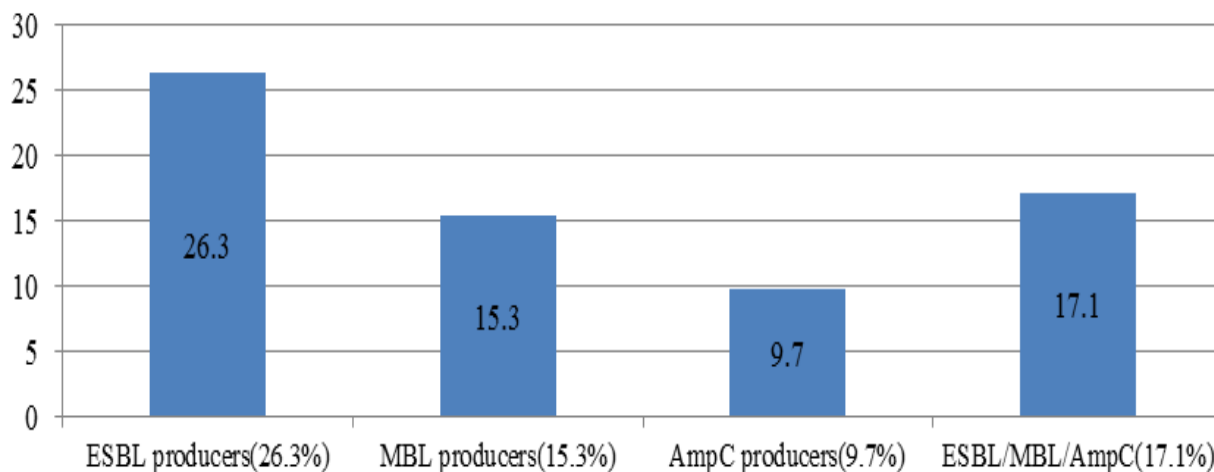


Fig.no. 2: Distribution of beta-lactamases production

Most of the isolates were resistant to beta lactam and non-beta lactam antibiotics in this study. This is because of over reliance on beta lactams and other higher antibiotics for the treatment of infections caused by gram negative organisms, empirically. Table 1 shows the antibiotic resistant pattern of beta lactamase producing isolates. Those strains which were producing beta-lactamase enzymes were also showing resistance to ciprofloxacin (76.2%), levofloxacin (68.5%), gentamycin (72.6%) and amikacin (61.8%). Almost all the multiple drug resistance strains were however sensitive to Polymyxin B and Colistin.

Table 1: Antibiotic resistance pattern of betalactamase producers

Name of the Antibiotics	Resistance(in%)
Amikacin	61.8
Gentamycin	72.6
Piperacillin-tazobactam	35.2
Ampicillin-sulbactam	39.2
Cefotaxime	85.9
Ceftazidime	88.1
Ceftriazone	73.1
Cefipime	85.2
Cefoxitin	73.4
Cefpodoxime	89.0
Ciprofloxacin	76.2
Levofloxacin	68.5
Imipenem	58.6
Cotrimoxazole	58.5
Colistin	9.1
Polymyxin	8.5

Discussion

The morbidity and mortality rate in the hospital setting has been alarmingly increasing since the infections associated with multidrug resistant bacteria that produce various beta-lactamases enzymes have been increasingly reported. The most common strains which are beta lactamase enzymes producers were found in *Klebsiella spp.* and in *E.coli* however, these enzymes are now produced by all members of Enterobacteriaceae and other gram negative bacilli.⁷ The prevalence rate of ESBL, AmpC, MBL beta lactamases production of our study were compared with the studies done by other authors and it is elicited in Table no.2 below.

Table 2: Prevalence rate of various beta lactamases enzymes

	ESBL	AmpC	MBL	ESBL+AmpC	ESBL+MBL	AmpC+MBL
Present study	26.3%	9.7%	15.3%	5.7%	7.5%	3.9%
Loveena et al.	35.1%	5.4%	10.9%	6.5%	8.7%	3.6%
Kolhapure et al.	38.5%	10.3%	9.2%	9.7%	4.8%	6.2%
Pramodhini et al.	47.6%	20.4%	50.5%	3.9%	10.8%	3.4%
Ibadin et al.	21%	7.8%	8.1%	2.9%	15.9%	2.6%

The present study demonstrated the prevalence of ESBL, AmpC and MBL in commonly found gram negative bacilli. The ESBL producers are found in 26.3% isolates which was in the range to the study done by Ibadin et al. (21%) and Shukla et al.(30.1%). 9.7% isolates were AmpC producers which was in close with the study done by Kolhapure RM et al.(10.3%) and less compared to Pramodhini et al (20.4%). MBL producing isolates are found in 15.3% which was similar with the study done by Bandekar et al. who reported 15.7% MBL producers.¹³⁻¹⁶

The co existence of ESBL and AmpC was detected in 5.7% isolates which was similar with the study done by Loveena et al. (6.5%). ESBL and MBL co production was observed in 7.5% which was lesser than the study done by Ibadin et al (15.9%) and higher than the study done by Kolhapure RM et al. (4.8%). AmpC and MBL co existence was seen in 3.9% bacterial isolates which are similar with the studies done by Loveena et al. (3.6%) and Pramodhini et al (3.4%).

Other studies have shown ESBL prevalence rates ranging from 27.3% to 98.51%, AmpC producers varied from 14.8% - 52.1% and the presence of multiple enzymes varied from 1.3% - 42.7%.¹⁷ This difference could be due to the factors like antibiotic usage pattern causing gene mutation leading to beta lactamases production. The additional factor could be variations in the normal flora due to cultural, nutritional and ethnic difference in various populations. Different phenotypic methods in various studies could also be another reason.¹⁸

The finding of multiple beta lactamases enzymes producing strains in our study emphasizes the need for the routine detection of these isolates by simple phenotypic methods in order to formulate the proper antibiotic policy. These pathogens are usually found to be multidrug resistance isolates however susceptible to Polymyxin B and Colistin in our study. At this point of we need to understand the importance of screening out these multiple enzymes coexistence in order to curb the development and dissemination of these multiple drug resistant strains.

Conclusion

The phenotypic tests done in our study to detect various beta lactamases enzymes producers were simple and cost effective and it can be incorporated in routine method to monitor the prevalence of these strains for epidemiological purpose as well as implementation of proper hospital infections control policies.

Gram negative bacterial isolates that produced single or multiple beta lactamases enzymes were resistant to multiple antibacterial agents. Judicious use of antibiotics and

continuous surveillance for detection of resistant strains in hospital and community settings will reduce the burden produce by these strains.

Conflicts of Interest: None.

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