Original Research Article

Prevalence of extended β lactamases, Amp C and metallo beta lactamases among gram negative clinical isolates at a tertiary care centre in South India

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Abstract

Background: Resistance of Gram negative bacteria to broad spectrum antibiotics is posing a clinical threat during the last decade. These ESBL and MBL producing organisms have a potential for rapid dissemination and hence their early detection is necessary to establish appropriate antibiotic therapy. **Aims:** This study aims to determine the prevalence of ESBL, AmpC and MBL producers among gram negative clinical isolates. **Material and Methods:** Four hundred and seventy four gram negative bacilli were isolated from various clinical specimens. Sample processing and identification was done by standard microbiological techniques. The antibiotic sensitivity test was performed and all the gram negative isolates were tested for ESBL by DDST and PCDDT method, AmpC and MBL production. **Observation and Results:** 474 gram negative bacilli were isolated from various clinical samples and the commonest isolate was *E.coli* (40.2%), *Klebsiella pneumoniae* (21.5%), *Pseudomonas aeruginosa* (19.6%). ESBL detection rate was found more by PCDDT. AmpC production was seen in *Acinetobacter* (14.8%), *Enterobacter* spp. (11.1%), *Pseudomonas* (9.6%.) Majority of MBL producers were *Pseudomonas aeruginosa* (18.2%) and *Acinetobacter* (17%). **Conclusion:** Incidence of β lactamases producing enzymes is tremendously increasing hence laboratory detection of these organisms producing ESBL, AmpC and MBL is becoming more important. Proper infection control practices should be adopted to curtail the spread of infections produced by organisms producing beta lactamases enzymes.

Key Words: ESBL, Amp C, MBL, Infection control.

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INTRODUCTION

Resistance of Gram negative bacteria to broad spectrum antibiotics is posing a clinical threat during the last decade. Resistance to ß-lactam antibiotics such as penicillins and cephalosporins is due to Extended-spectrum ß-lactamases (ESBLs) enzymes which cleaves the ß-lactam ring.[1] Among the family *Enterobacteriaceae*, *E. coli* and *Klebsiella pneumoniae* are the most common ESBL producers.[2] Enterobacteriaceae are resistant to most β-lactams except for cefepime and carbapenems due to Ambler class C cephalosporinases (AmpCs).[3] Infections caused by ESBL-producing organisms are treated by Carbapenems but metallo-ß-lactamase (MBL), a carbapenemase enzymes has been reported[4]. The MBL enzymes can hydrolyze all ß-lactams except monobactam and belong to the molecular class B family, or functional group 3 ß-lactamases.[5] Mechanism of resistance to antibiotics

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include decreased outer membrane permeability, mutation in porins, loss of outer membrane porins, increased efflux systems, alteration of penicillin binding proteins and carbapenem hydrolyzing enzymes carbapenemases.[6,7] These ESBL and MBL producing organisms have a potential for rapid dissemination and hence their early detection is necessary to establish appropriate antibiotic therapy and initiate necessary effective infection control measures to prevent their dissemination.[8] Currently most of the clinical laboratories test for production of ESBLs and MBL but do not detect AmpC β-lactamases in routine susceptibility tests. This study aims to determine the prevalence of ESBL, AmpC and MBL producers among gram negative clinical isolates.

MATERIAL AND METHODS

This study was carried out in the Department of Microbiology of Kamineni Institute of Medical Sciences, Narketpally over a period of one year. Four hundred and seventy four gram negative bacilli were isolated from various clinical specimens, including urine (163), sputum (119), blood (24), pus (98), Endotracheal Tube (42) and body fluids (28), both from out-patients and in-patients, were processed. All the clinical isolates other than gram negative bacilli were excluded from the study. Sample processing and identification was done by standard microbiological techniques.[9] The antibiotic sensitivity test[9] was performed by modified Kirby Bauer disc diffusion method according to CLSI guidelines.[10] The antibiotics tested were (Discs obtained from Himedia Laboratories Pvt. Ltd. Mumbai.) ampicillin (10 µg), piperacillin (30µg), cefuroxime (30 µg), ceftazidime (30 µg), cefazoline (30 µg), cefotaxime (30 µg), cefepime (30 µg), aztreonam (30 µg), amoxicillin–clavulinic acid (20/10 µg), piperacillin–tazobactam (100/10 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), gentamicin (10 µg), amikacin (30 µg), meropenem (10 µg), imipenem (10 µg), trimethoprim–sulfamethoxazole (1.25/23.75 µg). Norfloxacin (10 µg) and nitrofurantoin (300 µg) discs were tested against the isolates obtained from urine samples only. All the gram negative isolates were tested for ESBL, AmpC and MBL production.

