International Journal of Microbiology Research
ISSN: 0975-5276 & E-ISSN: 0975-9174, Volume 4, Issue 9, 2012, pp.-322-325.
Available online at http://www.bioinfopublication.org/jouarchive.php?opt=&jouid=BPJ0000234

PREVALENCE OF METALLO-β-LACTAMASES PRODUCING *Pseudomonas* AND *Acinetobacter* SPECIES IN TERTIARY CARE TEACHING HOSPITAL, GUJARAT

AHIR H.R.[1]*, PATEL P.H.[1], BERRY R.A.[1], PARMAR R.[1], SONI S.T.[1], SHAH P.K.[1], VEGAD M.M.[1] AND PATIL S.[2]

[1]Department of Microbiology, B.J. Medical College, Ahmedabad-380016, Gujarat, India.
[2]Department of PSM, Pad. Dr. D.Y. Patil Medical College, Navi Mumbai-400706, MS, India.
*Corresponding Author: Email- dr_hitesh84@yahoo.com

Received: November 01, 2012; Accepted: November 10, 2012

Abstract-

Background & Objective: Metallo-β-lactamases (MBLs) are metalloenzymes of Ambler class B and are resistant to clavulanic acid.. They require zinc as co-factor for enzymatic activity and their activity is inhibited by ethylene diamine tetra acetic acid(EDTA) and other metal ion chelating agents. The first plasmid-mediated MBL was reported in *Pseudomonas aeruginosa* in Japan in 1991. Since then many countries including few reports from India are available regarding the prevalence of MBLs. The present study was conducted to determine the prevalence of MBLs in *Pseudomonas spp.* and *Acinetobacter* spp.

Methods: A total number of 2912 clinical isolates of *Pseudomonas spp.* (n=2162) and *Acinetobacter spp.* (n=750) obtained over a period of one year, were screened for MBL production by CDST & DDST.

Results: A total of 325 out of 2912 isolates were positive for MBL production; of which 247 (11.42%) and 78(10.40%) were for Pseudomonas spp and Acinetobacter spp. respectively.

Conclusion: MBL producing *Pseudomonas aeruginosa* and *Acinetobacter baumannii* group are present in this institution, although in low prevalence. However, to prevent the increase in the incidence of these multidrug resistant organisms and to prevent their dissemination, it is important to detect them and make judicious use of antibiotics based on their susceptibility patterns.

Keywords- *Pseudomonas* spp., *Acinetobacter* spp., Metallo-β-lactamase (MBL), MBL detection, Combined disk synergy test (CDST), Double disk synergy test (DDST), Imipenem, Ethylenediamine tetra-acetic acid (EDTA)

Citation: Ahir H.R., et al (2012) Prevalence of Metallo-β-lactamases Producing *Pseudomonas* and *Acinetobacter* species in Tertiary Care Teaching Hospital, Gujarat. International Journal of Microbiology Research, ISSN: 0975-5276 & E-ISSN: 0975-9174, Volume 4, Issue 9, pp.-322-325.

Introduction

The most serious life threatening infections are caused by a group of drug resistant bacteria that have been labeled the ESKAPE pathogens because they effectively escape the effects of antibacterial drugs. According to CDC (Centre for Disease Control) the six ESKAPE bacteria (*Enterococcus faecium, S. aureus, K. pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter spp.*) cause two third of all hospital acquired infections. *Pseudomonas and Acinetobacter* have emerged as important nosocomial pathogens. They are widely distributed in nature and their presence in the hospital environment puts debilitated patients, especially those in intensive care units at risk of opportunistic infections by these multidrug resistant pathogens [1]. *Pseudomonas aeruginosa* is the most commonly encountered multiresistant gram negative pathogen. *Acinetobacter baumanii* traditionally infects patients in ICUs and burn units but is now being seen in general hospital population and nursing homes. Gram-negative bacteria with acquired metallo-β-lactamases production have been increasingly reported in some countries, necessitating their detection.

