



Original Research Article

## Prevalence of Metallo- $\beta$ -Lactamase Producers among Selected Isolates in A Tertiary Care Teaching Hospital

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### ABSTRACT

**Background:** The emergence of metallo-beta-lactamase ( MBL ) producing isolates is a challenge to routine microbiology laboratories, since there are no standardized methods for detecting them.

**Aim:** To study the prevalence of MBL producing Gram negative bacteria and non-fermenters and compare and evaluate the use of two phenotypic methods, double-disk synergy test ( DDST ) and a combination disk diffusion test ( CDDT ) method from various clinical samples.

**Materials & Methods:** A prospective study was done to test for MBL production by DDST and CDDT from select isolates that were Carbapenem and Ceftazidime resistant by Kirby Bauer disc diffusion test.

**Results:** Of the 108 isolates 48 (44.4%) were resistant to Imipenem or Meropenem or both and among them 14 (29.1 %) were metallo-beta- lactamase producers. Of the MBL producers, *Acinetobacter* species 5( 35.7 % ) was the most common followed by *Pseudomonas aeruginosa* 4 ( 28.5 % ), *Klebsiella pneumonia* 3 ( 21.4% ) and *E. coli* 2 ( 14.2 % ).DDST was found to be a technically easier screening method over CDDT method of evaluation.

**Conclusion:** Routine screening by DDST of isolates for MBL production should form an integral part of solving therapeutic failures, longer hospital stay and significant morbidity and mortality seen with these clinical isolates and thereby enhance the efficiency of the hospital infection control programme of any reputed health care facility.

**Key Words:** Combination disk diffusion test, Carbapenem resistance, Double Disk Synergy Test, Metallo- $\beta$ -Lactatamase.

### INTRODUCTION

Antibiotic resistance is an increasing menace with many modes of resistance described to any given antibiotic. Beta lactam group of drugs is the most commonly used antibiotic. As expected the resistance also is high in various species. Carbapenem class of drugs was introduced as broad

spectrum antibiotics. The activity was high against gram positive, gram negative and anaerobic bacteria.<sup>[1]</sup> However, the possession of carbapenamase enzyme which hydrolyses this drug causes resistance. Carbapenemases increasingly have been reported in enterobacteriaceae in the past 10 years. <sup>[2]</sup> In this study we highlight the

prevalence of MBL producers by screening resistant isolates to Imipenem, Meropenem or both and Ceftazidime from clinical samples in our set up and evaluate the pros and cons of two disc diffusion methods the DDST and CDDT.

## **MATERIALS & METHODS**

This prospective study was conducted in a 1050 bedded tertiary care hospital after ethical clearance for a period of three months from June to August 2012. 108 bacterial strains isolated from various clinical samples from wards, intensive care units and from outpatient cases were subjected to Kirby Bauer disk diffusion method. Those isolates found resistant to Imipenem, Meropenem or both and Ceftazidime were further subjected to screening test for MBL production by DDST and the results were compared with CDDT format. The demographic, clinical and laboratory details of the patients under the study were also noted. The obtained variables or parameters were analyzed statistically for prevalence of MBL for various species.

### **DDST Procedure Outline:**

A 0.5 McFarland bacterial suspension of the test isolate was lawn cultured onto Mueller-Hinton (MH) agar plate ( Hi Media, India ). Imipenem ( IMP 10 µg ) or Meropenem ( MER 10 µg ) and Ceftazidime ( CAZ 30 µg ) disks were aligned around blank filter disks, which contained 4µl of 0.5 M EDTA ( Sigma, Germany ) ( 750µg), added directly on the disk already placed on the MH agar plate. 0.5 M EDTA solution was prepared by dissolving 186.1 g of disodium EDTA ( Junsei Chemical, Tokyo, Japan) in 1,000 ml of distilled water and adjusting it to pH 8.0 by using NaOH. The mixture was sterilized by autoclaving. The following distances between the inhibitor and the substrates was 20 mm ( from

center to center ). A positive result was declared when enhancement of zone of inhibition between Imipenem or Meropenem and Ceftazidime disc with EDTA blank disc  $\geq 5$  mm indicating MBL production.

