

# Metallo- $\beta$ - Lactamase Producing Clinical Isolates in a Tertiary Care Hospital

S. Basireddy<sup>1</sup>, Singh M, Reddy H<sup>2</sup>, Ali S, Kabra V<sup>3</sup>

**Abstract:** Metallo- $\beta$ - lactamase (MBL) producing carbapenem resistant clinical isolates are an increasingly difficult problem in health care settings because of the treatment difficulties and rapid spread of these infections. The present study aims in detecting the MBL production among carbapenem resistant clinical isolates. 54 non repetitive clinical isolates of carbapenem resistant gram negative bacteria were studied to detect the Metallo- $\beta$ - lactamase production. Among them 20(37%) were MBL producers. MBL producers are predominantly observed among Enterobacteriaceae members with *E.coli* topping the list (81.8%) followed by *Klebsiella* (77.8%). The least number of MBL producers were observed among *Acinetobacter* isolates (9.6%). Timely detection of these infections along with the stringent infection control practices will prevent the spread of these bacteria in health care settings.

**Keywords:** Metallo- $\beta$ - lactamase, E strips, carbapenem resistance, enterobacteriaceae, acinetobacter

## 1. Introduction

Carbapenems are one of the most important groups of drugs useful for the treatment of multidrug resistant gram negative bacteria. Though the resistance to carbapenems was considered rare earlier, recently there has been an increase in the incidence of carbapenem resistant gram negative bacterial isolation from the clinical setup.<sup>(1)</sup> There are several mechanisms for carbapenem resistance among gram negative bacteria such as carbapenamase production, lack of drug penetration due to mutation in porins, loss of certain outer membrane proteins and efflux mechanisms.<sup>(2)</sup> Among these carbapenamase production, is the most frequently encountered mechanism of resistance. Carbapenamases are beta lactamases which include serine  $\beta$  - lactamases and Metallo- $\beta$ - lactamase (MBL). Metallo- $\beta$ - lactamase require metal ion Zinc for their activity which is inhibited by metal chelators like EDTA and Dipicolinic acid.<sup>(3)</sup> Resistance due to MBL production has the potential for rapid dissemination as it is often plasmid mediated and is the most feared in any hospital because of the ability to hydrolyse all the  $\beta$ - lactam drugs and also because of their frequently associated resistance to aminoglycosides and fluroquinolones.<sup>(4)</sup> For this, the rapid detection of MBL production is necessary to initiate effective infection control measures and to prevent their dissemination. The present study aims to detect incidence of Metallo- $\beta$ - lactamase production among the carbapenem resistant gram negative bacteria isolated in our hospital by using the E test based methodology.

## 2. Materials and Methods

A total of 54 non duplicative carbapenem resistant isolates obtained during Jan 2014 to Aug 2014, which are resistant to both Meropenem and Imipenem, isolated from various clinical samples like blood, sputum, ET secretions, urine were included in this study. All these microorganisms were identified by standard laboratory methods and antimicrobial susceptibility was done by Kirby Bauer disc diffusion methodology as recommended by CLSI.<sup>(5)</sup> All these isolates were screened for production of Metallo- $\beta$ -lactamase by using MBL E-strips (Hi-media). *E coli* ATCC 25922, *Pseudomonasaeruginosa* ATCC 27853 and a

laboratory confirmed strain of MBL producing *Klebsiella* were used as control strains.

