Detection of different ß - lactamases and their co-existence in gram negative bacteria isolated from clinical samples at a Tertiary care centre

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Abstract

Introduction: Infections due to Gram-negative bacilli are increasing worldwide. The extended spectrum β-lactamases (ESBLs), AmpC β-lactamases and metallo β-lactamases (MBLs), have emerged as a cause of antibacterial resistance in Gram negative bacteria (GNB). β -lactamase producing GNB presents significant diagnostic and therapeutic challenge in the management of infection. So the present study was conducted to know the antibiogram of Gram negative bacterial isolates, to detect the different β-lactamases and their co-existence, to guide clinician to start appropriate antibiotic therapy for the management of infection.

Materials and Methods: A total of 150 Gram negative clinical isolates were identified and antibiotic susceptibility testing was done according to Clinical and Laboratory Standards Institute (CLSI) guidelines. Detection of ESBL was done by the combined disk diffusion method as per CLSI guidelines. Detection of AmpC β-lactamase was done by phenyl boronic acid test. MBL was detected using EDTA disc potentiation test.

Result: Among 150 Gram negative bacteria studied, 26(17.34%) were pure ESBL producer, 50(33.34%) were pure AmpC producer. ESBL and AmpC co-existed in 16(10.67%) isolates and AmpC and MBL co-occured in 16(10.67%) isolates.

Conclusion: Along with routine antibiotic sensitivity testing, special tests should be done to detect "hidden" resistance mechanisms for the effective management of infection.

Keywords: Antibiogram, β-lactamases, Extended spectrum β-lactamases (ESBLs), AmpCβ-lactamases, Metalloβ-lactamases (MBLs), Co-existence.

Introduction

Gram-negative bacteria pose a therapeutic problem in the hospital settings and also in the community as they have acquired resistance to multiple antibiotics.[1,2] An increase use of - β lactam antibiotics in hospitals and community has created drug resistant pathogens leading to increased morbidity, mortality and health-care costs. Of the several mechanisms of resistance, the most widespread and most important is the cleaving of the β -lactam ring, which is mediated by β -lactamases.[3]

In Gram negative organisms, β-lactamases namely extended spectrum β-lactamases (ESBLs), AmpC β-lactamases and metallo β-lactamases (MBLs) are the major cause of β-lactam resistance.[4] ESBLs are enzymes that are able to hydrolyze a wide variety of penicillin and cephalosporin including third‑generation cephalosporin and monobactams. However, the ESBL producing bacteria are susceptible to cephamycins, beta‑lactam plus beta‑lactamase inhibitor combination, and carbapenems.[5] In addition; they often exhibit resistance to other classes of drugs such as aminoglycosides, co-trimoxazole, tetracycline and fluoroquinolones.[6,7] Presence of AmpC in the same strains renders them resistant to cephamycins and beta‑lactam plus beta‑lactamase inhibitor combinations, Thus, they limits the therapeutic options.[5,6] Recently, metallo -lactamases (MBLs) mediated resistance have emerged as one of the most feared resistance mechanisms due to their ability to hydrolyze virtually all β -lactam agents including carbapenems. Its spread on highly mobile gene elements in nosocomial pathogens limits the therapeutic options.[6-8]

The presence of ESBLs and AmpC beta‑lactamases in a same isolate decreases the effectiveness of the beta‑lactam–beta‑lactamase inhibitor combinations, while MBLs and AmpC beta‑lactamases confer resistance to carbapenems. Often, these enzymes are co‑expressed in the same isolate.[9,10]

β -lactamase producing Gram-negative organisms presents significant diagnostic and therapeutic challenge in the management of infection. This emphasizes the need for the detection of isolates that co-produce β-lactamases and prevent therapeutic failures and nosocomial outbreaks and to decrease the length of stay in a hospital, to reduce health-care costs and to formulate an effective antibiotic policy. So, the present study was undertaken to detect ESBL, AmpC beta‑lactamase, and metallo‑beta‑lactamase in gram negative bacteria.[11,4,12]

Aims and Objectives of the Study

1. To isolate and to study the antibiogram of gram negative bacterial isolates
2. To detect the different β-lactamases and their co-existence by using different substrates and inhibitors by disc potentiation method in Gram-negative bacteria isolated from various clinical samples.
3. To guide clinician to start appropriate antibiotic based on antibiogram and by knowing their beta lactamase production.

