Original article:

Evaluation of phenotypic and genotypic detection method of metallo beta lactamase producing pseudomonas *aeruginosa*

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Abstract:

Metallo-beta-lactamase producing *Pseudomonas aeruginosa (P.aeruginosa)* is an important emerging pathogen in the intensive care units (ICUs) of a health care setup. The aim of this study was to evaluate MBL production in *P. aeruginosa* by two phenotypic methods namely Combined disk synergy test IMP (CDST - IMP) and Double disk synergy test IMP (DDST IMP) along with genotypic method, PCR. Over a period of one year 167 isolates of *P.aeruginosa* were screened for MBL production by disk diffusion method using Carbapenems and Ceftazidime disk. Out of the 100 *P.aeruginosa* strains, which were suspected MBL producers were further evaluated for MBL production by Combined disk synergy test IMP (CDST IMP) and Double disk synergy test IMP (DDST IMP) and PCR. It was found that CDST- IMP detected MBL production in 74% whereas by DDST-IMP 70% were MBL producers. VIM gene was only gen detected by PCR in 29% of the isolates, but none of these were positive for SPM and IMP gene. Therefore identification of the drug resistance pattern and detection of MBL production in *P.aeruginosa* is of great importance in the prevention and control of Hospital acquired infections particularly in the ICUs. Of the two phenotypic methods, CDST-IMP was superior to DDST-IMP in the present study. Though detection of MBL gene by the genotypic method-PCR is the gold standard method, but it is not possible to perform this test in most of the laboratories at present.

Keywords: *P.aeruginosa,* Metallo-beta-lactamase, CDST, DDST, PCR

INTRODUCTION:

Pseudomonas aeruginosa is a common cause of Hospital associated Infections, including pneumonia, bloodstream, urinary tract and surgical-site infections (CDC, 2013) [1]. Drug resistance is very common in the nosocomial isolates of *P. aeruginosa.* Recently Multi Drug Resistant (MDR) *P.aeruginosa* have been found which are resistant to nearly all antibiotics including aminoglycosides, cephalosporins, fluoroquinolones and carbapenems. Consequently treatment options are narrowed down to only a few antibiotics[1]. Carbapenems are the antibiotics of choice for several pseudomonas infections. However resistance to this novel antibiotic is increasing worldwide. As Carbapenem resistance in *P.aeruginosa* is most commonly due to production of metallo-beta-lactamases (MBL), its over-use has led to a rise in MBL producing *P.aeruginosa*[2]. MBL producing *P.aeruginosa* are the leading cause of nosocomial infections in intensive care units (ICUs,) [3]. They are responsible for 3-7% of blood stream infections and high mortality rates 27-48% in the critically ill patients[4] . The different phenotypic methods available for MBL detection are (CDST-IMP),(DDST-IMP), Ceftazidime-EDTA Combined disk synergy test (CDST-CAZ),

Ceftazidime-EDTA Double disc synergy test (DDST-CAZ), Epsilo-meter Test (E-Test) and Modified Hodge test (MHT), but not a single one of these is recommended by CLSI guidelines.

Though different researchers have used either one or two or more phenotypic methods for MBL detection, most of these methods have not been standardized. The polymerase chain reaction (PCR) is considered as the gold standard for MBL detection in *P.aeruginosa*. Though it is highly accurate and reliable, its accessibility is often limited to reference and research laboratories. [4]

The Present study was carried out with an aim to detect MBL production in *P. aeruginosa* isolated from various ICUs in a tertiary care hospital setup. Comparative evaluation of two phenotypic methods currently in use namely DDST-IMP and CDST-IMP along with detection of MBL gene production by using PCR was an important part of this study.

Methods:

A prospective study was carried out at G. B. Pant Institute of Post Graduate Medical Education & Research,(PGIMER) New Delhi, a tertiary care hospital in North India over a period of one year (from January 2016 to December 2016). One sixty seven(167) Consecutive non-duplicate isolates of *P. aeruginosa* isolated from various clinical samples of patients admitted in various ICUs were included in this study. Permission for the study was granted by the institutional research board. Informed consent was also taken from the patients. Identification of *P. aeruginosa* was done as per standard laboratory procedures [5]. Antimicrobial sensitivity testing was performed for all the *P. aeruginosa* isolates using commercially available disks (HI- Media Mumbai, India) on Mueller-Hinton agar(MHA) plates by Kirby-Bauer disk diffusion method [6] .The following antibiotics were tested by Kirby-Bauer disk diffusion method in accordance with the CLSI guidelines [7] ie Piperacillin/ Tazobactam (100µg/ 10µg) (TZ) , Ceftazidime (30µg)(CAZ), Amikacin (30µg) (AK), Ciprofloxacin (5µg) (CF), Imipenem (10µg) (IMP), Gentamicin (10µg) (GM), Netilmicin (30µg) (NET), Meropenem 10µg) (MEM), Colistin (10µg) (CT), Levofloxacin(5µg)(L),Ticarcillin+Clavulanic acid(75/10µg)(TC), Tobramycin(10 µg) (TB)(and Cefepime (10 µg) (CPM). *P. aeruginosa* **ATCC 27853** was used as a quality control strain. Zone of inhibition for each antibiotic was measured and interpreted according to CLSI 2016 guide lines.

Screening for MBL production.

Resistance to Imipenem(IMP), Meropenem(MEM) and Ceftazidime(CAZ) was employed as a screening test to select suspected MBL producing *P. aeruginosa* . The strains showing resistance either to Imipenem(10ug), Meropenem(10ug) or Ceftazidime(30ug) or to any of the two antibiotics or resistance to all the three antibiotics were considered as MBL producers.

