

Detection of Metallo beta - lactamases among Enterobacteriaceae isolates at a tertiary care hospital, south India

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Background:

The spread of carbapenem resistant bacteria has caused grave concern due to the limited choice in antibiotics for treating infections caused by them. The emergence of metallo beta-lactamase (MBL) producing gram negative bacilli poses a therapeutic challenge and is of serious concern for infection control in hospital environment.

Materials & Methods:

A total of 475 non repeat clinical isolates of family *Enterobacteriaceae* were included in the study. Resistance to imipenem was determined in isolates by disc diffusion & minimum inhibitory concentration (MIC) method. Imipenem resistant isolates were tested for MBL production by combined disc diffusion test and modified Hodge test.

Results:

Out of the 475 *Enterobacteriaceae* strains, 20 showed resistance to imipenem. MBL activity was detected in all 20 (4.2%) isolates by combined disc diffusion test, in 18 isolates by modified Hodge test. The MBL producing isolates included clinical strains of *Klebsiella spp* (45%), *E. coli* (40%), *Citrobacter spp* (15%). Majority of the MBL isolates were from Intensive care unit (65%), from patients with comorbid conditions and with invasive devices. MBL producing isolates showed high level of resistance to aminoglycosides and fluoroquinolones but all were susceptible to colistin.

Conclusion:

The need of the hour is to detect MBL producing isolates for better patient outcomes, to

execute prompt infection control measures and decrease the escalation of resistance.

Keywords: *Imipenem resistance, Phenotypic methods, Colistin sensitivity*

Introduction:

Carbapenems are the most effective agents for treatment of serious infections caused by multiresistant *Enterobacteriaceae*, particularly those producing extended spectrum beta-lactamase (ESBL) and AmpC beta-lactamase enzymes.^{1,2} Currently, the spread of carbapenem resistant bacteria has caused grave concern due to the limited choice in antibiotics for treating infections caused by them.³ Resistance in gram negative bacteria to carbapenem is mainly due to the production of carbapenem hydrolyzing enzymes called carbapenemases.^{1,4} Another important cause among carbapenem resistant *Enterobacteriaceae* is hyperproduction of AmpC beta-lactamase enzyme in organism with porin loss.⁵

A consistent number of acquired carbapenemases have been identified during the

past few years, belonging to three of the four known classes of beta-lactamases, either Ambler molecular class B (metallo beta-lactamases) or Ambler molecular classes A and D (serine carbapenemases). Amongst these, the carbapenemases which are clinically significant are class B enzymes. Class B enzymes comprise the metallo beta-lactamases (MBL), such as the Imipenemases (IMP) family of carbapenemases, the Verona integron-encoded metallo beta-lactamases (VIM), Seoul imipenemase (SIM), German imipenemase (GIM) and the New-Delhi-metallo beta-lactamases (NDM) enzymes.^{1,4} The IMP or VIM series of metallo beta-lactamase enzymes have been reported globally. The New Delhi metallo beta-lactamase 1 (NDM-1) has received worldwide attention since it was first reported in *Klebsiella pneumoniae* recovered from a Swedish patient previously hospitalized in India.^{6,7}

MBL confer resistance to all beta lactam antibiotics except monobactams. They are zinc-dependent beta-lactam hydrolysing enzymes & are characterised by resistance to beta-lactamase inhibitors like clavulanic acid, sulbactam & tazobactam. They are distinct from other beta-lactamases in that they do not compete with penicillin binding proteins for their mode of action.⁸ MBL enzymes, whose genes can be chromosome or plasmid-borne are often situated in integrons and pose a serious risk of substantial dissemination among the gram negative fraternity.⁹

High morbidity & mortality is associated with invasive infections caused by MBL producing gram negative isolates. The emergence of metallo beta-lactamase (MBL) producing gram negative bacilli poses a therapeutic challenge and is of serious concern for infection control in hospital environment.^{3,10} In our health care setting carbapenem resistant *Enterobacteriaceae* strains were increasingly being isolated and hence this study was taken to know the prevalence of MBL among *Enterobacteriaceae*.

Materials and methods

This was a crosssectional study undertaken in the Microbiology Department, at our tertiary care hospital, South India. A total of 4536 samples were received for aerobic culture in the microbiology laboratory from the patients attending or admitted to hospital during the study period of June to December 2016. The bacterial isolates obtained from various clinical samples were identified according to standard microbiological procedure.¹¹ All isolates belonging to *Enterobacteriaceae* family were included in the study.

