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Research Article

METALLO BETA LACTAMASE MEDIATED RESISTANCE IN CARBAPENEM RESISTANT GRAM-NEGATIVE BACILLI: A CAUSE FOR CONCERN

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ABSTRACT

Introduction: The emergence of acquired metallo-β-lactamases (MBL) in Gram-negative bacilli is becoming a therapeutic challenge, as these enzymes usually possess a broad hydrolysis profile that includes carbapenems, extended-spectrum β-lactams. **Aim**: To detect Extended spectrum β-lactamases and metallo-β-lactamase in carbapenem resistant Gram negative clinical isolates from various clinical specimens and to evaluate their antibiotic susceptibility patterns. **Material and Methods**: A total of 100 non duplicates imipenem resistant isolates were tested for the presence of extended spectrum β-lactamases by phenotypic confirmatory test, metallo-β-lactamases by Double disk synergy test with various distances from edge to edge (10mm,15mm,20mm), between the IPM and EDTA and combined disc test. **Result:** Of the 100 IMP resistant isolates screened 30 (30%) were MBL positive by phenotypic methods, i.e., double disk synergy test and combined disc test. Co-existence of Extended spectrum β-lactamases and MBL were detected in 3 (30%). All the 30 MBL positive isolates had shown synergy at (100%) at 10 mm distance, 27 (90%) isolates had shown synergy at 15 mm distance and 13 (43.4%) isolates were shown synergy at 20 mm distance. All the 30 MBLs producers were multidrug resistant and 27 (90%) were sensitive to colistin (CL). All MBL positive *Pseudomonas aeruginosa* were sensitive to polymyxin B (100µg). **Conclusion:** Microbiologists are now facing a challenge of drug resistance due to MBL production. Although CLSI guidelines do not quote about the ESBL detection in Pseudomonas aeruginosa MBLs and ESBL have to be detected in them. The use of combination tests would increase the sensitivity to detect the presence of MBL among the clinical isolates of Gram-negative bacilli. The spread of MBL producing Gram negative organism can be prevented if they are detected in all isolates and routinely adopted in all laboratories.

Keywords: Carbapenem, CDT, DDST, EDTA, ESBL, Gram negative bacilli

INTRODUCTION

Gram negative bacilli resistant to penicillin and cephalosporin can be treated by carbapenems, but the enzyme carbapenemase can hydrolyse most of the beta–lactamases (extended- spectrum and Amp C beta – lactamases).[1] Three major groups of such enzymes are usually distinguished, class C cephalosporinases (AmpC), Extended spectrum β-lactamase (ESBL) and different types of β-lactamases with carbapenemase activity of which so called metallo -β-lactamases (MBL), are of great concern. [2] ESBLs are still considered as a threat since they are coded by plasmid and can be easily transmitted between species. ESBL producing organisms are highly effective in inactivating penicillins, most cephalosporins and aztreonam. [3]

We are facing a threat of the use of carbapenems especially against the Ambler class B MBLs. Many reports across the globe have shown high level resistance to all beta- lactams.[4]

Genes like IMP, VIM and others code for these Class B enzymes, for which divalent cation like zinc is required for the enzymatic activity. MBL genes seem to have disseminated from Pseudomonas aeruginosa to other members of family enterobacteriaceae. [5] World wide prevalence of MBL is seen with Pseudomonas aeruginosa and also recently among Gram negative bacilli. [6,7,8] The infection with these MBL strains remains a challenge for treatment and can lead to morbidity and mortality. Polymerase chain reaction is the gold standard for MBL detection, but it may not be available in all laboratory setups. Other non molecular methods are available depending on the chelating agents such as Ethylene diamine tetra acetic acid (EDTA) or 2-mercaptopropionic acid for enzyme detection may be used.[9]

Some of these tests like the double-disk synergy tests (DDST) using EDTA with Imipenem (IPM) or ceftazidime (CAZ), 2- mercaptopropionic acid with IPM or CAZ, the Hodge test a combined disk test (CDT) using EDTA with CAZ or IPM, the MBL 'E' test and a micro dilution method using EDTA and 1, 10- phenanthroline with IPM are available. [4]

Since the infection caused by Gram negative bacilli producing MBL is difficult to treat, detection should be carried out.

Therefore the present study was undertaken to detect MBL in carbapenem resistant gram negative bacilli by two phenotypic methods i.e., the Double disk synergy test (DDST) and Combined disc test (CDT) with EDTA . The ideal distance between the IPM and EDTA discs in the DDST was also carried out to look for the optimal critical distance between the discs. An attempt was made to detect ESBL among the MBL positive isolates.

MATERIALS AND METHODS

This prospective study was conducted in the Department of Microbiology, ESIC-MC-PGIMSR, from May 2011 to January 2012.