Double Disc Synergy test[6] (DDST) for ESBL Detection[10]: In DDST, on the lawn culture of test strain a disc of amoxyclav (20µg +10 µg) was placed in the centre and the 3rd generation cephalosporin i.e. ceftazidime or cefotaxime discs were placed 15mm apart from the central amoxyclav disc. After overnight incubation at 37˚C, there was an inhibition zone around the test antibiotic which showed a clear extension towards augmentin disc in case of ESBL producer.

Phenotypic Confirmatory Disc Diffusion Test[6] (PCDDT) for ESBL Detection[11]: In PCDDT, both cephotaxime (30µg) and ceftazidime (30µg) disc alone and in combination with clavulanic acid (30µg) were applied to the lawn culture of the test strain. After overnight incubation at 37^0C if an increase in zone diameter of ≥ 5 mm for ceftazidime and cephotaxime, tested in combination with clavulanic acid versus its zone when tested alone, was considered as ESBL producer. For quality control of ESBL test, *Klebsiella pneumoniae* ATCC 700603 and *E. coli* ATCC 25922 were used as positive and negative controls for ESBL production.

AmpC Beta lactamase detection: In Gram negative isolates if cefoxitin zone diameter was less than 18 mm and resistance to 3rd generation cephalosporins, then they were tested for AmpC enzyme production by AmpC disc test.

AmpC Disc test[12]: On the Mueller Hinton agar plate, *E.coli* was lawn cultured. A sterile disc (6mm) moistened with sterile saline (20 µl) was inoculated with several colonies of test organism. Inoculated disc was then placed beside a cefoxitin (30µg) disc on the inoculated plate. After overnight incubation at 35^0C, flattening of the cefoxitin inhibition zone in the vicinity of test disc indicates positive and undistorted zone negative.

Metallo-Beta lactamase detection[13]: MBL production in Gram negative bacilli was tested by imipenem-EDTA combined disc test. Organism was lawn cultured on Mueller-Hinton agar and 10 µg of imipenem disc and imipenem-EDTA (750 µg) were placed on the agar plate. After overnight incubation, enhancement of zone of inhibition of Imipenem + EDTA disc compared to that of Imipenem disc alone by ≥ 7mm was considered positive for MBL production.

OBSERVATIONS AND RESULTS

A total of 474 gram negative bacilli were isolated from various clinical samples like blood, pus, urine, sputum, body fluids from inpatient and outpatient departments. Out of 474 isolates, commonest isolate was *E.coli* (40.2%), *Klebsiella pneumoniae* (21.5%), *Pseudomonas aeruginosa* (19.6%) (Table 1). Table 2 shows antibiotic resistance profile of the isolated gram negative organisms. Table 3 shows ESBL production among gram negative bacilli. ESBL detection rate was found more by PCDDT. 4 cases each of *E.coli* and *Klebsiella pneumoniae* were missed by DDST method of ESBL production, 3 cases in *Pseudomonas aeruginosa,* 1 case of *Proteus* spp., 2 each in *Citrobacter* spp., *Enterobacter* spp. and *Acinetobacter* spp. respectively (Table 3). AmpC production was seen in *Acinetobacter* (14.8%), *Enterobacter* spp. (11.1%), *Pseudomonas* (9.6%.) (Table 4)**.** Majority of MBL producers were *Pseudomonas aeruginosa* (18.2%) and *Acinetobacter* (17%)(Table 5)**.**

Table 1: Various Gram negative isolates from clinical samples (n=474)

Organism isolated	Number of isolates (%)
E. coli	191 (40.2%)
Klebsiella pneumoniae	102 (21.5%)
Pseudomonas aeruginosa	93 (19.6%)
Acinetobacter spp.	47 (9.9%)
Proteus spp.	27 (5.7%)
Enterobacter spp.	9 (1.9%)
Citrobacter spp.	5 (1.05%)