Metallo-β-lactamases (MBLs) are metalloenzymes of Ambler class B and are resistant to clavulanic acid. They require zinc as co-factor for enzymatic activity and their activity is inhibited by ethylene diamine tetra acetic acid (EDTA) and other metal ion chelating agents. The first plasmid-mediated MBL was reported in *Pseudomonas aeruginosa* in Japan in 1991 [2].

Pseudomonas spp. and *Acinetobacter spp.* are the most important nosocomial pathogens with multiple drug resistance (MDR) [3]. Their high prevalence is of great concern because of their intrinsic and acquired resistance mechanisms, limiting the treatment options. Carbapenems are the drugs of choice for penicillin& cephalosporin resistant *Pseudomonas spp. and Acinetobacter spp*. infections. However, this scenario is changing with emergence of MBL

producing strains.

We determined the prevalence of acquired metallo-beta lactamases (MBL) producing non fermenting Gram negative bacilli in our tertiary care hospital on 2162 Pseudomonas and 750 *Acinetobacter* isolates.

Materials and Method

The present study was conducted from July 2011 to June 2012 over a period of one year. A total number of 2162 Pseudomonas and 750 Acinetobacter isolates were isolated from various clinical samples like swab, urine, sputum, pus, pleural fluid, ascitic fluid and blood samples received from indoor patients of hospital including those admitted to intensive care and acute medical care units. The data regarding samples were obtained from the Microbiology Department and the clinical data was obtained from the respective units and wards of the patients.

Samples were cultured on Nutrient agar, MacConkey agar and Blood agar. Confirmation of Pseudomonas and Acinetobacter spp were done by standard biochemical identification tests [4,5]. Kirby Bauer disc diffusion method was used to detect Imipenem resistance and then the isolates were further tested for MBL production by the combined disc synergy test (CDST)[6,7]and the double disc synergy test (DDST)[6,7] The antibiotic susceptibility profile was determined in accordance to CLSI guidelines using Kirby Bauer Disc diffusion method [8,9]. Antibiotics tested were gentamicin, amikacin, tobramycin, cefotaxime, ceftazidime, cefepime, ciprofloxacin, levofloxacin, mezlocillin and piperacillin-tazobactam and aztreonam. For *Acinteobacter* the antibiotics tested were Cefaclor, Cefotaxime, cefepime, ampicillin-Sulbactam, gentamicin, amikacin, moxifloxacin, tetracycline, cotrimoxazole and chloramphenicol.

- The combined-disk synergy test (CDST) was done using the IPM-EDTA (750-10µg) Combination [6,7].
- The Double disk synergy test (DDST) was done using IPM (imipenem)750µg -EDTA 10µg (ethylene diamine tetra acetic acid) [6,7].

Imipenem- EDTA Combined Disc Synergy Test (CDST)

The Imipenem- EDTA combined disc test (CDST) was preformed as described by Yong et al. The test organisms were inoculated on Mueller Hinton agar as recommended by the CLSI. A 0.5 M EDTA solution was prepared by dissolving 18.61 g. of EDTA in 100 ml of distilled water and adjusting its PH 8.0 by using NaOH. The mixture was sterilized by autoclaving. Two imipenem (10ug) discs were placed on the surface of an agar plate at distance of 25 mm and 4 ul EDTA solution was added to one of them to obtain a desired concentration of 750ug. The inhibition zones of imipenem and imipenem- EDTA discs were compared after 16 to 18 hrs. of incubation in air at 37°C [6] In the combined disc test, if the increase in inhibition zone with the imipenem and imipenem- EDTA disc was ≥7 mm than the imipenem alone, it was considered MBL positive [6,7] [Fig-1].

Imipenem - EDTA Double-Disc Synergy Test (DDST)

The test organisms were inoculated on to plates with Mueller Hinton agar as recommended by the CLSI[8]. An imipenem (10ug) disc was placed 20 mm center to center from a blank disc containing 4 ul of 0.5 M EDTA (750 ug). Positive results were documented when enhancement of zone of inhibition between imipenem and EDTA disc was ≥5mm [7].