A total of 100 clinically significant, non duplicate, IPM resistant, gram negative clinical isolates obtained from pus/ wound swab, sputum, blood, catheter tip and urine. The specimens were received from both inpatients and out- patients.

The 100 IPM resistant isolates included *Acinetobacter spp.* (n= 40), *Pseudomonas aeruginosa* (n= 34), *Klebsiella pneumoniae* (n=10), *Escherichia coli* (n= 9), *Proteus spp.*(n=3), *Enterobacter spp* and , *Providencia spp*. (n=2) each.

Standard microbiological procedure was carried out to speciate all the clinical isolates.[10] Antimicrobial susceptibility testing was done by using commercially available disc (Himedia, Mumbai, India) in accordance with Kirby Bauer's disc diffusion method. [11]

Piperacillin-tazobactam 100/10µg (PT), gentamicin 10µg (GEN), amikacin 30µg (AK), ciprofloxacin 5µg (CIP) , trimethoprim-sulfamethoxazole 1.25/23.75µg (COT), ceftazidime 30 µg (CAZ), ceftriaxone 30 µg (CTR), cefotaxime 30 µg (CTX), imipenem 10µg (IPM), meropenem 10µg (MR) , aztreonam 30 µg (AZT), colistin 25 µg (CL), and polymyxin B 300U(PB) were used in the antibiotic susceptibility tests..

Results were recorded and interpreted as per Clinical and Laboratory Standards Institute (CLSI) guidelines. [12] and for colistin for Enterobacteriaceae results were recorded as per Galani *et al.* [13] *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 strains were used for quality control.

The minimum inhibitory concentration (MIC) of IPM was determined by Etest, according to the manufacturer's recommendations (Biomerieux SA, France). *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 strains were used as quality control.

Phenotypic detection of MBL: In all the 100 IPM resistant GNB, MBL was detected by DDST and CDT.

DDST: Bacterial suspension corresponding to 0.5 McFarland was inoculated on a Mueller- Hinton Agar (MHA) plate. IPM (10 µg) disk was placed next to a blank filter paper disc (6mm in diameter) at a distance of 10mm, 15mm, and 20mm apart from edge to edge. 10µl of 0.1 M (292 µg) EDTA was added to the blank disc. After incubation for 16-18 hours at 37° C, an enhancement of the zone of inhibition between IPM disc and EDTA disc was considered positive for MBL. [9] (Figure .1)

CD Test: A 0.5 McFarland bacterial suspension was inoculated on MHA plate. Two 10 µg IMP discs were placed on the inoculated plate in which 10µl of 0.1M (292µg) EDTA was added to one of the IMP discs.

After incubation for 16-18 hours at 37° C, an increase in zone diameter of > 4mm around the IPM- EDTA disc as compared to IPM disc alone was considered positive for MBL. [9] (Figure .2)

ESBL detection: Phenotypic confirmatory test [12] was used to evaluate all isolates resistant to ceftazidime (30 µg) for ESBL production.

Bacterial suspension corresponding to 0.5 MacFarland's was spread on an MHA plate. Aseptically ceftazidime (30 µg) and ceftazidime / clavulanic acid (30 µg/ 10 µg) discs were placed on the agar plate. 15mm distance was maintained between the two discs (edge to edge). Overnight incubation of these culture plates was done. Confirmation for the production of ESBL was done by noting ≥ 5mm increase in the zone diameter for the antimicrobial agent which was tested in combination with clavulanic acid, versus its zone diameter when tested alone. The increase in the zone diameter was due to the inhibition of the β lactamse by clavulanic acid.

RESULTS

100 consecutive GNB resistant to IMP (10 ug) and Caz (30 ug) by the double disc diffusion method and confirmed by doing MIC by IMP Etest strip were obtained from clinical samples (one isolate per patient).

Of the 100 IPM resistant Gram negative clinical isolates screened for MBL, 30 (30%) isolates were MBL producers by both DDST and CDT and 70 (70%) isolates were non MBL producers.

The predominant source of the 30 MBL positive strains was from pus /wound swab12 (40%), followed by urine 7 (23%), catheter tip 5 (17%), sputum 3 (10%), blood 2 (7%), and fluids 1 (3%). (Figure.3) Of these, 26 (86.6%) isolates were from inpatients, and 4 (13.3%) isolates were from outpatient department. Among the isolates from inpatient department, highest numbers of strains were isolated from ICU 10 (38.4%) followed by post operative ward 7 (26.9%), surgical ward 6 (23%), medical ward 5 (19.2%), and pediatric ward 2 (7.7%).