Table 2: Resistance pattern gram negative bacilli

Antibiotic	E.coli n=191 (%)	Klebsiella n=102 (%)	Pseudomonas n=93 (%)	Acinetobacter n=47 (%)	Proteus n=27 (%)	Enterobacter n=09 (%)	Citrobacter n=05 (%)
Ampicillin	191 (100)	--	--	--	--	--	--
Piperacillin	98 (51.3)	53 (51.9)	47 (50.5)	42 (89.3)	--	--	--
Cefazoline	185 (96.8)	94 (92.1)	--	--	--	--	--
Cefuroxime	182 (95.2)	91 (89.2)	--	--	--	--	--
Ceftazidime	163 (85.3)	61 (59.8)	84 (90.3)	43 (91.4)	11 (40.7)	4 (44.4)	3 (60)
Cefotaxime	142 (74.3)	87 (85.2)	--	44 (93.6)	13 (48.1)	5 (55.5)	3 (60)
Cefepime	63 (32.9)	55 (53.9)	37 (39.7)	27 (57.4)	9 (33.3)	3 (33.3)	2 (40)
Aztreonam	134 (70.1)	69 (67.6)	46 (49.4)	--	--	--	--
Amoxyclav	164 (85.8)	64 (62.7)	--	--	--	--	--
Piperacillin/ Tazobactum	78 (40.8)	48 (47)	45 (48.3)	34 (72.3)	12 (44.4)	4 (44.4)	3 (60)
Ciprofloxacin	87 (45.5)	56 (54.9)	64 (68.8)	23 (48.9)	10 (37)	5 (55.5)	3 (60)
Levofloxacin	69 (36.1)	41 (40.1)	57 (61.2)	21 (44.6)	8 (29.6)	4 (44.4)	2 (40)
Gentamicin	117 (61.2)	69 (67.6)	73 (78.4)	36 (76.5)	17 (62.9)	5 (55.5)	4 (80)
Amikacin	83 (43.4)	53 (51.9)	51 (54.8)	19 (40.4)	14 (51.8)	3 (33.3)	2 (40)
Meropenem	49 (25.6)	49 (48)	33 (35.4)	17 (36.1)	11 (40.7)	3 (33.3)	2 (40)
Imipenem	37 (19.3)	23 (22.5)	28 (30.1)	13 (27.6)	9 (33.3)	2 (22.2)	1 (20)
Cotrimoxazole	107 (56)	78 (76.4)	--	31 (65.9)	--	--	--
Nitrofurantoin	53 (27.7)	46 (45)	--	--	--	--	--
Norfloxacin	62 (32.4)	49 (48)	68 (73.1)	--	11 (40.7)	3 (33.3)	2 (40)

Ampicilln, first and second generation cephalosporins showed highest resistance for all gram negative isolates.

Table 3: ESBL production among Gram negative isolates (n=474)

Organism (n)	ESBL producer	
	DDST (%)	PCDDT (%)
E. coli (191)	76 (39.7%)	80 (41.8%)
Klebsiella pneumonia (102)	38 (37.2%)	42 (41.1%)
Pseudomonas aeruginosa (93)	31 (33.3%)	34 (36.5%)
Proteus spp.(27)	5 (18.5%)	6 (22.2%)
Acinetobacter spp.(47)	9 (19.1%)	11 (23.4%)
Enterobacter spp.(9)	1 (11.1%)	3 (33.3%)
Citrobacter spp.(5)	1 (20%)	3 (60%)
Total	**161 (33.9%)**	**179 (37.7%)**

ESBL: Extended spectrum β-lactamase, DDST - Double Disc Synergy test, PCDDT - Phenotypic Confirmatory Disc Diffusion Test

Table 4: Amp C production among Gram negative isolates (n=474)

Organism	AmpC producer
E. coli (191)	16 (8.3%)
Klebsiella pneumonia (102)	6 (5.8%)
Pseudomonas aeruginosa (93)	9 (9.6%)
Acinetobacter spp.(47)	7 (14.8%)
Enterobacter spp.(9)	1(11.1%)
Citrobacter spp.(5)	0
Proteus spp. (27)	0
Total	**39 (8.2%)**

Table 5: MBL production among Gram negative isolates (n=474)

Organism	MBL producer
E. coli (191)	16 (8.3%)
Klebsiella pneumonia (102)	6 (5.88%)
Pseudomonas aeruginosa (93)	17 (18.2%)
Acinetobacter spp.(47)	8 (17%)
Enterobacter spp.(9)	1 (11.1%)
Citrobacter spp.(5)	0
Proteus spp. (27)	0
Total	**48 (10.1%)**