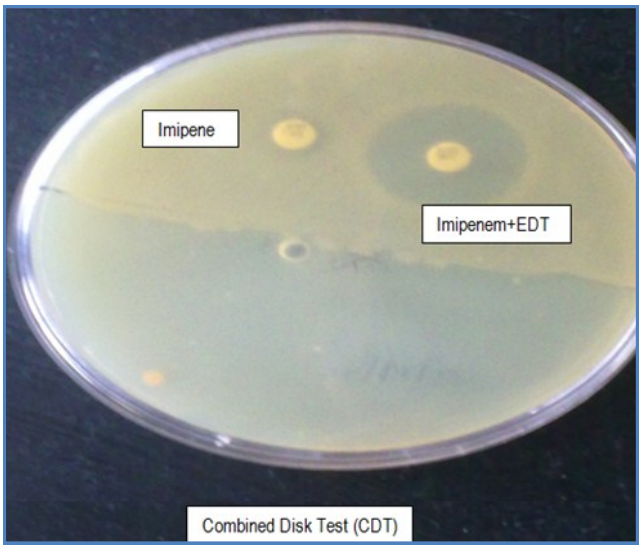

Fig. 1- Combined Disc Synergy Test showing enhancement of zone diameter of >7mm around Imipenem-EDTA disc

Result

A total of 2162 Pseudomonas spp. isolates and 750 of *Acinetobacter* spp. were tested for particular antibiotic sensitivity patterns against a panel of antibiotics. Out of them multidrug resistant including imipenem resistant isolates were tested by CDST and DDST [6,7].

In our study maximum number of MBL positive *Pseudomonas* spp. and *Acinetobacter* spp. were isolated from swab samples [Table-1, Fig-2].

Table 1- Sample-wise distribution of MBL

Sample	*Pseudomonas* (MBL +VE)	*Acinetobacter (MBL+VE)*
Swab	90	40
Sputum	15	7
Urine	88	8
Pleural Fluid	28	7
Pus	8	5
Blood	10	6
Other Body Fluid	8	5
Total	247	78

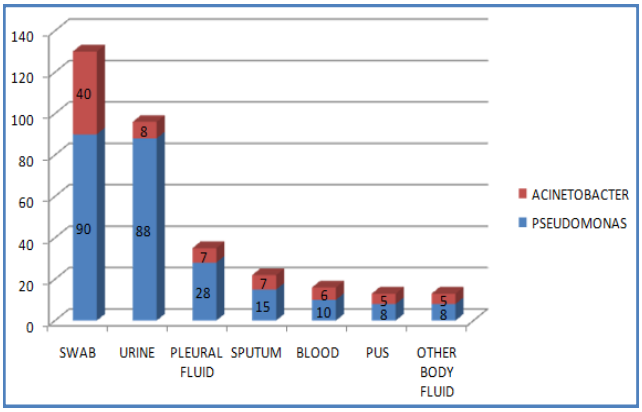

Fig. 2- Sample-wise distribution of MBL

247(11.42%) of pseudomonas and 78(10.40%) Acinetobacter isolates were confirmed MBL producers phenotypically [Table-2], [Fig-3].

Table 2- Prevalence of MBL in Pseudmonas and Acinetobacter spp.

Organism	Total	MBL Positive
Pseudomonas	2162	247(11.42%)
Acinetobacter	750	78(10.40%)

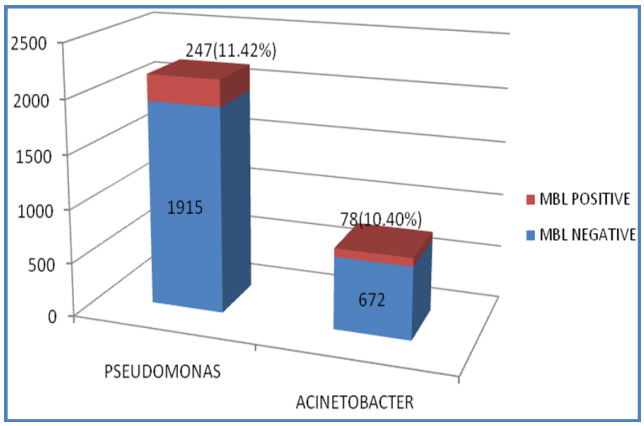

Fig. 3- 2 Prevalence of MBL in *Pseudmonas* and *Acinetobacter* spp.