The commonest organism was *Klebsiella pneumoniae* 10 (33.3%), followed by *Pseudomonas aeruginosa* 9 (30%), *Acinetobacter spp. 5* (16.6%), *Escherichia coli* 3 (10%), *Enterobacter spp.* 2 (6.6%) and *Providencia spp.* 1 (3.3%). (Figure .4)

Antimicrobial susceptibility of MBL producers showed that all of them were multidrug resistant, with resistance to 4 or more drugs (aminoglycosides, quinolones, third generation cephalosporins, and carbapenems). In the present study, 29 (96.6%) MBL positive isolates were resistant to aztreonam and 3 (10%) isolates were resistant to colistin. (Figure .5)

Besides these antimicrobials, polymyxin B was used for *Pseudomonas aeruginosa* and showed 100% sensitivity.

Coexistence of ESBL and MBL was seen in 3 (10%) of the 30 MBL isolates.

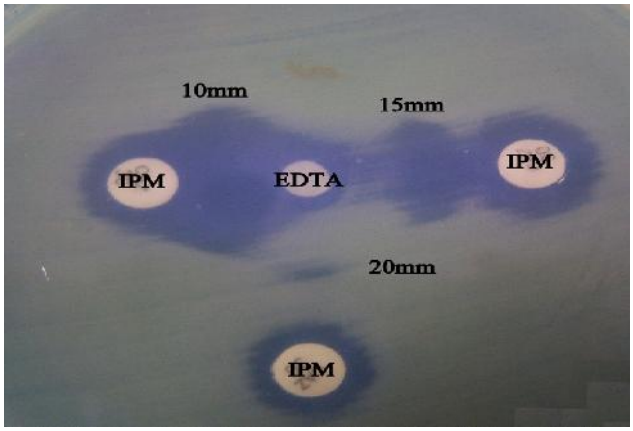

Fig 1: Double Disk Synergy Test

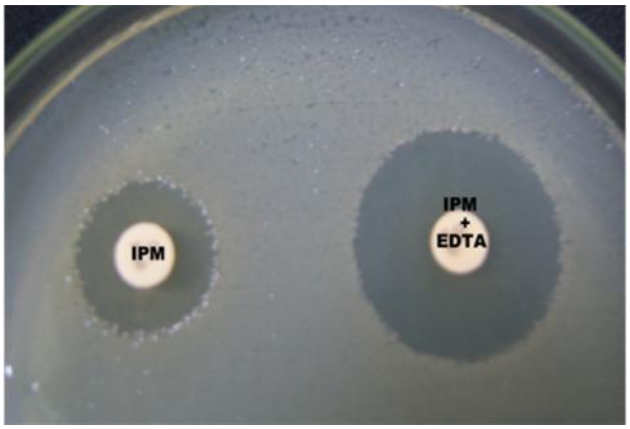

Fig 2: Combined Disc Test

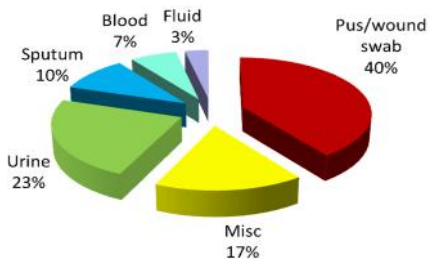

Fig 3: Sample wise distribution of metallo beta-lactamase positive strains

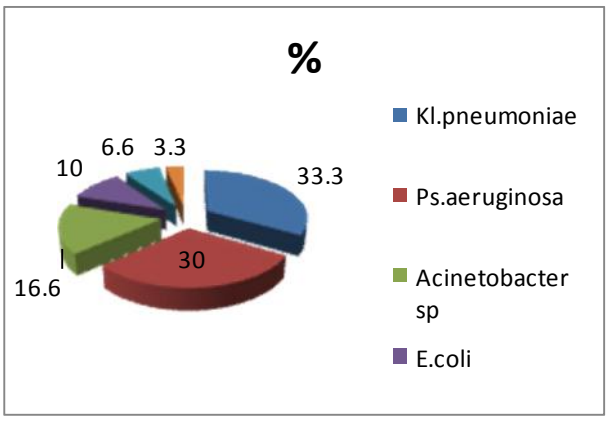

Fig 4: Organism wise distribution of MBL isolates

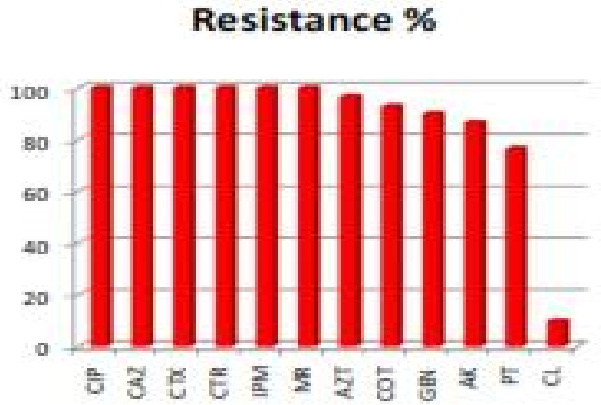

Fig 5: Antibiotic resistance profile of MBL isolates

In the present study, we tried to alter the distance between the IPM and EDTA discs to see the synergy between IPM and EDTA discs. Of these 30 MBL producers, all 30 (100%) isolates showed synergy at 10 mm distance, 27 (90%) isolates showed synergy at 15mm distance and 13 (43.4%) isolates showed synergy at 20mm distance. (Table number.1)