Antimicrobial susceptibility testing:

The antimicrobial susceptibility testing was done by Kirby Bauer's disc diffusion method on Mueller-Hinton agar (Himedia Laboratories Pvt. Ltd, Mumbai, India), as per Clinical Laboratory Standard Institute (CLSI) guidelines.¹² The antibiotics tested were: Ampicillin (10mcg), Amoxycylav (20/10mcg), Cefuroxime(30mcg), Cefotaxime (30mcg), Ceftazidime (30mcg), Cefepime (30mcg), Gentamicin (10mcg), Netilmicin (30mcg), Amikacin (30mcg), Ciprofloxacin (5mcg), Piperacillin (10mcg), Piperacillin+Tazobactam (100/10mcg), Imipenem (10mcg), Colistin (10mcg) (Himedia Laboratories Pvt. Ltd, Mumbai, India). In strains with reduced susceptibility to imipenem, minimum inhibitory concentration (MIC) was determined by agar dilution method. The *Enterobacteriaceae* strains with MIC values $\geq 4\text{mcg/ml}$ for imipenem were tested for MBL production by phenotypic methods

Metallo beta-lactamase detection:

Combined disc diffusion test:

MBL production was detected by combined disc diffusion test as described previously by Yong et al¹³. Briefly, test strains with 0.5 Mcfarland turbidity standard were inoculated onto Mueller Hinton agar plates. On the Mueller Hinton agar plate two 10 mcg imipenem discs were placed and to one of them 10mcl of 0.5 M EDTA solution was added to obtain the desired concentration (750mcg disodium EDTA dihydrate per disc). After 16-18hrs of incubation in air at 35°C the inhibition zones of imipenem and imipenem-EDTA discs were compared. The isolate was considered as positive for MBL production if there was increase in inhibition zone diameter of $\geq 7\text{mm}$ with imipenem and EDTA disc when compared to imipenem disc alone.

Modified Hodge test (MHT):

The Modified Hodge test was performed as described previously by Lee et al¹⁴. Indicator organism *Escherichia coli* ATCC 25922 of 0.5 McFarland turbidity standard (1:10 dilution) was lawn cultured on Mueller Hinton agar. Imipenem disc (10 mcg) was placed in the centre of the plate after drying. Then the test strains were heavily inoculated in a straight line from the edge of the disc to periphery of the plate and incubated at 35°C overnight. Following incubation, presence of clover leaf shaped indentation along the streak line of the test isolate was interpreted as a positive for carbapenemase production. While absence of indentation was interpreted as negative for carbapenemase production.

Results

A total of 475 non repeat clinical isolates belonging to family *Enterobacteriaceae* were obtained during the study period. Among these bacterial strains, 20 isolates were found resistant to imipenem according to CLSI breakpoints. Most of the imipenem resistant isolates (10/20) showed MIC of 16 mcg/ml (Table No 1).

Metallo beta-lactamase activity was demonstrated using combined disc diffusion test in all of the 20 imipenem resistant isolates. While the modified Hodge test detected 18 strains as carbapenemase and metallo beta-lactamase producer. The combined disc diffusion test was able to detect two more strains that were not detected by the modified Hodge test.

The metallo beta lactamse producing isolates included clinical strains of *Klebsiella spp* (45%), *E. coli* (40%), *Citrobacter spp* (15%). Urine was the predominant source of MBL producing isolates (Table No 2). None of the isolates with MBL activity were obtained from patients attending outpatient department, they were isolated mainly from Intensive care unit (13/20) followed by Surgery ward (5/20) and Medicine ward (2/20). Most of the patients infected with MBL producing *Enterobacteriaceae* had comorbid conditions (Table No 3). Nine patients had a documented history

of diabetes mellitus while each of the four patients had heart disease or neurological disorders. Indwelling devices were also common among the patients infected with *Enterobacteriaceae* with MBL activity, 12 of the patients had a urinary catheter in place, two had a central venous catheter, two were on ventilator and one had drain in place.

All the MBL producing isolates were resistant to Ampicillin, Amoxycylav, Piperacillin, Piperacillin + Tazobactam, Cefuroxime, Cefotaxime, Ceftazidime, Cefepime. For non-beta lactam antibiotics, nine (45%) MBL producing isolates were susceptible to Amikacin, four (20%) were susceptible to Gentamicin and Netilmicin and two (10%) were susceptible to Ciprofloxacin and Levofloxacin. All MBL isolates were susceptible to Colistin (Figure No 1).