Materials and Methods

Present study was done at Shimoga Institute of Medical Sciences, Shivamogga, for four month duration from September 2018 to December 2018, after obtaining institutional ethical committee clearance.

The study was conducted on 150 Gram negative bacilli isolates from various clinical samples of patients received at Microbiology laboratory from McGann teaching hospital, attached to Shimoga institute of medical sciences. These samples were processed on blood agar, chocolate agar, and MacConkey agar media and incubated at 37°C under aerobic conditions. The organisms were identified as per standard conventional methods.[13]

Antimicrobial susceptibility testing of isolates were done on Muller-Hinton agar by Kirby-Bauer disc diffusion method, according to the Clinical and Laboratory Standards Institute (CLSI, 2018) guidelines.[7] Susceptibility testing was carried out using the following antibiotics: Ampicillin (AMP), Ampicillin/sulbactam (A/S), Cefuroxime (CXM), Ceftazidime (CAZ), Co-trimoxazole (COT), Gentamicin (G), Chloramphenicol (c), Ciprofloxacin (CIP), Imipenem (IMP), Meropenem (MRP), Piperacilllin-tazobactam (PT), Amikacin (AK), Cefoxitin (cx), Cefepime (CPM), Aztreonam(AT).[14]

Detection of ESBL

Isolates showing decreased susceptibility to ceftazidime (zone diameter of ≤22mm) were selected for ESBL production. The CLSI double-disk diffusion test was performed for the confirmation of ESBL. Disk of ceftazidime (30µg) and ceftazidime/clavulanic acid (30/10 µg) were placed Mueller Hinton agar plate lawn inoculated with a 0.5 McFarland turbidity adjusted suspension of the test strain. After incubation, an enhanced zone of inhibition ≥5mm around ceftazidime/clavunic acid in comparison with ceftazidime disc alone was confirmed as ESBL producer.[4,11]

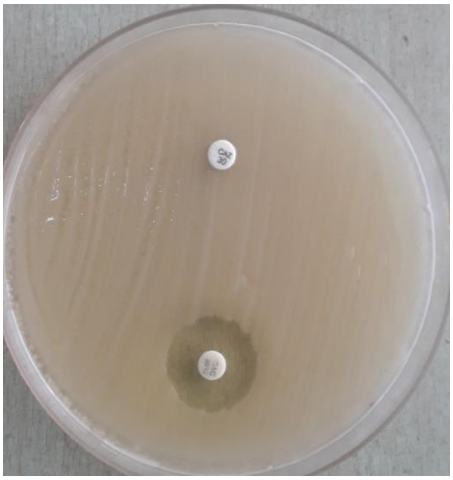

Fig. 1: Showing ESBL detection with combined disc diffusion method

Detection of AmpC β-lactamases

Isolates resistant to cefoxitin (30µg) were suspected to be AmpC producers. These isolates were subjected to phenyl boronic acid (PBA) disc enhancement method. In this method, two cefoxitin discs (30µg) were placed on Mueller Hinton agar plate lawn inoculated with a 0.5 McFarland turbidity adjusted suspension of the test strain. To one of the discs, 400 µg phenyl boronic acid was added. After overnight incubation at 37°C, enhancement of zone by 5mm around a cefoxitin disc with PBA, in comparisonwith a disc with cefoxitin alone, was taken as a positive result for AmpC production.[4]

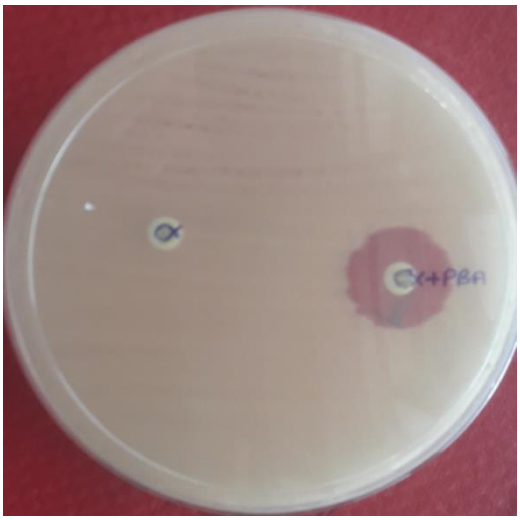

Fig. 2: Showing the detection of Amp C by Phenyl-boronic acid test

Detection of Metallo- β-lactamases

The isolates resistant to imipenem were suspected to be MBL-producer. Confirmation for the detection of MBL was done by disc potentiation test with EDTA- impregnated imipenem discs. An increase in the zone size of atleast 7mm around the imipenem-EDTA disc compared to plain imipenem disc was considered as an MBL producer.[4]