Table 1: Metalo beta lactamase detection by Double Disk Synergy Test, with various distances between the imipenem and EDTA

Total Number	EDTA and IPM		
	10mm distance	15mm Distance	20mm Distance
30	30(100%)	27(90%)	13(43.4%)

DISCUSSION

Simple and rapid phenotypic methods are required to screen and detect the MBL producing GNB which are high in prevalence in many regions. These MBLs if not detected and treated can disseminate in a hospital. [1]

In the present study, of the 100 IPM resistant isolates screened for MBL production, 30 (30%) were MBL producers and remaining 70 (70%) were non MBL producers. Other mechanisms of resistance like reduced permeability of pores or active efflux associated with class C ß-lactamases being endogenously over produced could be the reason for resistance to carbapenems seen in 70 MBL non producers.

In earlier studies resistance of 12% was noted to IPM and meropenem respectively in *Pseudomonas aeruginosa* in hospitalized patients. [14]

In various studies, across the world the prevalence of MBLs among the carbapenem resistant cases ranged from 44.5- 96.3 %. [15, 16, 17]

In our results, all 30 MBL producers were positive by both DDST and CD method. Accurate results may not be obtained by any single test. Hence we undertook these two techniques for screening purpose as they were was simple to perform, the materials used were cheap, nontoxic, easily available and helped the results to be interpreted well. The use of combination tests would increase the sensitivity, to detect the presence of MBL among clinical isolates of GNB.

Since 24 (86.6%) MBL producing strains were isolated from inpatients, this points to the fact that MBL are largely a problem of hospitalized patients who share numerous risk factors. A similar observation was noted by Prashanth et al where the major MBL producers were from the ICU and higher prevalence of infection was associated with the length of time the patient stays in the hospital. [5]

All the MBL producers showed very high resistance to all antimicrobials (beta lactams, aminoglycosides, and fluroquinolones, ranges from 76.6% to 100%) and also revealed (96.6%) resistance to aztreonam, showing association with other types of resistance mechanism like ESBL or Amp C. Association between MBLs and ESBLs appear to be a rare event. However, in our study co-existence of MBL and ESBL was noted in 3 (30%) of the MBL positive isolates. In our study the isolates tested showed less resistance to antibiotics like polymyxin and colistin. All MBL producing gram negative bacilli showed (90%) sensitivity to colistin. *Pseudomonas aeruginosa* isolates showed (100%) sensitivity to polymyxin B.

In the present study, we have evaluated the different distances between IPM and EDTA. In DDST the 10

mm distance between the IPM and EDTA disc exhibited excellent synergy, increasing the distance of the discs to 20mm resulted in the reduction of synergy. Therefore, we found that 10mm to be optimal as described by Arakawa *et al.* [18]

The problem of broad spectrum resistance of these MBLs also poses a problem because the location of MBL genes encoded on plasmids also encodes resistance to other antibiotics. Hence strains positive for MBL shows resistance to betalactams, aminoglycosides, and fluroquinolones. However, they usually remain susceptible to polymyxins. [18]

Although there are no guidelines for MBL detection, in *Providencia sp,* isolate was found positive for MBL by both methods, in the present study. However, this number is too small to attribute significance and more isolates need to be studied to correlate the same.

In the absence of therapeutic MBL inhibitors, polymyxins have been shown to be effective in the treatment of MDR *P.aeruginosa*. Polymyxin may not be very toxic as initially quoted. However, they should not be used in monotherapy. A combination therapy must be preferred.[19]

In our study, 3(10%) *Proteus spp* showed resistance to colistin. However colistin is not the treatment option for *Proteus spp.* as they are inherently resistant to them. [20]

CONCLUSION

For infections caused by MBL producing GNB therapeutic options are limited. The implementation of simple and accurate laboratory method to detect MBL production in Gram negative bacilli is useful, particularly in countries where MBL strains are increasingly reported. Our study highlights the resistance mechanism in carbapenem resistant Gram negative isolates. MBL producers may also be associated with ESBL. This poses serious problem choosing the right antibiotic for treatment. In order to prevent MBL to emerge in a hospital/health care setup and also to have a check on their spread, MBL should be detected in all microbiology laboratories.

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