

## Study of Metallo-beta lactamase production in imipenem-resistant gram-negative bacteria in surgery and ICUs in tertiary care hospital.

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### Abstract:

Broad spectrum antimicrobial resistance, where resistance to multiple, or even all available antibiotic classes, is a key global healthcare problem. Gram negative bacilli account for the majority of bacterial pathogens isolated from clinical specimens. The incidence of infections is due to gram negative bacilli resistant to  $\beta$  lactam agents. Metallo beta lactamase (MBL) producing gram negative bacteria have been recognized to be among the most important nosocomial pathogens. MBLs have been identified with increasing frequency to cause serious infections. **Aim:** This study was conducted to identify phenotypically for the presence of metallo-betalactamase producing isolates in surgery and ICUs. **Materials and Methods:** A total of 134 imipenem resistant microorganisms were isolated in Surgery and various ICUs. All the isolates were tested for anti-microbial susceptibility (Hi-Media Mumbai) for Imipenem by Kirby-Bauer disk diffusion method on Muller-Hinton agar. Imipenem resistant isolates were further tested for Metallo-betalactamase production by Combined disc diffusion test. **Results:** Out of 134 isolates, 67 (50%) were *Klebsiella spp.*, 26(19.4%) *E.coli*, 33 (24.6%) *Pseudomonas spp.*, 8(5.9%) were *Acinetobacter spp.* 77 isolates shows MBL production. Among 77 MBL producing isolates *Klebsiella spp.* comprised of 40/77 (51.9%), *Pseudomonas spp.* 21/77(27.2%), *E.coli*11/77(14.2%), *Acinetobacter spp.* 5/77(6.4%). Out of 77 MBL producers, 31 were isolated from ICUs and 46 were from Surgical ward. **Conclusion** The detection of MBL resistant organism showed importance of hospital environment in the ICUs and surgical wards are vicious due to their invasive procedures and enormous usage of antibiotics.

**Keywords:** - Imipenam, Metallo-beta-lactamase, Microorganisms.

### Introduction:

Multi Drug resistant microorganisms are now becoming a global threat. The burden is more in such countries where unrestrained use of antibiotics and due to extensive use of antibiotic over the counter treatment along followed by self medication.

Carbapenem is a broad-spectrum beta-lactam ( $\beta$ -lactam) antibiotic group, developed as one of the last resort antibiotics for treatment of serious and life threatening infections caused by multitude of multidrug resistant (MDR) gram

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negative bacterial infections.<sup>[2]</sup> Carbapenemases are diverse enzymes that vary in their ability to hydrolyze carbapenems and other beta-lactams. Detection of carbapenemase is a crucial infection issue which is associated with antibiotic resistant and treatment failures.

Gram negative bacilli account for the majority of bacterial pathogens isolated from clinical specimens.<sup>[3]</sup> The incidence of infections is due to gram negative bacilli resistant to  $\beta$  lactam agents. The resistance to monobactams and carbapenems is due to production of AmpC and Metallo-beta-lactamases respectively. The genes coding for the  $\beta$ -lactams are carried on plasmids facilitating rapid spread between micro-organisms and often are co-expressed in the same isolate.<sup>[4]</sup>

MBL producing gram negative bacteria have been recognized to be among the most common nosocomial pathogens. The present study was conducted to evaluate the prevalence of MBLs imipenem resistant isolates in surgery and ICUs.

### Material and Methods:

The present prospective study was carried in AMC MET Medical College, Ahmedabad. It was approved by the institutional review board. A total of 134 samples such as endotracheal secretions, pus, urine, blood sputum, CSF were collected from various ICUs & Surgical ward and tested for imipenem resistant by Kirby Bauer disc diffusion method.

Gram staining & direct microscopy of various samples except blood was done in the Microbiology Department. The specimens were cultured on Blood agar & Mac-Conkey agar and incubated at 37 °C for overnight. All the isolates were tested for anti-microbial susceptibility (Hi-Media Mumbai) by Kirby-Bauer disk diffusion method on Muller-Hinton agar.<sup>[5]</sup> Imipenem resistant isolates were further tested to Imipenem-EDTA disc method for MBL detection.

### Combined Disc test (Imipenem-EDTA disc method):

Test organism was inoculated on Muller-Hinton agar and two 10 $\mu$ g imipenem disc were placed. 10 $\mu$ l solutions (750  $\mu$ g) of Ethylene Diamine Tetra Acetic Acid (EDTA) were added to one of them & incubated the plate at 37 °C for 16-18 hours. Metallo- $\beta$ -lactamase positive result considered, if zone of inhibition of imipenem+ EDTA disc was >7mm than that of imipenem disc alone.<sup>[6]</sup>