DISCUSSION

Globally, threat due to antibiotic resistance is growing. Extensive use of β-lactam antibiotics to treat various infections has resulted in emergence of β- lactamase mediated resistance. In our study, a total of 474 gram negative bacilli were isolated from various clinical samples and commonest isolate was *E.coli* (40.2%), *Klebsiella pneumoniae* (21.5%), *Pseudomonas aeruginosa* (19.6%) (Table 1). Kaur N *et al*[14] also reported 41.6% *E.coli*, 24% *Klebsiella pneumonia* and 17.7% *Pseudomonas* spp from various clinical specimens. Our findings are comparable with Kaur N *et al*. Table 2 shows antibiotic resistance profile of the isolated gram negative organisms. Multidrug resistant bacteria are becoming an increasing threat due to antibiotic overuse. ESBL producers have limited therapeutic options. In our study, among the 474 gram negative isolates, 33.9% ESBL producers were detected by Double Disc Synergy test (DDST) method and 37.7% ESBL producers by Phenotypic Confirmatory Disc Diffusion Test (PCDDT). Maximum ESBL production was detected in *E.coli* (39.7% by DDST and 41.8% by PCDDT), followed by *Klebsiella pneumoniae* (37.2% by DDST and 41.1% BY PCDDT) and *Pseudomonas aeruginosa* (33.3% by DDST and 36.5% BY PCDDT). ESBL detection rate was found more by PCDDT (Table 3). Our finding are comparable to Rudresh *et al*[15] who reported 40.2% E.coli and 33.1% K. pneumonia in their clinical samples. ESBL enzyme being plasmid coated might also carry genes for coresistance for other antibiotics. These enzymes spread fast, hence rapid detection of ESBL is essential. AmpC β-lactamases are class C or group 1 cephalosporinases, resistant to penicillins, cephalosporins, cephamycins and monobactams. This enzyme is not affected by ESBL inhibitor clavulanic acid, sulbactam and tazobactam. Patients have a higher mortality and morbidity if infection is caused by AmpC positive bacteria.[16] In our study, AmpC production was seen in *Acinetobacter* (14.8%), *Enterobacter* spp. (11.1%), *Pseudomonas* (9.6%.) (Table 4)**.** Ogefere *et al*[16] reported 37% *Pseudomonas*, 14.2% *Klebsiella*, 10.5% *E.coli* Amp C producers from various clinical specimens. Many laboratories do not test for AmpC producers. These are generally associated with multi drug resistance, limiting the therapeutic options. Hence, screening of Amp C producers should be routinely done in clinical microbiology laboratories. Metallo- -lactamases (MBLs) hydrolyze penicillins, cephalosporins and carbapenems except aztreonam. Imipenem- EDTA combination disc can be used for quick, sensitive detection of MBL producing isolates.[17] Majority of MBL producers were *Pseudomonas aeruginosa* (18.2%) and *Acinetobacter* (17%) (Table 5). Our findings are comparable with Kaur *et al*[14] who reported 34.8% K. pneumonia, 34.6% Pseudomonas aeruginosa and 28% Acinetobacter MBL producers from various clinical specimens. MBL producers may carry genes for multidrug resistance for various antibiotics and thus limit the therapy options. Higher antibiotics such as Polymyxin B or colistin have to be used for treatment of such infections. Hence, early detection of MBL producers is essential to treat these infections and reduce the mortality and morbidity.

CONCLUSION

Overuse of antibiotics has lead to emergence of multidrug resistant organisms producing β lactamases posing a greater threat to the community. Incidence of β lactamases producing enzymes is tremendously increasing hence laboratory detection of these organisms producing ESBL, AmpC and MBL is becoming more important. Higher antibiotics such as carbapenems should be used as reserved drugs as these are still effective against ESBL producing strains. Antibiotics should be used judiciously. Every hospital should prepare its antibiotic policy depending on the local hospital antibiogram to curtail the overuse of antibiotics. Mortality rates among patients can be reduced by continuous surveillance of β lactamases producing Gram negative organisms. Prompt and proper infection control practices should be adopted to curtail the spread of infections produced by organisms producing beta lactamases enzymes.

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