Confirmation of MBL production.

MBL production by *P.aeruginosa* was further confirmed by **CDST**-IMP and **DDST**-IMP method**.** The Imipenem disks were supplemented with 0.5M EDTA. EDTA solution was prepared by dissolving 186.1gm of EDTA in 1 litre of distilled water, pH was adjusted to 8.0 using NaOH and sterilized by autoclaving. Then 10µl of this solution was added to the Imipenem disk[8].

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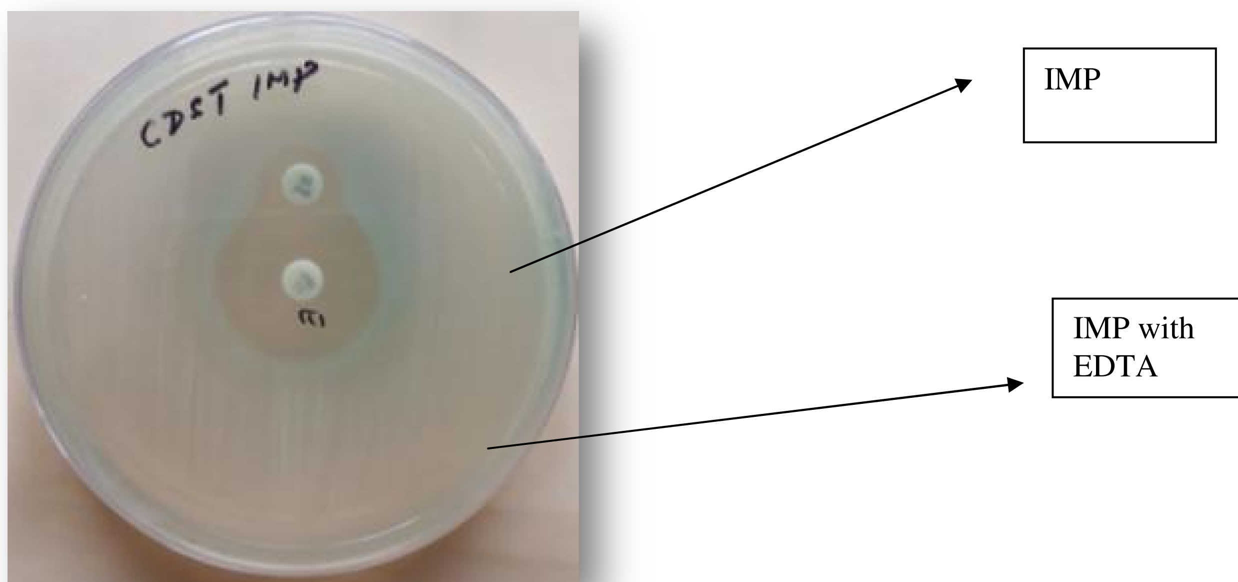
(I) Imipenem-EDTA Combined disk synergy test (CDST - IMP):

This method was done as described by Yong D et al [8]. Test Organism was inoculated in 5 ml of sterile peptone water and incubated for 30 minutes approximately to obtain 0.5 Mc Farland's standard (measured by turbidity meter). Lawn culture of the isolate was made on the Muller Hinton Agar plate and allowed to dry.

Two imipenem discs, one with 0.5M EDTA and the other plain Imipenem discs were placed on the surface of a MHA agar plate . The plate was incubated for 16 to 18 hours at 37˚C.

If the zone of inhibition of imipenem - EDTA disc was equal to or more than7 mm to the zone of inhibition of Imipenem disc alone, it was considered as MBL positive.

Figure of CDST IMP



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(II) Imipenem-EDTA Double disk synergy test (DDST-IMP)

The test was performed as per the method of Lee K et al [9]. Test isolate was inoculated in 5 ml of sterile peptone water and incubated at 37°C for 30 minutes to obtain a turbidity of approximately 0.5 McFarland's standard which was measured with a turbidity meter. Lawn culture was done on MHA plates.

The Imipenem disc (10µg) was placed on the surface of an MHA plate, 10 mm edge to edge, from a blank disc containing 10µl of 0.5 M EDTA solution (750µg). The plate was incubated for 16 to 18 hours at 37˚C. Enhancement of the zone of inhibition in the area between imipenem and EDTA disc in comparison to the zone of inhibition on the far side of the disc was interpreted as a positive result for MBL production.

