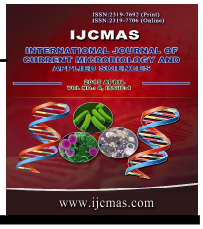
Int.J.Curr.Microbiol.App.Sci (2015) 4(4): 955-961

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 4 (2015) pp. 955-961**
http://www.ijcmas.com



Original Research Article

Prevalence of Metallo-β-Lactamases Producing *Pseudomonas aeruginosa* among the Clinical isolates: A study from tertiary care hospital

P.Vasundhara Devi*, P.Sreenivasulu Reddy and Maria Sindhura John

Department of Microbiology, Narayana Medical College, Nellore, AP, India
**Corresponding author*

Keywords

Pseudomonas aeruginosa, Nosocomial, MBL, EDTA disk synergy

A B S T R A C T

Pseudomonas aeruginosa is a clinically troublesome gram negative pathogen that causes a wide range of opportunistic infections and nosocomial outbreaks. They are responsible for 16% of nosocomial pneumonia,12% hospital acquired pneumonia,8% wound infections and 10% blood stream infections. Metallo beta lactamase have recently emerged one of the most worrisome resistance mechanisms to hydrolyse all beta lactam agents including carbapenems. The main objective was to asses the prevalance of Metallo β lactamases producimg strains among multidrug resistant *Pseudomonas aeruginosa* isolated from various clinical samples. The study was carried out during June 2013 to June 2014.During the study,50 non repetitive multidrug resistant strains of P.aeruginosa were taken for study. Isolation and identification of P.aeruginosa was done according to standard guidelines. Antibiotic sensitivity was done by Kirby Bauers Disk Diffusion method. EDTA Disk synergy test was performed to detect Metallo beta lactamase production among P.aeruginosa. Among of 50 samples, cathetertip(40%) and pus (40%)showed high percentage of MBL producers. P.aeruginosa showed highest percentage of sensitivity to Polymixin B and next to Gatifloxacin. Out of 50 samples processed,18 isolates were positive for MBL production. Most percentage of MBL's are highly sensitive to Polomyxin B. In the light of the above study multiudrug resistant strains of P.aeruginosa in a teritiary care hospital were tested for their antibiogram and Metallo beta lactamase production. Therefore,the reliable detection of the MBL producing strains is essential for the optimal treatment of infected patients and to control the nosocomial spread of resistance.

Introduction

Pseudomonas aeruginosa is a gram negative, non sporing, motile, aerobic bacilli cause of serious wound and surgical infections(1). Bacteria appear to possess a limitless ingenuity in avoiding the effects of antimicrobial agents, as well as in finding new ways to invade the compromised host. Moreover mutations conferring resistance to one antibiotic can at a stroke render a whole drug family impotent(2).

The mechanism of resistance to beta lactam antibiotics includes, the production of beta lactamase, reduced outer membrane permeability, the altered affinity of target penicillin binding proteins, plasmid mediated resistance involving modifying enzymes(3)

Metallo beta lactamases are class B beta lactamases. These require zinc or another

heavy metal for their catalytic activity and their activities are inhibited by metal chelating agent such as EDTA and thiol based compounds(4).Class B beta lactamases confer resistance to a wide range of beta lactam compounds, including cephalosporins and carbapenems. Class B beta lactamases are resistant to inactivation by clavulanate, sulbactum and tazobactam(5). Metallo beta lactamases have the ability to hydrolyse a wide variety of beta lactam agents such as penicillins, cephalosporins and carbapenems. The majority of matallo beta lactamases are chromosomally encoded and their expression may be constitutive and inducible(6).

Metallo beta lactamase producing strains pose a serious threat to patients, mandating careful antibiotic stewardship and infection control programs(7).

Currently no standardized method for MBL detection has been proposed, despite PCR being highly accurate and reliable(8).To facilitate the screening for MBL producers in the clinical microbiology laboratory, phenotypic tests based on the principle of disc diffusion for detection of MBL producing isolates have recently been proposed. They are EDTA disc synergy test, Hodge test, Double disk synergy test, Simple micro dilution test, Commercial methods MBL E test.(9,5,4,10). In the absence of novel agents for the treatment of infections caused by multi resistant gram negative bacteria, the uncontrolled spread of MBL produces may lead to treatment failures with increased morbidity and mortality(4). The main objective of the present study was to assess the prevalence of metallo beta lactamase producing strains among multi drug resistant pseudomonas aeruginosa isolated from various samples. Therefore the reliable detection of the MBL producing strains is essential for the optimum treatment of infected patients and to control the nosocomial spread of resistance.

Materials and Methods

The present study was carried out on *P.aeruginosa* obtained from various clinical samples of admitted patients of Narayana General and Superspeciality Hospital during the period June 2013 to June 2014.

