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Research Article

PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITY PROFILE OF METALLO-β-LACTAMASES (M-β-L) PRODUCING *PSEUDOMONAS AREUGINOSA* FROM RURAL HOSPITAL: COMPARISON OF TWO DISK DIFFUSION METHODS

***Kotgire Santosh A and Tankhiwale Nilima**
[1]*Department of Microbiology Dr. Ulhas Patil Medical College Jalgaon, Maharashtra*
[2]*Department of Microbiology Jawaharlal Nehru Medical College Datta Meghe Institute of Medical Sciences University Sawangi(M) Wardha, Maharashtra, India*
**Author for Correspondence*

ABSTRACT
A study of metallo-β-lactamases(MβL) and their susceptibility pattern was done in clinical isolates of *Pseudomonas areuginosa.* Isolates were tested for MβL by double disc diffusion test, combined disc method and agar microdilution method and comparison between two disc diffusion test were carried out. Combined disc method was better than double disc diffusion method and it co-relates well with microdilution method. 13.65% of isolated strains were MβL producer and were 100% sensitivie to polymixin B. Presence of MβL producer *P.aeruginosa* is cause of concern. Simple combined disc method can helpful for monitoring and treating such MβL strains

Key Words*: Metallo-β-Lactamases (MβL), Combined Disc Method, Pseudomonas Aeruginosa*

INTRODUCTION

Pseudomonas aeruginosa is one of most common pathogens that encountered in hospital settings and shows variety of drug resistant profile causing serious noscomial infections worldwide, it exhibits resistance to variety of antimicrobials including beta-lactams. Purva *et* a*l* (2008) Carbapenems (e,g. Imipenem, Meropenem) are often used as antibiotics of choice for such resistant *Pseudomonas areuginosa*. However, carbapenem resistance is been increasingly seen worldwide and also in India, and the most acquired form of resistance is mediated by Metallo Beta lactamase (MβL).These enzyme requires zinc for their catalytic activity and are inhibited by metal chelator such as EDTA (Ethylene-diamine-tetra-acetic acid) and thiol based compound (sodium mercaptoacetic acid, 2mercaptoetanol) Purva *et* a*l* (2008), Hemalatha *et* a*l* (2005). Such MβL producing strains of *Pseudomonas aeruginosa* in hospital setting poses not only a therapeutic problem but is also serious concern for infection control management. Therefore detection of MβL producing *Pseudomonas aeruginosa* is crucial for optimal treatment of patients particularly in critically ill and hospitilized patients to control the spread of resistance. Hemalatha *et* a*l* (2005), Bhera *et* a*l* (2008).

There is not much information available regarding such MβL producing strains from central part of India and also there is no CLSI (Clinical and Laboratory standard institute) guidelines exist for MβL detection and reporting .Bhera *et* a*l* {2008}Various methods have been studied for detection of MβL strains such as double disk synergy test, combined disk method, modified Hodge technique, dilution methods, E (epsilometer)- test and PCR but not a single method is sensitive and specific for detection of MβL Johann *et al* (2005) .

So the present study was undertaken to find out prevalence of MβL producing *P.aeruginosa* and to carry out antimicrobial susceptibility of isolated MβL producing *P.aeruginosa* .The present study also assess the two disk diffusion method for detection of MβL strains.

MATERIALS AND METHODS

The study was conducted at the Department of Microbiology, J.N. Medical College Wardha a rural based hospital situated in central part of Maharashtra from period of July 2009 to June 2010

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Bacterial isolates

A total 117 consecutive isolates of *P. aeruginosa* obtained from various clinical samples(urine, pus, tracheal aspirate ,blood, sputum, CSF) of hospitalized patients. The isolates will be identified as *Pseudomonas aeruginosa* by standard and recommended procedure.

Antimicrobial susceptibility

Antimicrobial susceptibility of all isolates was performed by the disc diffusion method according to CLSI (2006) guidelines using, Amikacin, Gentamicin, Ciprofloxacin, Chloramphenicol, Cefoxitin, Ceftazidime, Imipenem, Piperacillin and *P. aeruginosa* ATCC (27853) strain will be used as quality control.

MβL detection

The isolates resistant to Imipenem are further processed for MβL production by

Double disk synergy test (DDST)

The imipenem-EDTA double disk synergy test was performed as described by Lee *et al* (2003).
test organisms were inoculated on to plates with Mueller Hinton agar as recommended by the CLSI. An imipenem(10mcg) or ceftazidime(30mg) disc was placed 20 mm centre to centre from blank filter paper disc(6mm in diameter, whatman filter paper no.2) containing 10µl of 0.5 M EDTA(750µg) and after overnight incubation, the presence of enlarged zone of inhibition was interpreted as MβL producer Lee *et* a*l* (2003).