### **\*\*CDDT Procedure Outline:**

Strains resistant to IMP or MER and CAZ and were also screened for MBL by CDDT method as described by Yong et al. Test organisms were lawn cultured onto plates of MH agar (Opacity adjusted to 0.5 McFarland opacity standards). Two 10 µg Imipenem ( IMP ) discs or two 10 µg Meropenem ( MER ) discs were placed at a distance of 25 mm and 4 µl of EDTA solution was added to one Imipenem or one Meropenem disc to obtain the desired concentration of 750µg. The zone of inhibition around Imipenem or Meropenem discs alone and those with EDTA was recorded and compared after 16-18 hrs incubation at 35°C. An increase in zone size of at least  $\geq 7$  mm around the Imipenem-EDTA disc or Meropenem-EDTA disc was recorded as a positive result for MBL.

To test the stability of the EDTA-added Imipenem discs, an EDTA solution was added to 10µg Imipenem disks to obtain a concentration of 1,000 µg. The disks were dried immediately in an incubator and stored at 4°C or at 20°C in airtight vials without desiccant. The inhibition zones produced for MBL positive and negative isolates were compared after storage of the disks. ATCC strains for the select isolates were used to check the quality of work undertaken. The inhibition zone for the control strain was within the acceptable range did not exhibit any zone size enhancement with EDTA impregnated imipenem discs.

## **RESULTS**

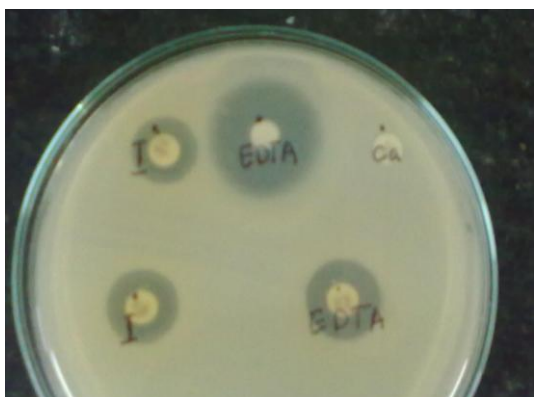
Of the 108 isolates 48 (44.4% ) were resistant to Imipenem or Meropenem or both and among them 14 ( 29.1 % ) were metallo-beta-lactamase producers. Of the MBL

producers, *Acinetobacter* species 5 ( 35.7 % ) was the most common followed by *Pseudomonas aeruginosa* 4 ( 28.5 % ), *Klebsiella pneumonia* 3 ( 21.4% ) and *E. coli* 2 ( 14.2 % ). The MBL isolates were mainly from the MICU and were non fermenters ( **Table 1** ).The two methods employed in testing for metallo- $\beta$ - lactamase

production were by DDST & CDDT. A non MBL producing strain is depicted in (**Fig:1**) and an MBL producer is shown in (**Fig:2**) evaluated by both methods. DDST was found to be technically simpler in testing for MBL production on a routine basis. The results using both techniques were the same for all the isolates tested.

**Table 1: MBL strains distribution among samples from wards or ICU's and Out patient dept.**

WARDS / ICU'S / OP	SAMPLES ( n=14 )	ISOLATES
BICU	Burns wound = 1	Acinetobacter species
	Burns wound = 1	Acinetobacter species
	Burns wound = 1	Pseudomonas aeruginosa
NICU	Umbilical tip = 1	Acinetobacter species
	Suction tip = 1	Klebsiella pneumoniae
	Endotracheal tip = 2	Klebsiella pneumoniae & P.aeruginosa
MICU	Sputum samples = 2	Acinetobacter species, E.coli
	Endotracheal tip = 2	Acinetobacter species & P. aeruginosa
	Wound swab = 1	Pseudomonas aeruginosa
WARDS	Urine = 1	E.coli
	Pus swab = 1	Klebsiella pneumoniae
OP	Nil	Nil