The samples from which the strains were isolated include Pus, Urine, Sputum, Catheter tip, Body fluids. All the samples were processed for isolation of and antibiotic sensitivity testing was performed on Muller Hinton Agar plates by Disk Diffusion method(CLSI).

Isolation of organism was done by streaking the samples on Macconkey's agar and Blood agar plates. Futher identification was done by gram staining(gram negative bacilli), catalase(positive), oxidase(positive), pigment production(positive), hanging drop preparation(motile) was done.

Antibiotic sensitivity testing method was performed by Kirby Bauer method. Antibiotics included in the study are piperacillin (100µg/disc), ceftazidime (30µg/disc), cefoperazone (75µg/disc), cefaperazone+sulbactum (75+30µg/disc), amikacin (230µg/disc), gentamicin (10µg/disc), tobramycin (40µg/disc), netilmycin (30µg/disc), polymixinB (100units/disc), imipenem (10µg/disc), meropenem (10µg/disc), gatifloxacin (5µg/disc).

Metallo beta lactamase testing

EDTA disk synergy test was performed to detect Metallo lactamase production among *P.aeruginosa.*

An overnight broth culture of the test strain with opacity adjusted to 0.5 MC farland standard was used and inoculated on a Muller Hinton agar after drying. A 10µg/ml imipenem disc and a blank filter paper disc, were placed 10 mm apart from edge to edge,10µl of 0.5 M EDTA solution was then applied to the blank disc with resulted in appropriate 1.5 mg/disc. After overnight incubation, presence of enlarged zone of inhibition was interpreted as EDTA synergy positive.

Result and Discussion

In the ptesent study 50 non repetitive multidrug resistant strains of *P.aeruginosa* were taken for study which were oxidase positive, gram negative, motile. The study is carried out from June 2013 to June 2014 in department of Microbiology, Narayana Medical College, Nellore.

Among all the clinical specimens catheter tip and pus showed high percentage of MBL producers. Among all the clinical specimens (50),18 isolates showed MBL producers. *P.aeruginosa* showed highest percentage of sensitivity to Polymixin B and next to Gatifloxacin.

Most percentage of MBL's are highly sensitive to Polomyxin B.

P.aeruginosa has been recognized as an important pathogen responsible for a vast majority of hospital acquired infections especially in teritiary care hospitals.

Carbapenems are beta lactam antibiotics, presently considered as the most potent agents of treatment of multidrug resistance pseudomonas infections due to the stability of these agents against majority of beta lactamases and their high rate of permeation through bacterial outer membranes.

Carbapenem hydrolyzing MBL's have been reported in several countries and have emerged as the most important mechanisms of carbapenem resistance.

Production of MBL by pseudomonas species has tremendous consequences, since these organisms also carry other multi drug resistance genes and the only viable treatment option remains potentially toxic polymyxin B. In this study we have used IPM-EDTA double disk synergy test with a cut off ≥7mm as positive MBL production test. 50 *P.aeruginosa* isolates from various clinical samples showing multidrug resistance were taken and tested for MBL production. 18(36%) were positive for MBL production(Table-1).

Peleg *et al*(11) have recently described a two year study from Alfred hospital, showing 55.8% MBL positive isolates(11), Doguen young *et al*(12) from Korea showed 50% of MBL production in Pseudomonas.

A study from European hospital detected multi drug resistant *P.aeruginosa* that were carrying bla-VIM MBL genes, were shown to be wide spread among 20% of all Pseudomonas isolates and 70% of Carbapenem resistant isolates(8).

From Vellore only 75% were found to be MBL producers by EDTA disc synergy test(13)

Another study from Banglore showed that 12% isolates were resistant to carbapenems and all are MBL producers(14).

Sarkar *et al*(15) in Uttaranchal used IMP-EDTA disk synergy test for distinguishing MBL producers from non MBL producers 54.5% were MBL producers.

Table.1 Prevalence of MBL producing strains

Clinical Specimens	No.of isolates	MBL producers
Urine	21	8(38%)
Pus	200	8(40%)
Catheter tip	5	2(40%)
Body fluids	2	-
Sputum	2	-
Total	50	18(36%)