Fig. 3: Showing detection MBL using EDTA disc potentiation test

Results

Present study was carried out in the department of microbiology, Shimoga Institute of Medical Sciences, Shivamogga. The observations made from the study are shown in following tables.

Graph 1: Organisms isolated from clinical samples

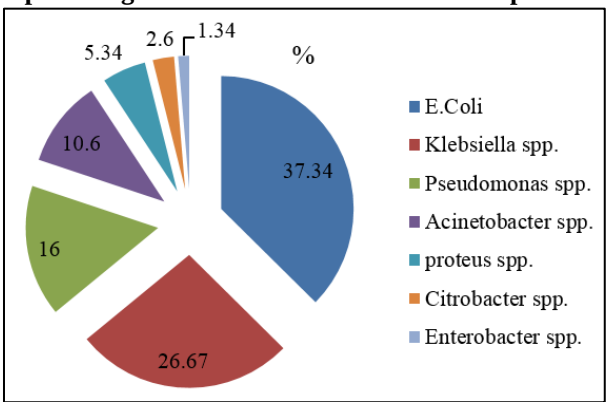

Table 1: Organisms isolated from clinical samples

Organisms	N (%)
Escherichia coli	56 (37.34)
Klebsiella spp.	40 (26.67)
Pseudomonas aeruginosa	24 (16.00)
Acinetobacter spp.	16 (10.67)
Proteus spp.	8 (5.34)
Citrobacter spp.	4 (2.60)
Enterobacter spp.	2 (1.34)
Total	150 ((100)

Table 2: Organism-wise distribution of different beta lactamases and their co-production

Organisms	Pure ESBL N (%)	Pure AmpC N (%)	ESBL+ AmpC N (%)	AmpC+ MBL N (%)	Non beta lactamase Producer N (%)	Total N (%)
Escherichia coli	18 (12.00)	14 (9.34)	6 (4.00)	6 (4.00)	12 (8.00)	56 (37.34)
Klebsiella spp	4 (2.60)	12 (8.00)	8 (5.34)	4 (2.60)	12 (8.00)	40 (26.67)
Pseudomonas aeruginosa	2 (1.34)	16 (10.67)	0	2 (1.34)	4 (2.60)	24 (16.00)
Acinetobacter spp.	2 (1.34)	6 (4.00)	2 (1.34)	2 (1.34)	4 (2.60)	16 (10.67)
Proteus spp.	0	0	0	0	8 (5.34)	8 (5.34)
Citrobacter spp	0	0	0	2 (1.34)	2 (1.34)	4 (2.60)
Enterobacter spp	0	2 (1.34)	0	0	0	2 (1.34)
	26 (17.34)	50 (33.34)	16 (10.67)	16 (10.67)	42 (28.00)	150 ((100)

Among 150 Gram negative bacteria studied 26(17.34%) were pure ESBL producer, 50(33.34%) were pure AmpC producer. ESBL and AmpC co-occured in 16(10.67%) isolates, AmpC and MBL co-occured in 16(10.67%) isolates.