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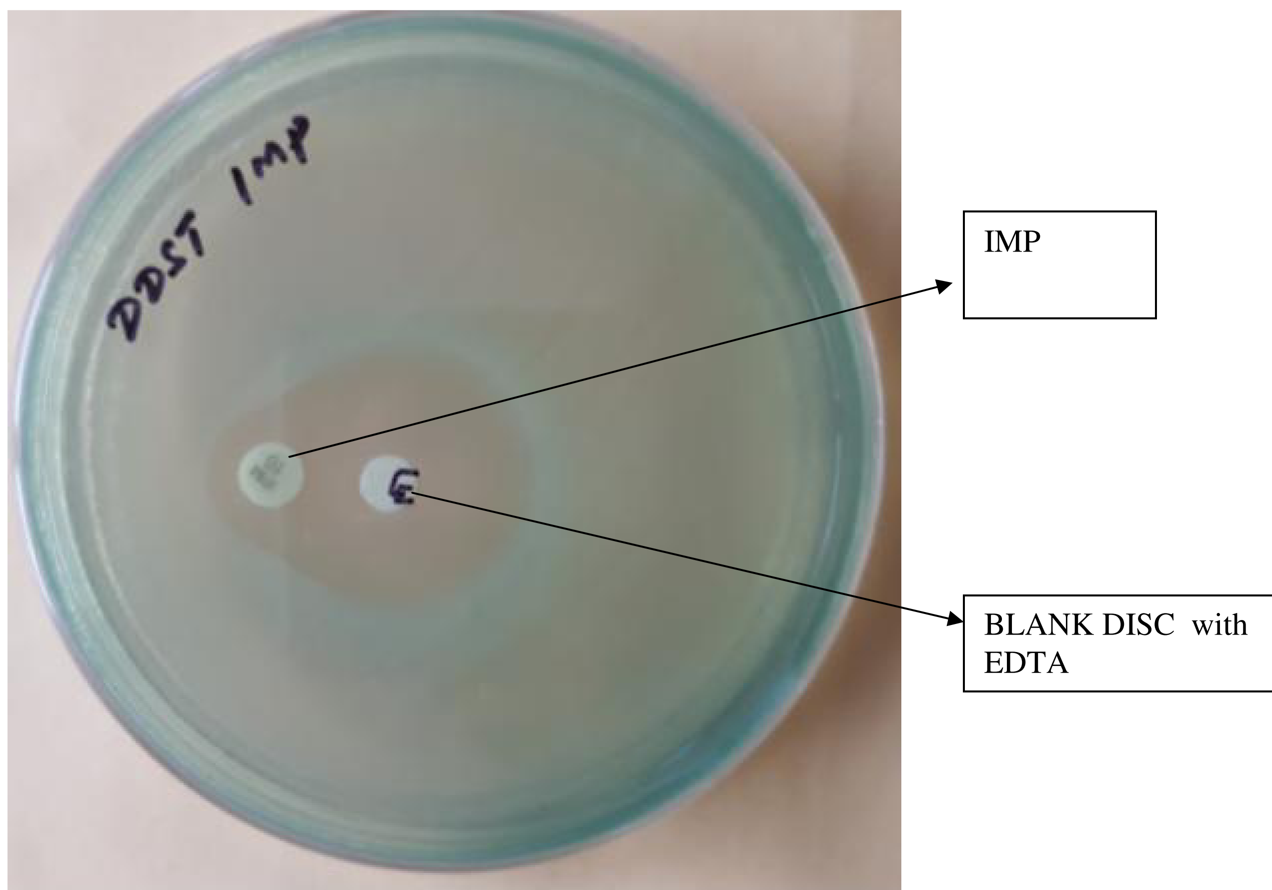
Figure DDST IMP



Detection of MBL gene by using PCR :

MBL producing isolates were ribotyped and screened for production of MBL genes by PCR using primers for *bla*$_{IMP,}$ *bla*$_{VIM}$ and *bla*$_{SPM..}$ Detection of MBL gene was performed as described by Khosravi A D et al, 2008 [10].

To perform the PCR reaction, 0.5ml of overnight grown culture was pelleted down and washed with equal volume of sterile double distilled water and centrifuged at 12000 rpm for 5 minutes. 50 µl of washed pellet was boiled at 100ºC with 50 µl distilled water for 10 minutes. Samples were further centrifuged at 12000 rpm for 5 minutes and 10 µl of supernatant was used as template for PCR.

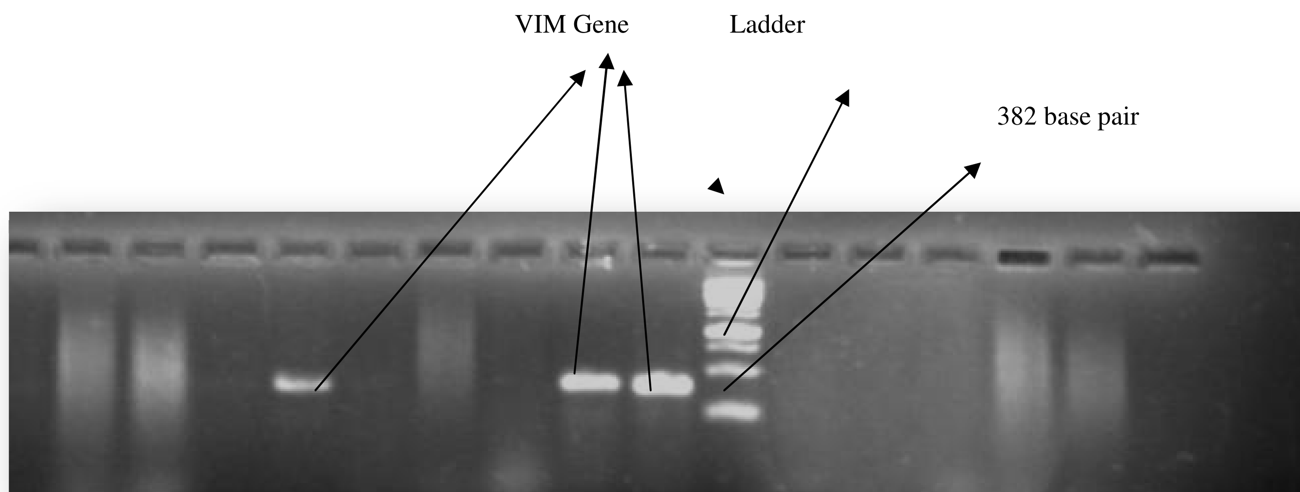
For MBL gene specific PCR amplification , the PCR reaction was performed in 25 µl reaction mixture which contained 1m*M* dNTPs mix (3 µl) , 5 ng/ µl forward and reverse primers (2.5 µl each). 2.0 unit Taq Polymerase (MBI, Fermentas, USA.), 2 µl DNA solution from *P.aeruginosa* (30 ng) 10x Taq reaction buffer (2.5 µl) and sterile double distilled water to make 25 µl . The PCR programme utilized the following thermal profile : 95ºC - 5 min (preheating) ; 94ºC-30 sec, 52ºC-1 min, and 72ºC-2 min, (32 Cycle) followed by 72ºC-10 min as final elongation step and 4ºC for storage until use. The amplified product was resolved in 0.7% Agarose gel (in Tri-Acetate -EDTA buffer) by horizontal gel electrophoresis. The presence of MBL gene specific band in amplified product lanes was

observed in ethidium bromide stained gel on a UV transilluminator and photographed with a Gel Documentation system. The size of individual bands in the amplified profile was determined by comparison with a co-migrating DNA ladder (100 bp DNA ladder plus, MBI Fermentas, USA.)