Table.2 Antibiotic sensitivity profile of pseudomonas from clinical isolates

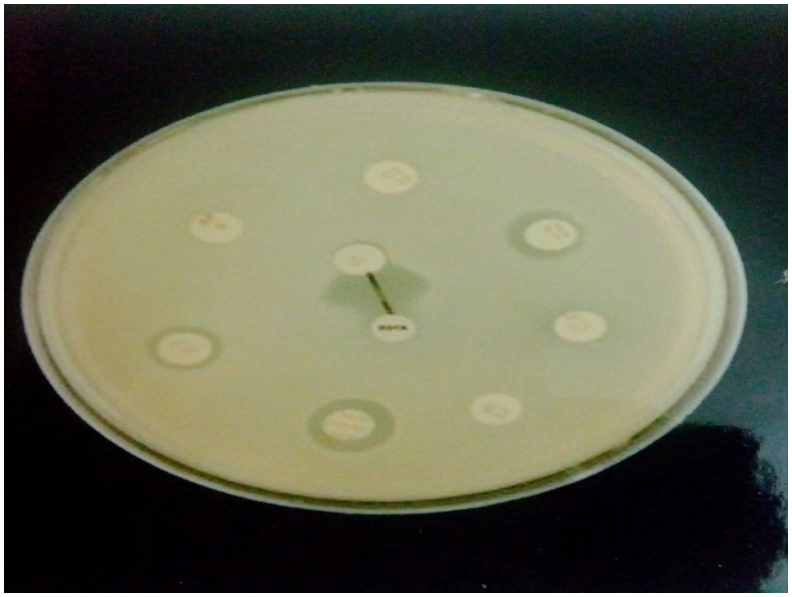
Antibiotics	*P.aeruginosa* isolates (n=50)		
	Sensitivity	Moderately sensitive	Resistance %
piperacillin	2	1	47(93%)
cefoperazone	2	9	39(78%)
cefoperazone+ sulbactam	6(12%)	7(14%)	37(74%)
cefotoxime	3(6%)	1(2%)	46(92%)
ceftazidime	5	4	41(82%)
aztreonam	5	10	35(70%)
imipenem	0	0	50(100%)
meropenem	0	0	50(100%)
polymixin B	10(20%)	36(72%)	4(8%)
ciprofloxacin	3(7%)	0	47(93%)
gentamicin	2(5%)	1(2%)	47(93%)
amikacin	7(14%)	4(8%)	39(78%)
tobramycin	1(2%)	1(2%)	48(96%)
netilmycin	1(2%)	1(2%)	48(96%)
gatifloxacin	8(16%)	2(4%)	40(80%)
chloramphenicol	1(2%)	2(4%)	47(93%)

Table.3 Sensitivity pattern of MBL producers to Various antibiotics

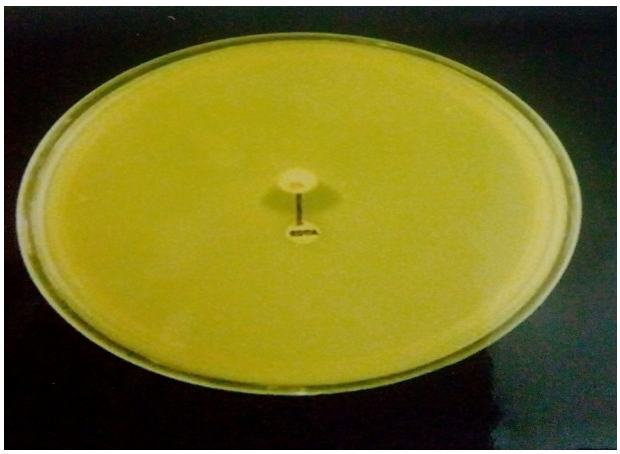
Sl. No	Antibiotics	Percentage sensitivity MBL's(n=18)
1	piperacillin	Nill
2	cefoperazone	2(11.1%)
3	cefoperazone+ sulbactam	1(5.5%)
4	cefotoxime	1(5.5%)
5	ceftazidime	2(11.1%)
6	aztreonam	7(38.8%)
7	imipenem	-
8	meropenem	-
9	polymixin B	11(61.1%)
10	ciprofloxacin	-
11	gentamicin	-
12	amikacin	7(38.8%)
13	tobramycin	1(5.5%)
14	netilmycin	1(5.5%)
15	gatifloxacin	1(5.5%)
16	chloramphenicol	-

Most percentage of MBL's are highly sensitive to Polomyxin B.

Multi drug resistance



IMP-EDTA Double disk synergy test positive



In our study 18(36%) of MBL producing Pseudomonas were isolated among 50 carbapenem resistant Pseudomonas. Among these 50 Pseudomonas aeruginosa isolates 21 from urine,20 pus,5 catheter tip, 2 body fluids,2 sputum.MBL production seen in 8(38%) from urine, 8(40%) from pus, 2(40%)catheter tip. There were no MBL producing strains among the Pseudomonas isolates from body fluids and sputum.

There was highest isolation of MBL producing Pseudomonas among the catheter tip isolates (40%)(Table-1). Antibiotic sensitivity pattern was checked for 18 MBL positive isolates and polymixinB was found to be the most sensitive 11(61%) followed by amikacin 7(38.8%) and aztreonam 7(38.8%).