**Fig 1:** Non MBL producing strain by DDST as shown above and CDDT done below.



**Fig 2:** MBL producing strain by DDST as shown above and CDDT done below.

## DISCUSSION

Carbapenem resistance was first identified in 1995.<sup>[3]</sup> The first carbapenemases described were from Gram-positive bacilli. Based on various studies carbapenemase is classified into four: Class A, B, C and D. Based on the requirement of divalent cations for enzyme activation, carbapenemases can be divided two groups: Metallo-carbapenemases ( Zinc-dependent class B ) and non-metallo-carbapenemases ( Zinc-independent class A, C and D ).<sup>[4]</sup> “Class A” carbapenemase belonging to group 2f has reduced susceptibility to Imipenem; contain an active serine amino acid in its active site.<sup>[5]</sup> “Class B” carbapenemase belongs to Metallo-Beta-lactamase type efficiently hydrolyze all  $\beta$ -lactams, except for aztreonam, in vitro.<sup>[6]</sup> Class C carbapenemases hydrolyze imipenem at levels similar to those of class A, B, and D carbapenemases.<sup>[4]</sup> Class D of OXA-48 was first described by Poirel et al<sup>[7]</sup> and OXA-51 by Brown et al.<sup>[8]</sup>

There are various methods available for detection of Class B carbapenamase which include: disk potential test, double disk synergy test and modified Hodge test. [9] Hodge test was not recommended for detection of metallo beta lactamases (MBL's) by John et al. Although Hodge test and E test are phenotypic detection methods, genetic probes for a molecular approach can be an alternative. [6,9,10] Acquired drug resistance has been a clinical challenge ever since therapeutic antibiotics has been initiated. Carbapenems have been introduced to overcome the  $\beta$ -lactam resistance. Imipenem, Meropenem and more recently Ertapenem have been widely used. The activity of carbapenem ensures a wide range of antibiotic cover. This has increased its use as an empiric drug. With extreme use of Carbapenems, the selection pressure on the bacteria has driven the emergence of Carbapenem resistant bacteria. [1,2]

Carbapenems are the newest members of  $\beta$ -lactam group of antibiotics. Their action remains the same as that of other members of the group. Carbapenems bind to penicillin binding proteins ( PBP ), and attack the transpeptidase or transglycolase enzyme system, mostly by competitive inhibition involved in formation of cell wall. Carbapenem resistance can be due to multiple factors. The drug may be destroyed by the carbapenamase enzyme or may be effluxed from the cell. [1,2,11]

Acquired carbapenamase can be class A ( KPC ), class B ( IMP, VIM, NDM ) or class D ( OXA-48, OXA-181 ) carbapenamases. Phenotypic detection methods cannot distinguish between the various subtypes. [5-8] The metallo- $\beta$ -lactamases belong to the IMP, VIM, SPM, GIM, and SIM families and have been detected primarily in *Pseudomonas aeruginosa*. The class D carbapenamases consist of OXA-type  $\beta$ -lactamases frequently detected in *Acinetobacter*

*baumannii*. However, there are increasing numbers of reports worldwide of this group of  $\beta$ -lactamases in the *Enterobacteriaceae*. [5]

Till date, there is no CLSI recommended guidelines for testing carbapenamase resistance. Several methods have been used by different studies. The most well accepted screening methods include double disk synergy test ( DDST ), [12] combination disk diffusion test ( CDDT ) by Yong et al. [6] EDTA may inhibit certain bacteria causing false positives. On the other hand, modified Hodge test which precludes the use of EDTA, detects only carbapenamase activity. It does not confirm the metal dependence of the carbapenamase. [13] Several studies have come up with a large variety of results. Different tests have been shown to be useful in multiple settings and consistent results have not been replicated. This possibly indicates that the detection method is strain dependent and the efficacy of method is variable based on the testing strain. More recently Carba-NP test has been described for testing carbapenamase producers. [14]

It is a widely known fact that Imipenem is more resistant to efflux mechanism compared to Meropenem. Efflux pumping is attributed to mexB, mexR, mexY, mexF, oprD and more. [15] Imipenem has a unique hydroxyl chain that possibly prevents it from efflux. Thus increasing the efflux pumps cannot decrease the activity of imipenem. However, Meropenem is not resistant to efflux. [1,2,15] This phenomenon is not absolute as certain efflux pumps have been believed to evolve against Imipenem. This concept is used as a rough guide to predict resistance mechanism. If both Imipenem and Meropenem show resistance, then the possible mechanism suggested is enzyme mediated. In contrast if only Meropenem resistance is seen, it could be because of efflux.