Primers used for MBL Producing gene (IMP,VIM, SPM) during study :

Primer Name	Primer sequence 5' to 3' (' = prime)
IMP Forward	5'GAAGGCGTTTATGTTCATAC3'
IMP Reverse	5'GTATGTTTCAAGAGTGATGC3'
VIM Forward	5'GTTTGGTCGCATATCGCAAC3'
VIM Reverse	5'AATGCGCAGCACCAGGATAG3'
SPM Forward	5'CCTACAATTCTAACGGCGACC3'
SPM Reverse	5'TCGCCGTGTCCAGGTATAAC3'

Amplified VIM Gene at 382bp



Amplification of VIM gene in various isolates of *P. aeruginosa*

Target Gene	First Denaturation	Denaturation	Annealing	Extension	Number of cycle	Final Extension	Base pairs
Bla IMP	95˚C -5 min	94˚C -30 sec	52˚C -1 min	70˚C -2 min	32	72˚C-10 min	587
Bla VIM	95˚C -5 min	94˚C -30 sec	52˚C -1 min	70˚C -2 min	32	72˚C- 10 min	382
Bla SPM	95˚C -5 min	94˚C -30 sec	52˚C -1 min	70˚C -2 min	32	72˚C- 10 min	600

RESULTS:

During the study period of one year, 167 strains of *Pseudomonas aeruginosa* were isolated from culture of various clinical samples of patients admitted in the various ICUs of the hospital. They were screened for MBL production by observing their resistance to carbapenems by the disc diffusion technique. Out of 167strains, 67 strains which were sensitive to Carbapenems and Ceftazidime discs were excluded from the study. One hundred *P.aeruginosa* which were suspected as MBL producers were included in the study for further evaluation.

The resistance pattern of the suspected MBL *P.aeruginosa* is shown in Table 1.

Table 1: Distribution of Carbapenem Resistance pattern of suspected MBL *P.aeruginosa* (n=100)

IMP	MEM	CAZ	IMP+MEM	IMP+CAZ	MEM+CAZ	IMP+MEM+CAZ
13	2	6	12	2	5	60

Abbreviations : Imipenem -IMP , Meropenem -MEM, Ceftazidime- CAZ.

Sixty percent of the isolates studied were resistant to IMP+MEM+CAZ followed by13 % to IMP alone,12% to IMP+MEM,6 % to CAZ alone, while two strains each were resistant to MEM & IMP+CAZ respectively.

Of the 100 isolates, maximum numbers were isolated from General Intensive Care Unit, 30, followed by Neurosurgery ICUs 29, Gastrointestinal surgery ICUs 20, Neurology ICUs 10, High Dependency Unit 4 and 3 each from Gastrointestinal medicine ICUs and CTVS ICUs respectively along with 1 from the Liver ICU.

Suspected MBL *P.aeruginosa* isolates from various clinical samples is shown in Table (2)

Table 2: Distribution of *Pseudomonas aeruginosa* in various clinical samples.

Pus	Tracheal aspirate	Endotr-acheal aspirate	Blood	CSF	Bile	Body fluids	Stent	Arterial line tip	Total
27	27	22	6	6	5	3	3	1	n=100

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Maximum numbers of suspected MBL *P.aeruginosa* isolates were from Pus & Tracheal aspirate. ie 27 each , followed by Endo Tracheal aspirate- 22, Blood and CSF 6 each , Bile-5, Body fluids and Stent 3 each with only one (1) from Arterial line tip.

Table 3: Sensitivity pattern of MBL *P.aeruginosa* to various groups of antibiotics

MBL PA	Number N=	AK	CPM	CF	NET	GM	TB	TC	TZ	L	CT
IMP+MEM+CAZ	60	12	1	0	5	5	10	2	10	2	60
IMP	13	5	0	1	4	4	6	1	7	1	8
IMP+MEM	12	11	4	5	8	9	9	3	5	7	9
CAZ	6	1	0	1	0	0	0	0	0	0	7
MEM+CAZ	5	4	2	2	2	3	3	2	4	2	4
MEM	2	0	0	0	1	0	0	0	0	0	0
IMP+CAZ	2	2	1	1	2	2	2	1	2	0	1
Total	100	35	8	10	22	23	30	9	28	12	89

Eighy nine percent (89%) of the suspected MBL *P. aeruginosa* were sensitive to Colistin(CT) followed by Aminogycosides ie Amikacin (AK) 35%,Tobramycin(TB) 30%,Gentamicin(GM) 23% & Netilmicin(NET) 22% respectively. Sensitivity to Piperacillin & Tazobactum(TZ) and Ticarcillin & Clavulanic acid (TC)were 28% and 9% respectively. Sensitivity to Quinolones ie Levoflox(L) was 12% Ciproflox(CF) was10% and to while Cefipime (CPM)sensitivity was seen in 8% of the isolates.