From this it can be inferred that Metallo beta lactamases producing Pseudomonas aeruginosa are some what lower in incidence in our Narayana General Hospital. In future this scenario may change because the gene responsible for MBL production is carried on mobile genetic element resulting in rapid spread to sensitive bacteria in the Hospital.
This emphasizes the necessity for recognition of Metallo beta lactamase producing isolates, rigorous infection control and restricted clinical use of broad spectrum beta lactamases including carbapenems.

P.aeruginosa is the commonest pathogen causing HAI among immunosuppressed patients.MBL production is the major cause for resistance to carbapenem group of antibiotics which are considered to be effective drugs for treatment of infections caused by *P.aeruginosa*. By using imipenem-EDTA double disk synergy test we can detect MBL production phenotypically in the laboratory. Routine detection of MBL's will ensure optimal patient care and timely introduction of appropriate infection control procedure.

References

1.Doggett R,G. Microbiology of Pseudomonas aeruginosa.In:Dogett R.G,ed.pseudomonas aeruginosa. Clinical manifestations of infection and current therapy. New York Academic press;1979;1-8.
2.IAN M.Gould, Antibacterial therapy, S.Peter Borriello, Patrick R.Murray and cuido Funke Topley and Wilson's microbiology and microbial infections,

Bacteriolofy 2005,volume 2 ASM Press,466-505.

3.Cristina Lagatolla et al. Endemic carbapenem resistant pseudomonas aeruginosa with acquired metallo beta lactamase determinants in European hospital;Emerging infectious disease.vol 10(3),2004:535-538.

4.Johann D. D Pitout et al., Molecular epidemiology of metallo beta lactamase producing pseudomonas aeruginosa in the Calgary health region:emergence of VIM-2 producing isolates.Journal of Clinical Microbiology 2007;2,294-298.

5.Joseph D, C.Yao and Robert C. Moellering J.R Antibacterial agents. Patrick R.Murray, Ellen Jo Baron; Jamas H.Jorgensem, Micheal A.Pfaffer, Robert H.Yolken Manual of clinical microbiology:8th edition vol1 asm press 2003:1039-1061.

6.Gerald B.Pier, Reben Ramphal. Pseudomonas aeruginosa. Gerald L.mandell,John E.Bennet, Raphael Dolin. Mandell Douglas and Bennett's principles and practices of infectious diseases.fourth edition.,vol.1,Churchill livingstone,2000:2587-2608;1074-1094;233-381.

7.H.Aboufaycal et al., bla VIM-2 and bla VIM-7 carbapenemase producing pseudomonas aeruginosa isolated detected in a teritiary care medical centre in the united states report from the mystic program. Journal of clinical microbiology 2007;45;no.2,pp:614-615.

8.Clare Franklin, Lisa Liotios, Anton Y.Peleg. Phenotypic detection of carbapenem susceptible Metallo beta lactamase producing gram negative bacilli in the clinical laboratory. Journal of Clinical Microbiology,2006;vol 44(9):3139-3144.

9.Isabelle Ziha-Zarifi et al., Infivo emergence of multidrug resistant mutants of pseudomonas aeruginosa over expressing the active efflux system Mex A-Mex B-opr M;Antimicrobial agents and chemo therapy,Feb 1999,vol43(2):287-291.

10.K.Le,Y.S Lim, D.Young, JH.Ym and Y.Chong Evaluation of the Hodge test and the Imipenem EDTA double disk synergy test for differentiating metallo beta lactamase producing isolates of Pseudomonas sps and Acinitobacter sps-Journal of Clinical Microbiology,2003,vol 41(10):4623-4629.

11.Peleg AY, Franklin C, Bell JM and Spelmann DW (2005). Dissemination of the metallo-betalactamase gene blaIMP-4 among gram-negative pathogens in a clinical setting in Australia. Clinical Infectious Diseases 41 1549-56.

12.Dongeun Yong et al., Imipenem EDTA disk method for differentiation of metallo beta lactamases producing clinical isolates of Pseudomonas sps and Acinitobacter sps. Journal of Clinical Microbiology,2002,vol 40(10),3798-3801.

13.Mary V.Jesudason, A.J.Kan dathil and V.Balaji comparision of two methods to detect carbapenemase and metallo beta lactamase production in clinical isolates. Indian J Med res 121 2004,June 2005:780-783.

14.Navaneeth BV, Sridaran D, Belwadi MR. A preliminary study on Metallo beta lactamase producing pseudomonas aeruginosa in hospitalized patients. Indian J Med Res 2002;116:264-267.

15.Sarkar B, Biswas D, Prasad R. A clinic microbiological study on the importance of *Pseudomonas* in nosocomially infected ICU patients with special reference to metallo-β-lactamase production. Indian J Pathol Microbiol. 2006;49:44–6.