Imipenem MIC value	No of <i>Enterobacteriaceae</i> isolates
32 mcg/ml	2
16 mcg/ml	10
8 mcg/ml	6
4 mcg/ml	2
Total	20

Table 1. Distribution of Imipenem MIC values among Imipenem resistant *Enterobacteriaceae* isolates

Sample	No of MBL producing isolates (%)
Urine	11 (55)
Pus	5 (25)
Endotracheal secretions	2 (10)
Blood	2 (10)
Total	20

Table 2. Distribution of metallo beta lactamase producing isolates from various clinical samples

Comorbid conditions	No of Patients (%)
Diabetes mellitus	9 (45)
Neurological disorders	4 (20)
Heart disease	4 (20)
Chronic obstructive pulmonary disease	1 (5)

Table 3. Distribution of comorbid conditions among the patients infected with MBL producing isolates.

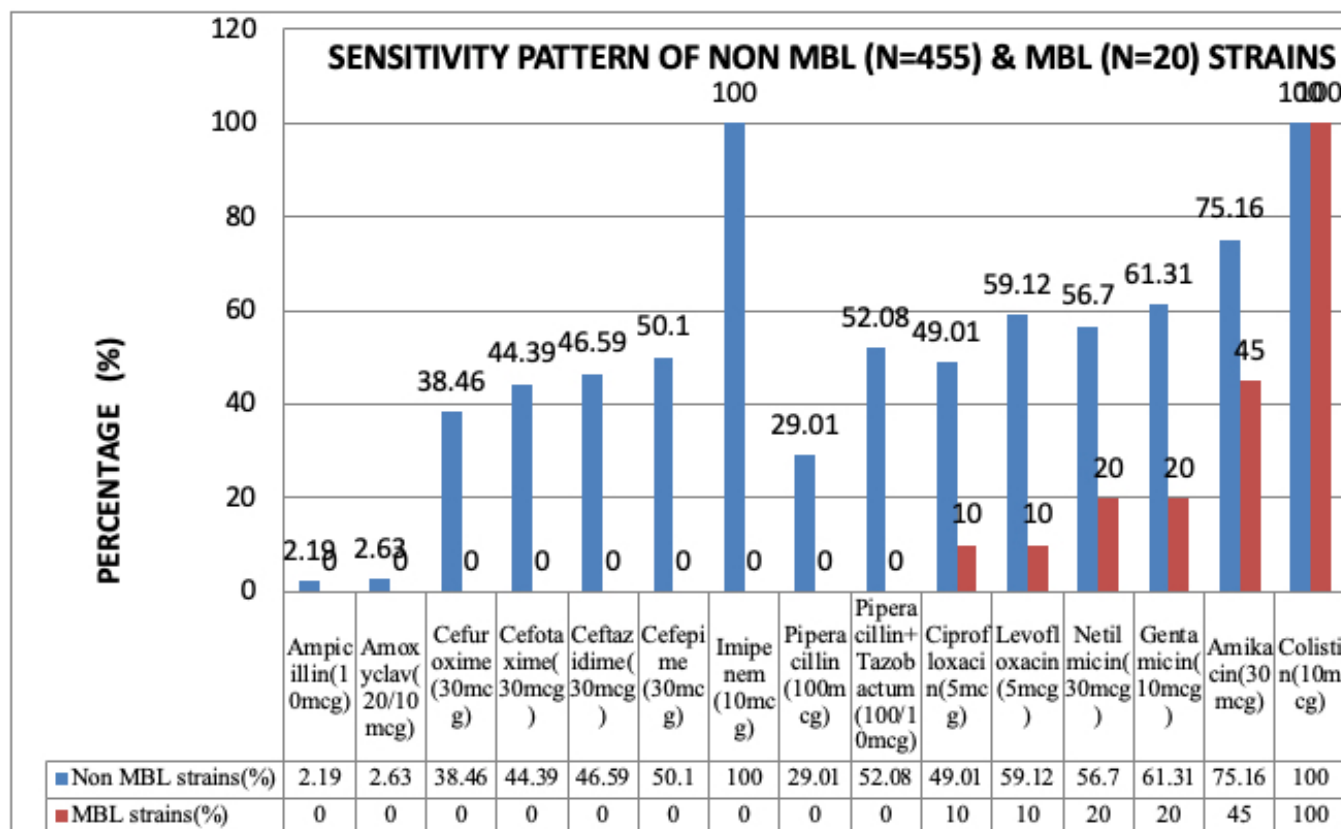


Figure 1. Sensitivity pattern of MBL & non MBL producing *Enterobacteriaceae* strains.