The confirmation of MBL production was done in all 100 suspected MBL producing *Pseudomonas aeruginosa* by two phenotypic methods namely CDST-IMP and DDST-IMP.

Of the total 100 isolates, 74 *P. aeruginosa* isolates were confirmed to be MBL producers by

CDST- IMP while 70 were found to be positive by DDST- IMP. Sixty five (65) of the isolates were detected to be MBL producers by both methods namely CDST-IMP & DDST-IMP while 21% of the strains were non MBL producers by both methods i.e. CDST-IMP and DDST-IMP. Nine percent (9%) of the strains which were positive by CDST-IMP were negative by DDST-IMP while Five (5 %) percent of the strains which were positive by DDST-IMP were negative by CDST-IMP.

All 100 strains which were positive by the screening methods were further tested for the presence of **MBL gene by the PCR method.** Of these only 29 isolates were positive for VIM gene. IMP gene and SPM gene were not detected in any of the isolates in this study. Correlation between the Genotypic and Phenotypic methods is shown in Table 4.

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Table: 4 Genotypic Vs Phenotypic methods

VIM +ve 29	VIM -ve 71
CDST-IMP +ve & DDST-IMP +ve 24	CDST-IMP +ve & DDST-IMP +ve 41
CDST-IMP +ve & DDST-IMP -ve 2	CDST-IMP +ve & DDST-IMP -ve 7
DDST-IMP +ve & CDST-IMP -ve NIL	DDST-IMP +ve & CDST-IMP -ve 5
CDST-IMP -ve & DDST-IMP -ve 3	CDST -ve & DDST -ve 18

Of the 29 VIM Positive cases, 24 were positive by both the phenotypic methods while 3 were negative by both methods. In Only Two (2) cases which were positive by CDST-IMP method were negative by DDST-IMP method. Of the 71 VIM negative cases, 41 were positive by both methods while 18 were negative by both methods. Seven (7) cases which were positive by CDST-IMP method were seen to be negative by DDST-IMP method. It was further observed that 05 strains which were positive by the DDST-IMP method were negative by CDST-IMP method.
Phenotypic method CDST-IMP and DDST IMP was compared with PCR and the result is shown in table 5 and 6 respectively.

Table 5: Comparative analysis of CDST-IMP vs PCR.

S: No	**Phenotypic test n= 100**	**PCR +ive**	**PCR -ve**
1.	**CDST-IMP (+ive) n= 74**	**25**	**49**
2.	**CDST- IMP (-ive) n= 26**	**4**	**22**

Table 6: Comparative analysis of DDST-IMP vs PCR.

S: No	**Phenotypic test n= 100**	**PCR +ive**	**PCR -ve**
1.	**DDST- IMP (+ive) n= 70**	**24**	**46**
2.	**DDST-IMP (-ive) n= 30**	**5**	**25**

Table 7: Specificity and Sensitivity of CDST-IMP **and DDST**-IMP

S.No:	**Test**	**Sensitivity**	**Specificity**	**PPV**	**NPV**	**Accuracy**
1.	CDST- IMP	**86.21%**	**30.99%**	**33.78%**	**84.62%**	**47%**
2.	DDST- IMP	**82.76%**	**35.21%**	**34.29%**	**83.33%**	**49%**

In the present study, Sensitivity of CDST-IMP was found to be 86.21% while the Specificity was 30.99%. Sensitivity of DDST-IMP on the other hand was 82.76% while its Specificity was 35.21%.

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DISCUSSION:

Pseudomonas aeruginosa is a versatile nosocomial opportunistic pathogen which is often multiresistant to various antibiotics. Over the last few decades MBL producing *P. aeruginosa* isolates have emerged in the hospital setup leading to poor therapeutic outcome and increased mortality. So the early detection of MBL production in *P. aeruginosa* is crucial for the optimal treatment of critically ill patients. This would help to initiate strict infection control measures and prevent their nosocomial spread. The present study was undertaken to evaluate two phenotypic methods namely CDST-IMP and DDST-IMP for detection of MBL in *P. aeruginosa* and to correlate it with PCR which is a genotypic method.

During the study period, 167 *P. aeruginosa* isolated from various clinical samples were screened for MBL production by observing their resistance to Carbapenems and Ceftazidime discs. Out of 167, 100 isolates (59.8%) were found to be suspected MBL producers. Imipenem resistance was detected in 87% of the isolates and Meropenem resistance in 79% while Ceftazidime resistance was observed in 73% isolates. Yongwei Le et al 2015[11]reported that 51.6% of the isolates were suspected MBL *P. aeruginosa* by using Cefazidime disc which is lower than that in the present study. They also reported that 73.3% of their strains were resistant to Imipenem and 63.9 % to Meropenem respectively. Fallah et al [12] used Imipenem disc for MBL screening and found that 83% of the isolates were resistant to Imipenem, a finding which is similar to the present study. Ahmed A et al [13] reported 25% resistance to Imipenem in their *P. aeruginosa* isolates.