Figure 1: Imipenem (IMP) EDTA combined disc test

The IMP-EDTA disk test was performed as described by Yong *et al* (2002). Test organisms were inoculated on to plates with Muller Hinton agar as recommended by CLSI. Two imipenem discs (10mcg) (Himedia , India) were placed in the plate and appropriate amount of 10µl of EDTA solution were added to one of them to obtain the desired concentration (750 µg) and after overnight incubation, an increases in

inhibition zone with imipenem and EDTA disc was ≥ 7mm than the imipenem disc alone then it was interpreted as MβL producer Yong *et* a*l* (2002) Figure 2.

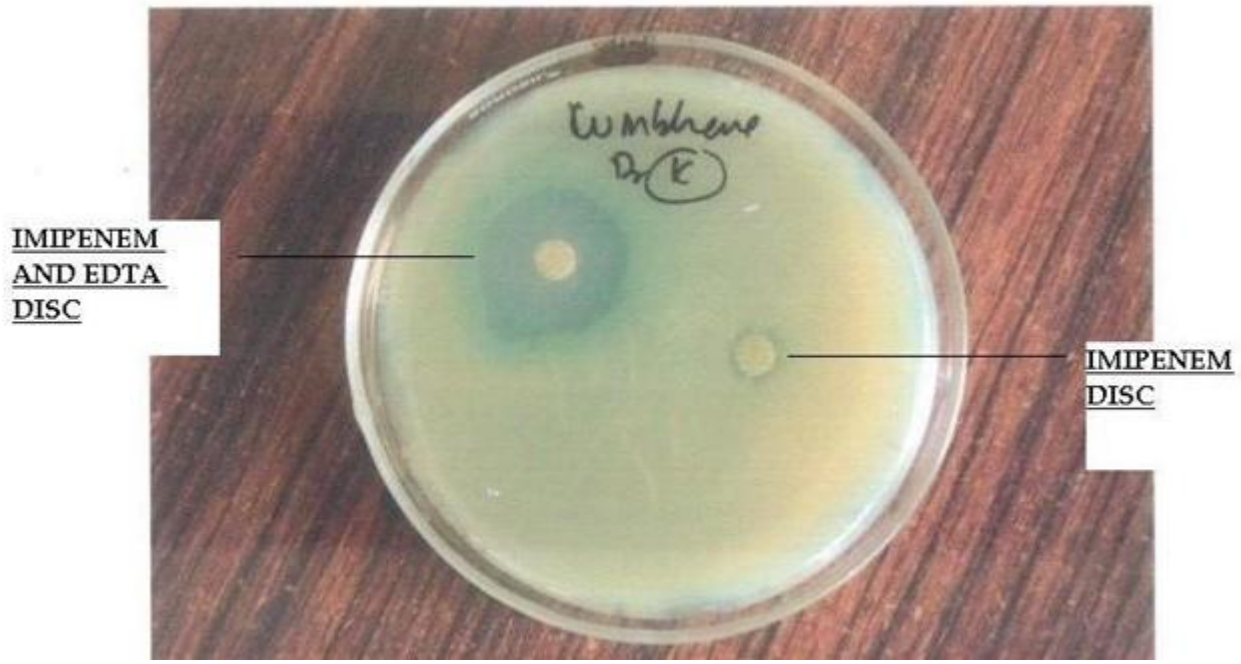

Figure 2: Imipenem (IMP) EDTA combined disc test.

MIC (Minimum inhibitory concentration) of Imipenem –EDTA combination

MIC of imipenem –EDTA combination was also performed on isolates resistant to imipenem. EDTA (1mi solution of 0.5 M) was added to 1ml 1 of imipenem solution spanning similar concentration. Each 2ml of EDTA and imipenem was added to 18ml of molten Muller Hinton Agar and poured on the plates that were allowed to set. A fixed inoculums of test strain was spot inoculated on these plates and reading was taken after overnight incubation. A minimum four fold reduction in MIC of these strains to MIC of imipenem alone, then it was interpreted as MβL producer Hemalatha *et* a*l* (2005), Bhera *et* a*l* (2008).