Discussion

MBL production by *Pseudomonas* spp. and other gram negative organisms limits the therapeutic options to thetoxic drugs like polymyxin B and colistin.

As MBLs hydrolyze virtually all classes of β-lactamase, their continued spread will be a clinical catastrophe [10]. With the global increase in the types of MBLs, early detection is crucial [13]. Over the last decade, most of the studies were on different methods of MBL detection in *Pseudomonas* and *Acinetobacter* species [11-14]. Though MIC detection remains the gold standard, DDST and CDST are comparable with the former and score well in terms of being simple. easy to perform, reliable and cheap [11-13]. Therefore, these tests can be used in a small laboratory set up also. Other methods for MBL detection used by other workers are: EDTA disc potentiation using ceftazidime, ceftizoxime, cefepime, cefotaxime and the MBL E test [13,16]. The high diversity and prevalence of MBL-producing *P. aeruginosa*, *Acinetobacter* spp., and *Enterobacteriaceae* isolates have necessitated the search for an accurate MBL screening test. The aim of this study was to evaluate the accuracy of the double-disk synergy test (DDST) and the combined disk (CD) assay to screen for MBL-producing isolates among *Pseudomonas spp*, *Acinetobacter spp* isolates that are producers of IMP, GIM, SIM, SPM, or VIM enzymes which ideally should be further confirmed by molecular diagnostic modalities like polymerase chain reaction(PCR). *Pseudomonas aeruginosa* and *Acinetobacter baumannii* were common MBL producing isolates in our study [Table-3]. The prevalence of MBLs in *Pseudomonas* was 11.42% and in *Acinetobacter* species it was 10.40%. Other studies have reported the prevalence of MBL in Pseudomonas as 30.3-36% [20,22].

Besides being resistant to imipenem, the MBL producers were characteristically resistant to third generation cephalosporins and quinolones[10,15] [Table-4], [Table-5], [Fig-4], [Fig-5] thus limiting the therapeutic options as polymyxin only which too must be used judiciously and not be used as monotherapy [10]. It can be combined with an appropriate aminoglycoside. Aztreonam is the drug of choice for MBL producing *Pseudomonas aeruginosa* [10]. For treatment of infections caused by multidrug resistant and MBL producing strains of Acinetobacter species a combination of Imipenem or meropenem along with ampicillin sulbactam remains available [15].

Table 3- Species-wise distribution of MBL

Pseudomonas spp.	MBL	*Acinetobacter* spp.	MBL
Pseudomonas aeruginosa	140	*Acinetobacter baumannii*	40
Pseudomonas mendocina	30	*Acinetobacter lwoffii*	20
Pseudomonas stutzeri	25	*Acinetobacter hemolyticus*	10
Pseudomonas putida	20	*Acinetobacter calcoaceticus*	8
Pseudomonas alkaligens	32	-	-
Total	247	Total	78

Table 4- Antibiotic resistance pattern in Pseudomonas spp.

Drugs	Pseudomonas spp (N=247)
Cefotaxime	224(90.68%)
Ceftazidime	180(72.87%)
Cefepime	190(76.92%)
Piperacillin-Tazobactam	90(36.43%)
Tobramycin	195(78.94%)
Amikacin	120(48.58%)
Ciprofloxacin	66(26.72%)
Levofloxacin	34(13.76%)
Mezlocillin	24(9.71%)
Aztreonam	12(4.85%)