In the present study maximum numbers of suspected MBL producing *P. aeruginosa* were sensitive to Colistin 89% followed by Aminogycosides ranging from 22- 35% ie Amikacin (AK) 35%,Tobramycin(TB) 30%,Gentamicin(GM) 23% & Netilmicin(NET) 22%. Sensitivity to Piperacillin & Tazobactum(TZ) and Ticarcillin+ Clavulanic acid (TC) were 28 % and 9% respectively. Sensitivity to Quinolones ie to Levofloxacin was 12% and to Ciprofloxacin was 10% while Cefepime sensitivity was observed in 8% of the isolates. Mehta A et al [14] In their study reported that in the Carbapenem resistant isolates Polymixin - B was the most effective antibiotic with 90% sensitivity. They also reported that sensitivity of *P. aeruginosa* to Aminoglycosides ie Amikacin was 6/20 (30%) and Gentamicin was 2/20(10%) while for Quinolones ie Ciprofloxacin was 2/20(10%) and for Piperacillin+Tazobactum was 8/20(40%) and for Cefepime 2/20(10%).These findings are almost consistent with our findings. Kali A et al [15] reported that IMP resistant *P.aeruginosa* showed 100% sensitivity to Polymixin B and Colistin in their study. All strains were resistant to Gentamicin ie 100% while 36.3% and 27.2% sensitivity was observed to Netilmicin and Amikacin respectively. They found a high degree of resistance to Gentamicin ie 100%,and Ciprofloxacin 99.1 % in the *P. aeruginosa* isolates while 36.3 % and 27.7 % sensitive was observed to Netilmicin and Amikaacin respectively. In a recent Indian study by John S et al[16] 2011 the resistance to Imipenem , Gentamicin ,Ciprofloxacin, Netilmicin,Piperacillin and Amikacin amongst MBL *P.aeruginosa* was 77.5%, 77%, 72.1%, 67.3%, 57.7% and 56.1% respectively**.** Tsakris A[17] et al 2009 and De AS et al[18] 2010 in their studies reported 100 % resistance to most of the drugs tested namely all Aminoglycosides, Beta-lactams and Quinolones.

Dwivedi M et al[19] 2009 found P. aeruginosa as the most common pathogen is 23.3% of all bacteria isolated from ICU infections. They also found 46.7% of these isolates were resistant to Piperacillin, 51.7% to Ceftazidime, 47.3%

to Gentamicin,32% to Amikacin, 47.3% to Ciprofloxacin, 28.3% to Piperacillin+ Tazobactum, 37% to Meropenem while 37.7% were resistant to Imipenem. All the isolates were uniformely sensitive to Colistin. Diverse resistance patterns have been described by different authors [16,17,19]. These variations reflect the antibiotic practices which are followed in different regional hospitals.

The prevalence of MBL in India has ranged from 7% to 65% among Carbapenem-resistant *P. aeruginosa*. Mary V Jesudason[20] from Vellore, India reported that 75 % of their *P. aeruginosa* were MBL producers by EDTA disc synergy test. The IMP disc diffusion screening test was found to be better than CAZ and MEM for selecting probable MBL producing strains.

In our study confirmation of MBL Production was done by CDST- IMP and DDST- IMP. 74% of the MBL *P.aeruginosa* were positive by CDST IMP while 70% were positive by DDST- IMP. 65% of these isolates which were positive by both the methods. These results are well comparable to that of Qu TT. et al, 2009 [21] who demonstrated CDST-IMP as the best method for screening for MBL producing *P.aeruginosa*. Kali A et al [15] reported that all of their strains (100%) were MBL producers by CDST-IMP method while only 72 % strains were MBL producers by DDST-IMP. Mehta A et al [14] found that strains positive for MBL production in *P. aeruginosa* were 60% and 40% by CDST-IMP and DDST-IMP respectively.Sulmaz reshi **[22]** detected MBL production by *P. aeruginosa* in 88.4% by CDST-IMP method and 84.4% by DDST-IMP method, which is little higher than the present study. Various group of researchers Ranjan S et al [23] 2015, Lee et al 2003 and [9] Pitout et al [24] 2005 found that DDST-IMP was more specific in detection of MBL production as compared to CDST-IMP whereas Qu TT et al [21] were of the view that CDST-IMP is the best method for screening for MBL production in *Pseudomonas aeruginosa* in their study. P Vasundhara Devi et al [25] used DDST-IMP method for MBL detection in their study and found that 36% of their strains were positive for MBL production.

DDST-IMP is a qualitative test while CDST-IMP is semi quantitative in nature. The high false positive reporting rate in CDST-IMP may be due to the EDTA used in the test. EDTA has a membrane permeability property and could have a harmful effect on *P. aeruginosa* as can seen in the extended zone size which is responsible for the difference observed between IMP and IMP-EDTA disc used in the test rather than the actual metal chelating effect of EDTA. [21]

In Asia, *bla*IMP and *bla*VIM are more prevalent while *bla*IMP is found mainly in China ,Iran, Brazil and Malaysia, [11,12,,28, & 29]. Sader H S et al [28], A researcher from Brazil found SPM -1-like gene in 55.6% , VIM-2 - like gene in 30.6% and IMP-1-like gene in 8.3% of the *P. aeruginosa* in their study.Yongwei Le et al [11] from China, reported in their study that 84.1% P. aeruginosa exhibited the presence of *blaVIM* genes while in 76.1% the presence of *blaIMP* genes were detected which corresponds to study by Pitout JD et al [24] . Khosravi Y. et al 2012[29] from Malaysia, found that of the 90 Imipenem resistant *P.aeruginosa,* MBL genes were detected by PCR in 32 isolates namely bla IMP-4,blaIMP-7, bla VIM-2 and bla VIM 11 in their study. In our study 29 out of 100 isolates were positive for the presence of VIM gene which was lower than that of the above researchers while *blaIMP and bla SPM* genes were not detected. In a study by Manoharan A. et al [30] from India, the rate of MBL production in *P.aeruginosa* was 42.6%. PCR for MBL gene was performed on 48 out of their 61 isolates. Of these 15 were *bla* VIM type which was the most common gene type. *blaIMP and bla SPM* genes were also not detected by them

which was similar to our study. In the study by Amudhan MS, et al [31] among their 61 isolates of *P. aeruginosa, bla* VIM gene was detected in only 36 isolates. Kali A et al. et al[15] found VIM - 2 gene positive MBL *P.aeruginosa* in their study.In a study by Castanheira M, et al 2009 [32] a total of 301 *Pseudomonas* isolates were collected from 10 different hospitals in India. Of these 107 isolates were Carbapenem resistant with MIC ≥ to 8 ug/ml for Meropenem and Imipenem . Ninety Seven (97) of these isolates were *P.aeruginosa* . *bla* VIM - 2 was the commonest gene type in 35 *P. aeruginosa* isolates followed by *bla* VIM 6 in 12 isolates. The other VIM genes detected in this study were VIM - 5, VIM -11 and VIM -18.