### MBL detection by E test

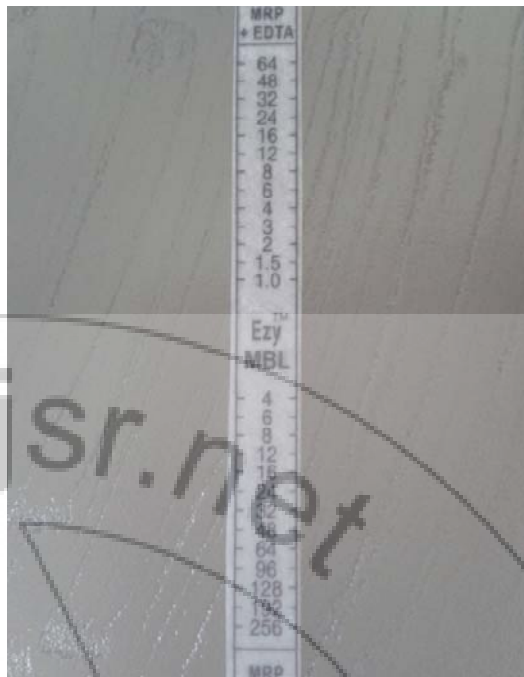
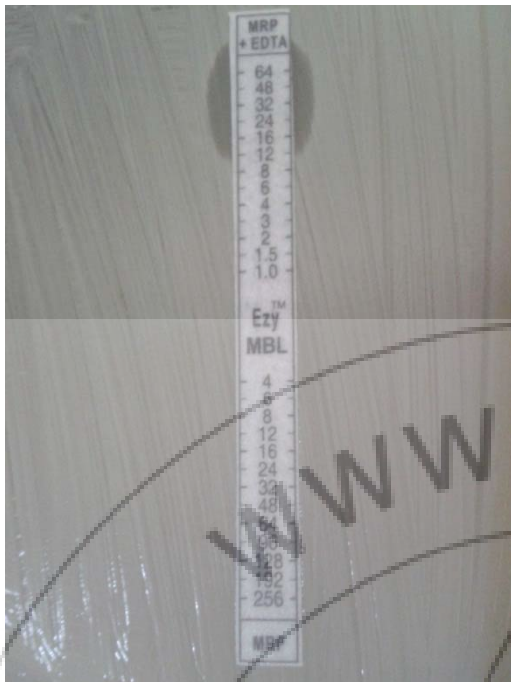
Metallo- $\beta$ - lactamase detection E- strips (Hi-media) with a double sided seven dilution range of meropenem (4 to 256  $\mu$ g/ml) on one half and meropenem overlaid with a constant gradient of EDTA on the other half (1 to 64  $\mu$ g/ml) were used for testing. Colony suspension of the test organism adjusted to 0.5 McFarland turbidity was prepared and inoculated onto Mueller Hilton agar plate, E-strip was placed onto the agar plate and incubated at 37<sup>o</sup> c for 16 to 18 hrs. Zones of inhibition were measured and compared. A reduction of meropenem MIC by  $\geq$  3fold dilution in the presence of EDTA was interpreted as Metallo- $\beta$ - lactamase positive.<sup>(2)</sup>

## 3. Result

Out of 54 carbapenamase resistant isolates included in our study, 31 were *Acinetobacterbaumannii*, 11 *E coli*, 9 *Klebsiella* and 3 *Pseudomonas aeruginosa*. Out of these 54 isolates 20 (37%) showed Metallo- $\beta$ - lactamase production by E test method. The highest Metallo- $\beta$ - lactamase production was observed among *E coli* isolates with 9 out of 11 (81.8%) isolates showing MBL production, followed by *Klebsiella* (77.8%) and *Pseudomonas* (33.3%). Out of 31 carbapenem resistant *Acinetobacterbaumannii* isolates only 3(9.6%) isolates produced Metallo- $\beta$ - lactamase.

Organism	Number of carbapenem resistant isolates	Metallo- $\beta$ -lactamase
A baumannii	31	3(9.6%)
E. coli	11	9(81.8%)
Klebsiella	9	7(77.8%)
Pseudomonas	3	1(33.33%)
Total	54	20(37%)

In the present study, all the *Acinetobacter* isolates had an MIC > 256  $\mu$ g/ml, MIC of *E.coli* and *Klebsiella* ranged between 32 to > 256  $\mu$ g/ml and *Pseudomonas* the MIC range was between 32 to 128  $\mu$ g/ml.