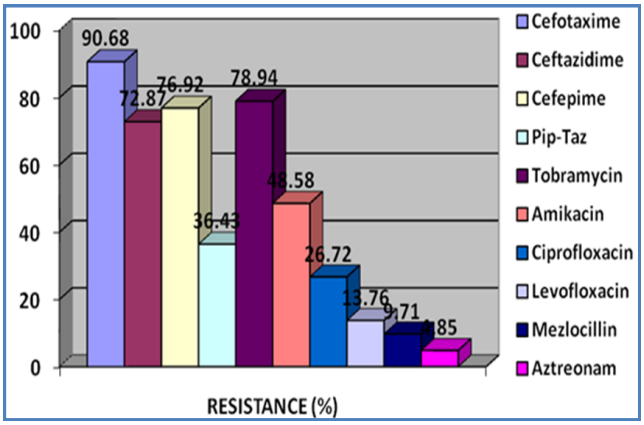

Fig. 4- Antibiotic resistance pattern in *Pseudomonas* spp.

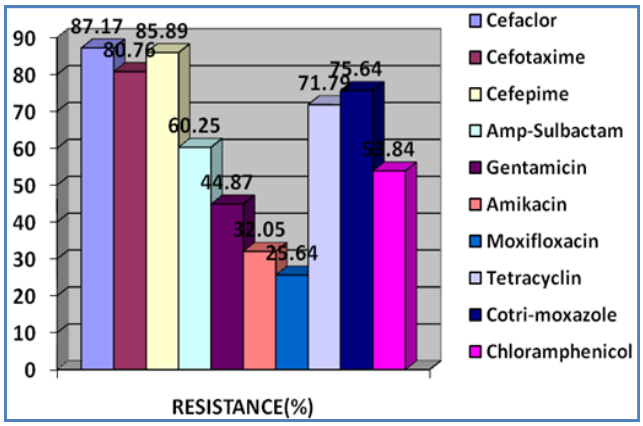

Fig. 5- Antibiotic resistance pattern in *Acinetobacter* spp.

Table 5- Antibiotic resistance pattern in Acinetobacter spp.

Drugs	*Acinetobacter* spp (N=78)
Cefaclor	68(87.17%)
Cefotaxime	63(80.76%)
Cefepime	67(85.89%)
Ampicillin-sulbactam	47(60.25%)
Gentamicin	35(44.87%)
Amikacin	25(32.05%)
Moxifloxacin	20(25.64%)
Tetracyclin	56(71.79%)
Cotri-moxazole	59(75.64%)
Chloramphenicol	42(53.84%)

In a Government hospital like ours, that provides health care facilities to the poorest of the poor, the cost constraints evaluation mandate the prescription of a drug if the drug is not available in the hospital formulary. Patient affordability is another factor which has to be kept in mind before the drug is prescribed.

Conclusion

MBL producing Pseudomonas aeruginosa and Acinetobacter baumannii group are found in wards, although in low prevalence. However, to keep them in check, regular detection of this bacteria and judicious use of antibiotics to which they are still susceptible is mandatory. Increased prevalence of carbapenem resistance being acquired by MBL is particularly reported for Pseudomonas(11.42%) and for Acinetobacter(10.40%). Rapid detection of MBL producing gram negative bacteria is necessary for therapy and to prevent their further dissemination. A high prevalence of MBL among Pseudomonas aeruginosa is a critical problem representing a practical therapeutic challenge. Emergence of MBL producing *P.*aeruginosa and *Acinetobacter* species in ICUs is alarming and reflects excessive use of carbapenems. In ICUs the selection pressure is the greatest which selects multi drug resistant strains over the competitive flora [17] Therefore a strict antibiotic policy should be followed in intensive care areas to prevent further spread of MBLs. Clinicians should prescribe antibiotics judiciously. Timely implementation of strict infection control practices and antibiotic resistance surveillance programs should be carried out from time to time [13]. Detection of MBLs by either CDST or DDST should be routinely performed in all microbiology laboratories for all imipenem-resistant isolates, which will help to reduce morbidity and mortality in these patients. Though it is desirable to detect MBL producers at the earliest by routine laboratory testing, one must exercise care while interpreting phenotypic results based on inhibitor synergy. PCR should be done to validate such results.

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