Graph 2: Distribution of different beta lactamases and their co-production

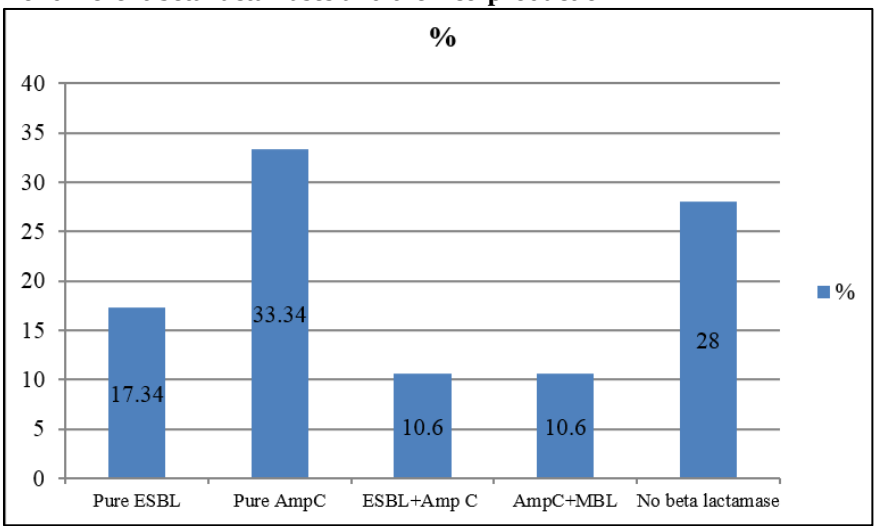

Table 3: Antibiotic sensitivity pattern of β-lactamase producing gram negative bacteria

Antibiotics tested	β-lactamase producers						
	Escherichia coli N-48(%)	*Klebsiella spp.* N-28(%)	*Acinetobacter spp.* N-10(%)	*Proteus spp* N-0	*Citrobacter spp* N-2(%)	*Enterobacter spp* N-2(%)	*Pseudomonas spp.* N-20(%)
Ampicillin	0	0	0	-	0	0	-
Gentamicin	28 (58.33)	10 (35.71)	6 (60.00)	-	0	1 (50.00)	6 (30.00)
Amikacin	36 (75.00)	15 (53.57)	7 (70.00)	-	1 (50.00)	1 (50.00)	10 (50.00)
Ampicillin/sulbactam	6 (12.50)	4 (14.28)	4 (40.00)	-	0	0	-
Cefuroxime	7 (14.58)	5 (17.85)	2 (20.00)	-	0	0	-
Cefoxitin	10 (20.83)	7 (25.00)	2 (20.00)	-	0	0	-
Cefepime	10 (20.83)	10 (35.71)	6 (60.00)	-	1(50)	1 (50.00)	6 (30.00)
Ciprofloxacin	24 (50.00)	15 (53.57)	4 (40.00)	-	2 (100)	0	10 (50.00)
Imipenem	42 (87.50)	22 (78.57)	8 (80.00)	-	1 (50.00)	2 (100)	18 (90.00)
Meropenem	16 (33.34)	18 (64.28)	4 (40.00)	-	1 (50.00)	1 (50.00)	16 (80.00)
Co- trimoxazole	16 (33.34)	7 (25.00)	0	-	0	0	-
Aztreonam	10 (20.83)	7 (25.00)	2 (20.00)	-	1 (50.00)	1 (50.00)	13 (65.00)
Ceftazidime	10 (20.83)	8 (28.57)	5 (50.00)		1 (50.00)	1 (50.00)	8 (40.00)
Chloramphenicol	36 (75.00)	17 (60.71)	4 (40.00)		2 (100)	2 (100)	-
Piperacillin- tazobactam	-	-	-		-	-	9 (45.00)

Table 4: Aantibiotic sensitivity pattern of non-β-lactamase producer

Antibiotics tested	Non-β-lactamase producers						
	Escherichia coli N- 8(%)	*Klebsiella spp.* N- 12(%)	*Acinetobacter spp* N- 6(%)	*Proteus spp* N- 8(%)	*Citrobacter spp* N- 2(%)	*Enterobacter spp* N-0	*Pseudomonas spp* N- 4(%)
Ampicillin	0	0	0	0	0	0	-
Gentamicin	7 (87.50)	8 (66.67)	4 (66.67)	4 (50.00)	2 (100)	0	3 (75.00)
Amikacin	6 (75.00)	8 (66.67)	4 (66.67)	4 (50.00)	2 (100)	0	3 (75.00)
Ampicillin/sulbactam	0	2 (16.67)	2 (33.34)	4 (50.00)	2 (100)	0	-
Cefuroxime	4 (50.00)	6 (50.00)	4 (66.67)	4 (50.00)	0	0	-
Cefoxitin	5 (62.50)	6 (50.00)	5 (83.34)	7 (87.50)	2 (100)	0	-
Cefepime	7 (87.50)	9 (75.00)	5 (83.34)	7 (87.50)	2 (100)	0	2 (50.00)
Ciprofloxacin	6 (75.00)	9 (75.00)	4 (66.67)	6 (75.00)	2 (100)	0	2 (50.00)
Imipenem	8 (100)	12 (100)	6 (100)	8 (100)	2 (100)	0	4 (100)
Meropenem	8 (100)	9 (75.00)	5 (83.34)	7 (87.50)	2 (1000	0	4 (100)
Co- trimoxazole	6 (75.00)	7 (58.33)	4 (66.67)	5 (62.50)	1 (50.00)	0	-
Aztreonam	7 (87.50)	10 (83.34)	4 (66.67)	7 (87.50)	2 (100)	0	4 (100)
Ceftazidime	4 (50.00)	9 (75.00)	2 (33.34)	6 (75.00)	2 (100)	0	2 (50.00)
Chloramphenicol	6 (75.00)	9 (75.000	5 (83.34)	7 (87.50)	2 (100)	0	-
Piperacillin-tazobactam	-	-	-	-	-	-	3 (75.00)