Our current study aimed to identify the width of the resistance mechanism involved in carbapenem resistance. As shown from our data, the resistant isolates were commonly isolated from wound swabs and respiratory samples which invariably is expected to be poly-microbial in nature. This is indicative of the good transmission and spreading of resistance among multiple bacteria. The percentage of Imipenem resistant strains were 44.4 %. Increasing the drug concentration and bioavailability is of use only in scenarios where the mechanism of resistance is efflux pumping. Our data thus suggests that higher doses of carbapenem use may be useful in our settings as only 29.1% isolates were carbapenemase producers.

Our general experience in routine testing states; most of the *Acinetobacter* and *Pseudomonas* isolates are pan drug or totally resistant phenotypes. When infections with this group are suspected carbapenems are used before the laboratory report is available. This correlates well with our data showing most of the resistance is contributed by non fermenters. The presence of *Klebsiella pneumonia* is indicative of an increasing trend of MBL induced resistance in the enterobacteriaceae group. This is consistent with other data globally.<sup>[2,3]</sup> ICU's are a potential storehouse from where drug resistant forms do emerge which was consistent with our findings. No community acquired MBL isolates were detected in our study. Carbapenems are often the last resort available in antibacterial therapy. Most clinician's prefer the use of carbapenems owing to its high activity when the nature of infection is unknown. Thus having been already started on empiric therapy with Carbapenems may predispose to spread of resistance as many isolates may escape the routine methods of MBL testing and environmental persistence of these isolates

in various ICU's can lead to subsequent patient colonization.

The laboratory identification and reporting has a turnaround time of at least 60 hrs ( 2-3 days ). De-escalation of antibiotics based on the antibiogram is important to reduce further selective evolutionary pressure on the organisms to evolve.<sup>[16]</sup> Our data suggests that there is an urgent requirement to strictly implement the principles of antimicrobial stewardship. The prevalence of MBL production among *P.aeruginosa* in our hospital was 28.5% which was in accordance with other Indian studies.<sup>[13]</sup> MBL production among *Acinetobacter* species was 74% in a study by Hakima et al<sup>[17]</sup> and in our study yielded 35.7%. MBL isolates among enterobacteriaceae was comparatively higher in our set up with *Klebsiella pneumonia* 21.4% and *E. coli* 14.2 % while other published articles by Agrawal G et al have shown a very low prevalence of 10 %.<sup>[18]</sup>

Some drawbacks in our study were its short duration and a small sample size which might not represent all possible variations in the given population. It may also not be a true reflection of all types of MBL's especially when most studies report it among non fermenters and very few discuss the emergence of it among enterobacteriaceae. Hence further evaluation is necessary. MBL producers may not always be carbapenem resistant. Since this study aimed at finding the prevalence of MBL producers alone, excluding carbapenem susceptible isolates the MBL producing carbapenem susceptible isolates would have been missed. The sensitivity and specificity of the CDDT screening test by Yong et al<sup>[6]</sup> claims a 95.7% sensitivity and 91% specificity. In our case however the sensitivity and specificity of these tests could not judged as we have not compared it

with the E-test and the gold standard PCR technique.

## CONCLUSION

In our opinion, the detection of MBL producing isolates is of crucial importance not only for institutions with a high prevalence of such isolates but also in those in which the phenotype of resistance has never been detected. In a scenario of a high frequency of MBL producing isolates, the detection of such strains would be important for the adjustment of empirical antimicrobial therapy and, probably, the reduction of morbidity and mortality rates for the infected patients. Early identification of carbapenemase producers in clinical infections, at the carriage state, or both, is therefore mandatory to prevent development of hospital-based outbreaks.

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