In the present study, 24 out of 29 VIM positive (82.7%) *P. aeruginosa* isolates were detected to be MBL Producers by both the Phenotypic methods namely CDST- IMP, DDST- IMP. In 3 of the isolates of *P.aeruginosa*, only the VIM gene was detected but MBL production was not detected phenotypically by both CDST-IMP and DDST-IMP methods. In only 2 of the isolates MBL production was detected by the genotypic method and CDST-IMP method but they were negative by DDST-IMP. Yongwei Li[11] reported that 84.1% of their P.aeruginosa were MBL producers by CAZ-EDTA and they also detected a VIM gene by PCR.

Sensitivity and specificity of CDST-IMP was 86.21% and 30.99% while that of DDST-IMP was 82.76% and 35.2% respectively as compared to PCR which was the gold standard method in the present study. The Sensitivity and Specificity of CDST-IMP in a study by Sachdeva et al 2017 [33] was 97.95% and 96.11% respectively. Khosravi Y. et al [29] from Malaysia found that the Sensitivity of all the three tests namely DDST,CDST and E.Test was 100% while Specificity of DDST-IMP was 96.6% followed by E.Test which was 62.1 % and that of CDST-IMP 43.1% respectively. They concluded that DDST-IPM was the most specific of the three tests

Thus from the present study it can be seen that both the phenotypic methods namely CDST-IMP and DDST-IMP had almost similar predictive values for detection of MBL production in *P. aeruginosa*, however while CDST detected it in 74% cases in comparison to DDST where MBL detection was observed in only 70% cases.

Conclusion:

Thus, the rapid dissemination of MBL producers in the hospital setup is an important cause of worry and necessitates the implementation not only of mere surveillance studies but also proper and judicious selection of antibiotics and its proper usage particularly Carbapenems in the treatment of infections due to MDR *Pseudomonas aeruginosa.* Though PCR is the gold standard method for detection of MBL gene in our study but the Phenotyping of MBL producing *P. aeruginosa* isolates by the CDST- IMP method remains the test of choice by virtue of the fact that it is cheap, cost effective and can be performed easily in any Laboratory setup and is devoid of visual misinterpretations.

References:

1. CDC Report: Antibiotic resistance threats in the United States, 2013.
2. Hammami S, Boubaker BBI, Ghozzi R, Saidani M, Amine S, Redjeb BS. Nosocomial outbreak of imipenem-resistant Pseudomonas aeruginosa producing VIM-2 metallo-beta-lactamase in a kidney transplantation unit. Diagn Pathol. 2011;6:106.
3. Mohanasundram KM. The antimicrobial resistance pattern In the clinical isolates of *Pseudomonas aeruginosa* in a tertiary care hospital; 2008-210 (A 3 year study). JCDR June 2011;5(3):491-494.

www.ijbamr.com P ISSN: 2250-284X , E ISSN : 2250-2858

4. Navneeth BV, Sridaran D, Sahay D, Belwadi MR. A preliminary study on metallobeta lactamase producing *Pseudomonas aeruginosa* on hospitalized patients. Indian J Med Res 2002;264-7.

5. Mackie & McCarteny Practical Medical Microbiology, 13th edition , Volume- 2, by J.G. College. J.P. Duguid, A.G. Fraser, B.P. Marmion, 1989. *Pseudomonas aeruginosa* 491-503:

6. Bauer AW, KirbyMM,Sheris JC,Jurek M.Antibiotic susceptibility testing by a standardized single method. Am J Clin Pathol 1966.;45:493-6.

7. Clinical And Laboratory Standards Institute: 26TH edition. January 2016. M1005 Performance Standards for Antimicrobial Susceptibility Testing. M02 andM07, 2BI; p62-63.

8. Yong D, Lee K, Yum JH, Shin HB, Rossolini GM, Chong Y. Imipenem-EDTA disk method for differentiation of metallobeta- lactamase-producing clinical isolates of *Pseudomonas spp*. and *Acinetobacter spp*. J Clin Microbiol. 2002;40:3798–801

9. Lee K, Lim YS, Yong D, Yum JH, Chong Y. Evaluation of the Hodge test and the imipenem-EDTA double-disk synergy test for differentiating metallo-beta-lactamase-producing isolates of *Pseudomonas spp*. and *Acinetobacter spp* . J Clin Microbiol. 2003;41:4623–9

10. Khosravi AD, Mihani F. Detection of metallo-β-lactamase–producing *Pseudomonas aeruginosa* strains isolated from burn patients in Ahwaz, Iran January 2008;1 (60):125–128.

11. Yongwei Li, Zhang X, Wang C, Hu Y, Niu X, Pei D, et al. Characterization by phenotypic and genotypic methods of metallo-β-lactamase-producing *Pseudomonas aeruginosa* isolated from patients with cystic fibrosis. Molecular Medicine Reports 2015;11:494-498.