Discussion

The β-lactamases, including extended-spectrum β-lactamases, Amp-C-β-lactamases, and metallo-β-lactamases, have emerged worldwide as a cause of antimicrobial resistance in Gram-negative bacteria (GNB).Often these enzymes are co-expressed in the same isolate and genes for all these three enzymes are often carried on plasmids, facilitating rapid spread between microorganisms.[9] Emergence of β -lactamase producing Gram-negative organisms presents major diagnostic and therapeutic challenge in the management of infection.

In our study among 150 gram negative bacteria studied 26(17.34%) were pure ESBL producer, 50(33.34%) were pure AmpC producer. ESBL and AmpC co-occured in 16(10.67%) isolates. AmpC and MBL co-occured in 16(10.67%) isolates. The present study showed a high prevalence AmpC beta lactamases among various Gram negative bacteria isolated in our Hospital. Probably the risk factors for the colonization or infection with these organisms are due to prolonged hospital stay, intensive care unit admission, urinary and intravenous catheterization and exposure to antibiotics including extended spectrum cephalosporins.[12]

Study done at Uttarakhand, in 2013 reported among 184 gram negative bacteria studied 30 (16.3%) were pure ESBL producer, 26(14.1%) were pure AmpC producer., 60(32.6%) were pure MBL producer, ESBL and AmpC co-occured in 42(22.8%) and AmpC and MBL co-occured in 16(8.6%) isolates.[2] Study done at Uttar Pradesh in 2007-2008 reported among 251 isolates studied, 138 (54.98%) were ESBL producers, 49 (19.52%) were AmpC producers and 45 (17.93%) were MBL producers.[7] Study done in

Hyderabad in November 2010 to October 2011 reported of a total of 200 Gram negative isolates, pure ESBL were seen in 50(25%) isolates and pure AmpC were seen in 35(17.5%) isolates. ESBL and AmpC co-existed in 38(19%) isolates. AmpC and MBL co-occurred in a single isolate.[4]

In our study Antibiotic resistance was high among β-lactamase producers when compared to β-lactamase non producers which are comparable with study done at Pondicherry in 2011 and at Hyderabad in November 2010 to October 2011. This may be due to the fact that plasmids carrying these enzymes may carry co-resistance genes for other antibiotics.[15]

The occurrence of multiple beta lactamases among gram negative bacteria limits the therapeutic options and poses diagnostic challenge to the microbiologist. The presence of ESBLs and AmpC beta-lactamases in a single isolate reduces the effectiveness of the beta-lactam–beta-lactamase inhibitor combinations, while MBLs and AmpC beta-lactamases confer resistance to carbapenems.[5,9] Detection of beta lactamases in GNB of great importance for epidemiological purposes as well as for infection control purposes and also helpful in management of infection with appropriate antibiotics.

Conclusion

If antibiotic susceptibility reports are being referred for treatment without testing of β-lactamases, it can result in serious therapeutic failures so along with routine antibiotic sensitivity testing, special tests should be done to detect these "hidden" resistance mechanisms. Detection of beta lactamases by phenotypic tests using various substrates and inhibitors are simple and easy to perform in a routine diagnostic laboratory.

Conflict of Interest: None.

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