Metallo- $\beta$ - lactamase positive Klebsiella Metallo- $\beta$ - lactamase negative Acinetobacter

#### 4. Discussion

Carbapenems are often used as a last resort in infections due to multi drug resistant gram negative bacilli. However, in the recent years there has been an alarming increase in the reports of carbapenemase mediated resistance among clinical isolates. The first Metallo- $\beta$ - lactamase was observed in *Pseudomonas aeruginosa* in Japan in 1988.<sup>(6)</sup> In India, Metallo- $\beta$ - lactamase was first reported in 2002.<sup>(7)</sup> For many years these Metallo- $\beta$ - lactamase production was restricted to only nonfermenters like *Pseudomonas* and *Acinetobacter* but now these mechanism has been widely dispersed to other bacterial members like Enterobacteriaceae which possess a potential threat in any hospital.

Various phenotypic methodologies have been described for the detection of Metallo- $\beta$ - lactamase production. Among these inhibitor based assays using EDTA are more widely used which include disc potentiation test, double disc synergy test and MBL E strip based methodologies. Though modified Hodge test has been described for detection of carbapenemase production it has a relatively low sensitivity in detection of Metallo- $\beta$ - lactamase production when compared to other modalities.<sup>(3,5)</sup>

In the present study, Enterobacteriaceae member have shown the highest Metallo- $\beta$ - lactamase production with *E coli* topping the list (81.8 %) followed by *Klebsiella* (77.8%). Similar findings were observed by Hodiwala et al<sup>(8)</sup> where 91% of *E coli* and 100% of *Klebsiella* were Metallo- $\beta$ -lactamase positive. Similarly in Nagaraj et al<sup>(9)</sup> study 80% of the carbapenem resistant Enterobacteriaceae members were positive for Metallo- $\beta$ - lactamase production by PCR based method.

In our study, only 9.6% of *Acinetobacter* isolates were Metallo- $\beta$ - lactamase producers. This is similar to Noyal et

al<sup>(10)</sup> study where only 6.5% of *Acinetobacter baumannii* were Metallo- $\beta$ - lactamase producers. In John et al<sup>(11)</sup> study the prevalence of Metallo- $\beta$ - lactamase production among *Acinetobacter baumannii* was 14.8%. In another Indian study on Meropenem resistant *Acinetobacter* species none of the isolates have produced Metallo- $\beta$ -lactamase<sup>(12)</sup>. It has been described that in *Acinetobacter* species resistance to carbapenems is mediated predominately by mechanisms other than MBL production like OXA carbapenemases, hyper production of Amp C  $\beta$ -lactamase or other mechanisms like loss of porins increase efflux pump activity.<sup>(13)</sup>

In contrast to *Acinetobacter*, in our hospital carbapenem resistance in *Pseudomonas* is extremely rare. In our hospital 27% of *Acinetobacter baumannii* isolates were carbapenem resistant while in only 2 % of the *Pseudomonas* isolates carbapenem resistance was observed. In the present study only 3 carbapenem resistant *Pseudomonas* were isolated and tested for MBL production and it is observed that MBL production is seen in only 1 out of 3 (33.3%) carbapenem resistant isolates, which is similar to John et al study where 27.7% of *Pseudomonas* isolates were MBL producers, but the number of isolates were too few in our study when compared to their study.<sup>(11)</sup>

The increasing reports of MBL producing Enterobacteriaceae especially NDM-1 producers have been a potential threat to global health as they are capable of colonizing the gut of patients and in turn serve as reservoirs for spreading the infection and contaminating the environment in health care setup. To control the spread of these infections stringent disinfection measures and strict infection control practices are absolutely necessary.

The implementation of a simple laboratory detection method for identifying MBL production which is quick,

sensitive, specific and reproducible is preferred. Though the EDTA disc potentiation test is most commonly used because of its cost effectiveness when compared to E test methodology, E test based methodologies are good in the performance and have the advantage of simultaneous detection of minimum inhibitory concentration.<sup>(2)</sup>

With the recent discovery of molecule like aspergillomarasmine A (under trials) which turns off the resistance mechanism of NDM-1 and thus makes bacteria once again sensitive to traditional antibiotics, detection of the MBL production among clinical isolates will gain more importance in future for formulating the treatment strategies.<sup>(14)</sup>

To conclude, routine surveillance for detection of MBL production should be carried out among clinical isolates, which helps not only for treatment purposes but also infection control practices which in turn prevent spread of these infections and decreases the mortality and morbidity.

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