Discussion

MBL producing *Enterobacteriaceae* infections are generally healthcare associated, although community associated infections are beginning to appear.¹⁵ Infections caused by ESBL and AmpC beta-lactamase producing *Enterobacteriaceae* are being treated with carbapenems which has caused a significant threat in increasing occurrence of MBL producing *Enterobacteriaceae*.^{1, 2}

Reports indicate that prevalence of metallo beta-lactamase producing *Enterobacteriaceae* isolated from clinical samples is increasing in the last few years.^{6, 16} The metallo beta-lactamase efficiently hydrolyzes all beta-lactams, *in vitro*, except aztreonam making the therapeutic options severely limited.¹⁷

The prevalence of MBL type carbapenemase among *Enterobacteriaceae* strains in our health care setup was 4.2% (20/475). Similar findings were found in studies by various authors from India. Datta et al¹⁸ have reported prevalence of 5.75% among *Enterobacteriaceae* strains producing MBL type carbapenemase. In a study conducted by Rai et al¹⁹ 7% of *Enterobacteriaceae* strains were MBL producers. Deshmukh et al²⁰ have reported low prevalence (1.25%) of MBL activity among *Enterobacteriaceae* strains. While Govindswamy et al¹⁶ have reported a high prevalence (65.1%) of MBL producing *Enterobacteriaceae*. In the present study combined disc diffusion test (20/20) was slightly better than MHT (18/20) to detect MBL production among *Enterobacteriaceae*. Limitation of our study was inability to compare the phenotypic methods with genotypic methods for detection of MBL production.

Majority of the MBL isolates were from Intensive care unit followed by Surgery and Medicine. Indwelling devices are frequently used in these areas, which can play a major role in the spread of

infective agents. Important risk factors for colonization with Carbapenemase producing *Enterobacteriaceae* (CPE) include prior antibiotic usage, long term healthcare exposure, presence of invasive devices and comorbid conditions.²¹ Indwelling devices were common among patients infected with carbapenem resistant *Enterobacteriaceae* in our study: 60% of patients had a urinary catheter in place, 10% had a central venous catheter in place and 10% were on mechanical ventilator. Gómez Rueda et al²² have also reported presence of indwelling devices like central venous catheter (77%), urinary catheter (67%) and mechanical ventilator (59%) among patients infected with carbapenem resistant *K. pneumoniae*.

In our study comorbid conditions were also frequent among patients infected with carbapenem resistant *Enterobacteriaceae*; 45% had a documented history of diabetes mellitus and 20% had either neurological disorders or heart disease. Guh et al²³ have reported at least one comorbid condition in 91.4% of individuals with carbapenem resistant *Enterobacteriaceae*. The most commonly reported co-morbid conditions by them included diabetes (44.3%) and neurological disorders (40.7%).

We observed that the carbapenem resistant organisms were isolated mainly from urine samples (55%) followed by wound discharge (25%), respiratory secretions (10%) and blood sample (10%). Rai et al¹⁹ observed that majority of carbapenem resistant *Enterobacteriaceae* isolates were from urine (89%) followed by blood sample (11%). While Deshmukh et al²⁰ reported majority of the MBL producers from pus (36.8%) and tracheal secretions (26.3%), and less number of isolates were from urine (15.9%). *Klebsiella spp* (45%) were the predominant MBL producers which is comparable with findings of Deshmukh et al²⁰ (31.6%).

MBL enzymes hydrolyse virtually all beta-lactams except aztreonam. A unique feature of MBL producing isolates is that it also shows broad spectrum resistance profile. In the present study MBL producing isolates were completely resistant to all beta-lactam antibiotics including third and fourth generation cephalosporins. These isolates also showed high level of resistance to aminoglycosides and fluoroquinolones. However all the MBL producing isolates were susceptible to colistin. This finding is substantiated by Deshmukh et al²⁰ (100% susceptible). MBL producing microorganisms are increasingly being isolated in whom colistin is the microbiological treatment of choice. The emergence of colistin resistance poses a realistic hazard compromising treatment choices and potentially the outcome of critically ill patients. Colistin should always be used in combination with other antimicrobials to have adequate activity and prevent resistance.²⁴

Conclusion

A significant number of *Enterobacteriaceae* isolates with MBL activity along with multiple drug resistance was found in our study. Few antibiotics retain activity against MBL producing *Enterobacteriaceae* due to the ability of MBL enzymes to hydrolyze most of the beta-lactam antibiotics as well as the frequent coexistence in MBL producing *Enterobacteriaceae* isolates of additional mechanisms of resistance against other antibiotic classes such as fluoroquinolones and aminoglycosides. The mobile genetic elements carrying the IMP and VIM-type enzymes aid their spread and compromise the future usefulness of carbapenems for the treatment of life threatening infections caused by *Enterobacteriaceae*. These facts highlight the need to precisely detect MBL producing isolates by clinical microbiologists, for better patient outcomes, to execute prompt infection control measures and decrease the escalation of resistance

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