Antimicrobial susceptibility of isolated MβL producers

Antimcrobial susceptibility of all MβL isolates was performed by the disc diffusion method using polymyxin B (300 units), colistin(10mcg), rifampin(5mcg), azetronem(mcg), pipercillin/ tazobactem (100/10) .

RESULT

A total of 117 clinical isolates of *P. aeruginosa* studied, 18 isolates were resistant to imipenem and were subjected for MβL production. Of total 18 imipenem resistant isolates , combined disk test showed ≥7mm zone size in 16 isolates while 13 isolates gave positive result by DDST.

While MIC by agar diffusion of these 18 isolates 16 showed four fold reduction while the two isolates resistant to imipenem by disk method, MIC value was found to be <4µg/ml indicating their susceptibility by dilution method. MIC value of this 18 isolates is shown in Table 1

There was complete correlation between combined disc method and MIC method of imipenem.

While remaining 99 isolates of *P.aeruginosa* were sensitive to imipenem. The susceptibility pattern of *Pseudomonas aeruginosa* is shown in Table 2. Antimicrobial susceptibility of MβL isolates is shown in Table 3

Table 1: MIC values of MβL isolates

	Sensitive MIC (µg/ml)				Resistant MIC (µg/ml)				
Number of IMP resistant	1	2	3	4	16	32	64	128	256
P.aeruginosa	-	-	-	2	4	3	3	4	2

IMP=imipenem MIC=minimum inhibitory concentration

Table 2: Antimicrobial susceptibility profile of *P. aeruginosa* n=117

Sr.no	Antibiotic disc	Sensitive	Percentage
1	Imipenem	99	84.61%
2	Ceftazidime	95	81.19%
3	Gentamicin	79	67.52%
4	Amikacin	76	64.95%
5	Ciprofloxacin	70	59.82%
6	Piperacillin	59	50.42%
7	Cefoxitin	45	38.46%

Table 3: Antimicrobial susceptibility profile of MβL isolates n=16

Sr.no	Antibiotic disc	Sensitive	Percentage
1	Polymyxin B	16	100%
2	Azetronam	12	75%
3	Colistin	10	62.25%
4	Rifampin	10	62.25%
5	Pipercillin/ Tazobactem	08	50.0%

DISCUSSION

Carbepenems are β-lactam antibiotics, presently considered as the most potent agents of treatment of multidrug resistant gram-negative bacterial infections. Carbapenems hydrolyzing MβLs have been reported in several countries and have emerged as the most important mechanisms of carbapenem resistance.

In this study prevalence of MβL producing *P.aeruginosa* is found to be 13.65% which correlates with 16% MβL production observed in the study of Hemalata *et al* (2005). Another study from south India reported 12% MβL mediated imipenem resistance Naveaneeth *et* a*l* {2006}.

In another study by Sarkar *et al*{2006} reports high 36% MβL production while in study of Agrawal *et al*{2006} and Mendiratta *et al*. (2005) shows MβL production of 8.04% and 8.62% respectively. However in literature the overall prevalence MβL production ranges from 7-65% Arakawa *et* a*l* (2000), Walsh *et* a*l* (2000), Hancock *et* a*l* (1999).

Since there is no standard guidelines for detection of MβL, different studies have reported the use of different methods Bhera *et* a*l* (2008), Hemalata *et al* (2005), Agrawal *et al* (2006). Both combined disk methods and DDST can be used for detection of MβL production. Our study shows that combined disk method is superior to DDST and is in accordance with the study carried out in south India Agrawal *et al* (2006). In another study carried out by Beher *et al* (2008) also confirms our findings. In this study combined disk diffusion method and MIC reduction give same results. MIC reduction is cumbersome and time consuming and laborious method .so we recommend that combined disc method should be used for detection of MβL producing strains.

We also carried out antimicrobial susceptibility testing of MβL strains and found that polymyxin B was most effective antibiotic recording 0% resistance and it is similar to study carried out by Sarkar *et* a*l*{2006}followed by 75% sensitivity to azetronam, 62.5% sensitivity to colistin while 50% sensitivity was shown to pipercillin/ tazobactem. So use of combination therapy of either polymyxin B or azetronam

with either aminogylcosides or fluroquinolones should be used and never as monotherapy Yoon *et* a*l* {2006}.

To conclude , our study found that combined disk method is superior than DDST and should be routinely used for screening MβL production this might useful in clinical laboratories to monitor the emergence of metallo-B lactamases enzymes for clinical and surveillance management.

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