12. Fallah F., Borhan RS., Hashemi A., Detection of bla(IMP) and bla(VIM) metallo-β-lactamases genes among Pseudomonas aeruginosa strains Int J Burn Trauma 2013;3(2):122-124

13. Ahmed W, EL-Shafei, EL-Mosallamy, Osman AS et al. Phenotypic and Genotypic Methods for Detection of MetalloBeta-Lactamase (MβL) Producing Pseudomonas Aeruginosa . Egyptian Journal of Medical Microbiology July 2015;3(24): 27-35

14. Mehta A, Tukaram P. Detection and characterization of metallo-β-lactamases producing Pseudomonas aeruginosa clinical isolates at a tertiary care hospital of Bhopal, India. Int J Res Med Sci 2016;4:4084-8.

15. Kali A, Srirangaraj S, Kumar S, Divya HA, Kalyani A, Devi US. Detection of metallo-betalactamase producing*Pseudomonas aeruginosa* in intensive care units. AMJ 2013; 6(12):686-693.

16. John S, Balagurunathan R. Metallo beta lactamase producing *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. Indian J Med Microbiol. 2011;29:302-4.

17. Tsakris A, Poulou A, Kristo I, Pittaras T, Spanakis N, Pournaras S, Markou F. Large dissemination of VIM-2- metallo-beta-lactamase-producing *Pseudomonas aeruginosa* strains causing health care-associated community-onset infections. J Clin Microbiol 2009;47:3524–3529.

18. De AS, Kumar SH, Baveja SM. Prevalence of metallo-betalactamase producing *Pseudomonas aeruginosa* and *Acinetobacter species* in intensive care areas in a tertiary care hospital. Indian J Crit Care Med. 2010;14:217-9.

19. Dwivedi M, Mishra A, Singh RK, Azim A, Baronia AK, Prasad KN. Nosocomial cross-transmission of pseudomonas aeruginosa between patients in a tertiary care unit. 2009;52(4):509-513.

20. Jesudason MV, Dathil AJK, Balaji V 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.

21. Qu TT, Zhang JL, Wang J, Tao J, Yu YS, Chen YG, et al. Evaluation of phenotypic tests for detection of metallo-betalactamase-producing Pseudomonas aeruginosa strains in China. J Clin Microbiol 2009;47:1136-42.

22. Reshi S, Bali N, Manzoor M, Farooq S, Kakru DK. Comparison of Various Phenotypic Methods for the Detection of Metallo-beta-lactamases in Pseudomonas aeruginosa. JAMMR 2017; 23(3): 1-8.

23. Ranjan S, Banashankari Gs, Babu Pr. Evaluation of Phenotypic tests and screening markers for detection of metallo-betalactamse in clinical isolates of Pseudomons aeruginosa: A prospective study.Med J DY Patil Univ 2015; 8: 599-605.

24. Pitout JD, Gregson DB, Poirel L, McClure JA, Le P, Church DL.Detection of Pseudomonas aeruginosa producing metallo-beta- lactamases in a large centralized laboratory. J Clin Microbiology 2005; 43: 3129-35.

25. Devi PV, Reddy PS, John MS. Prevalence of Metallo- Lactamases Producing Pseudomonas aeruginosa among the Clinical isolates: A study from tertiary care hospital Int.J.Curr.Microbiol.App.Sci 2015; 4(4): 955-961

26. Dong F. , Xi-Wei X, Wen-Qi S, Ping L, Hong YY, Zhuang SX. Characterization of multidrug-resistant and metallo-beta-lactamse-producing *Pseudomonas aeruginosa* isolates from a paediatric clinic in China. Chin Med J 2008;121: 1611-1616.

27. Franco MRG, Caiaffa-Filho HH, Burattini MN, Rossi F. Metallo-beta-lactamases among imipenem-resistant *Pseudomonas aeruginosa* in a Brazilian university hospital. Clinics 2010;65: 825-829.

28. Sader HS, Reis AO, Silbert S, Gales AC. IMPs, VIMs and SPMs: the diversity of metallo-β-lactamases produced by carbapenem-resistant *Pseudomonas aeruginosa* in a Brazilian hospital. Clin Microbiol Infect. 2005;11:73–6. 10.1111/j.1469-0691.

29. Khosravi Y, Loke MF , Chua EG, Tay ST, Vadivelu J. Phenotypic Detection of Metallo-**β**-Lactamase in Imipenem-Resistant *Pseudomonas aeruginosa.* Scientific World Journal. 2012; 2012: 654939.

30. Manoharan A, Chatterjee S, Mathai D. Detection and characterisation of metallo beta lactamase producing *Pseudomonas aeruginosa*. Indian J Med Microbiol 2010;28: 241-244.

31. Amudhan MS, Sekar U, Kamalanathan A, Balaraman S. *bla*IMP and *bla*VIM mediated carbapenem resistance in *Pseudomonas* and *Acinetobacter* species in India**.** *J Infect Dev Ctries* 2012; 6(11):757-762.

32. Castanheira,M, Bell JM, TurnidgeJD, Mathai D, Jonesi RN. Carbapenem Resistance among *Pseudomonas aeruginosa* Strains from India: Evidence for Nationwide Endemicity of multiple Metallo-beta-Lactamase Clones (VIM-2, -5, -6, and -11 and the Newly Characterized VIM-18) Antimicrobial Agents and Chemotherapy 2009;53(3): 1225–1227.

33. Sachdeva R, Sharma B, Sharma R. Evaluation of different phenotypic tests for detection of metallo-β-lactamases in imipenem-resistant Pseudomonas aeruginosa. J Lab Physicians 2017;9:249-53.