### Results:

**Table 1: Distribution of isolates in ICUs and Surgical ward**

Organism	No. of Imipenem resistant isolates	ICUs	Surgical ward
Klebsiella spp.	67(50%)	29(43.2%)	38(56.7%)
E.coli	26(19.4%)	4(15.3%)	22(84.6%)
Pseudomonas spp.	33(24.6%)	9(27.2%)	24(72.7%)
Acinetobacter spp.	08(5.9%)	3(37.5%)	5(62.5%)
Total	134	45(33.5%)	89(66.4%)

Among 134 isolates, 67 (50%) were *Klebsiella spp.*, 33 (24.6%) *Pseudomonas spp.*, 26(19.4%) *E.coli* and 8 (5.9%) were *Acinetobacter spp.* were found. *Klebsiella* were found at much higher percent rather than other organisms isolated from ICUs and surgical wards. It

was also observed that *Klebsiella* showed highest Imipenem resistance among all the isolates found in this present study.

**Table 2: Number of MBL producers**

Organism	No. of Imipenem resistant isolates tested	No. of MBL producers
<i>Klebsiella</i> spp.	67(50%)	40(51.9%)
<i>E.coli</i>	26(19.4%)	11(14.2%)
<i>Pseudomonas</i> spp.	33(24.6%)	21(27.2%)
<i>Acinetobacter</i> spp.	08(5.9%)	5(6.4%)
Total	134(100)	77(100)

**Table 3: Distribution of MBL producing isolates in ICUs and Surgical ward**

Organism	No. of MBL producers	ICUs	Surgical ward
<b><i>Klebsiella</i> spp.</b>	40 (51.9%)	21 (27.2%)	19 (24.6%)
<b><i>E.coli</i></b>	11 (14.2%)	2 (2.5%)	9 (11.6%)
<b><i>Pseudomonas</i> spp.</b>	21 (27.2%)	6 (7.7%)	15 (19.4%)
<b><i>Acinetobacter</i> spp.</b>	5 (6.4%)	2 (2.5%)	3 (3.8%)
<b>Total</b>	77 (100%)	31 (40.2%)	46 (59.74%)

#### Discussion:

All the 134 carbapenem resistance isolates were screened for MBL detection by using IMIPENEM-EDTA disc method. Among 134 isolates 77(57.4%) were MBL producers. Among 77 (57.46%) MBL producing isolates *Klebsiella* spp. comprised of 40/77 (51.9%), *Pseudomonas* spp. 21/77(27.2%), *E.coli* 11/77(14.2%), *Acinetobacter* spp. 5/77(6.4%).

Present study were found - *Klebsiella* spp. 40/67 (59.7%), *Pseudomonas* spp. 21/33 (63.6%), *E.coli* 11/26 (42.3%), *Acinetobacter* spp. 5/8(62.5%) while Pitout et.al Canada (2005) showed a prevalence of 46% MBL producing *Pseudomonas* spp.<sup>[7]</sup> and Dr.Wankhede S.V et.al reported similar to our study the rate of MBL production in *Pseudomonas* spp. are 23.78 %.

In the present study more number of MBL producer are noted in *Klebsiella* spp. (59.7%) where Dr.Wankhede S.V et.al reported low percentage (8.3%)<sup>[8]</sup> and Olowo-okereet et al reported 36.8%(11). 6.4% of *Acinetobacter* spp. are MBL producers which are similar to the study reported by Noyal et al<sup>[9]</sup> and Sowmya et al.<sup>[10]</sup>

Present study showed 57.4% MBL producers which was contrasting with the lower 4.2%, 8.5% and 14.4% MBL producing bacteria reported in Edo, Lagos and Kano, Nigeria, respectively( Oduyebo et al.,2015; Yusuf et.al.,2015; Jesumirhewe et al.,2017) and Higher than 73.1% reported in a multi centre study conducted in Kaduna and kano, northwest, Nigeria(Yusuf et.al.,2013).

Many factors may account for the isolation of carbapenem resistance in gram negative bacilli among patients with no history of previous exposure to carbapenem. Cross-resistance between carbapenem and other  $\beta$ -lactam antibiotics may result in independent emergence of carbapenem resistance in gram negative bacilli. Beta-lactam antibiotics are extensively used

in hospitals which may lead to resistance which has been well documented (Ibrahim et al., 2017; Olowo-okere et al., 2018).

The higher number of MBL producers in the present study are isolated from pus and urine. These organisms might have been acquired from hospital environment to patients. It reveals that proper hygienic conditions are required in hospital environment like personal health hand washing while attending the patients otherwise person to person transmission may occur.

The present study also revealed that most number of MBL producers were from Surgical wards (59.7%) comparative that of ICUs(40.2%). Lee et al reported an isolation of 31.7% of MBL producers from ICUs in Korean hospitals.<sup>[17]</sup> This showed importance of hospital environment in the ICUs and surgical wards were vicious due to their invasive procedures and enormous usage of antibiotics. ICUs and surgical wards were harboured by the MBL producing isolates- *Klebsiella spp.* 40(21/19), *Pseudomonas spp.* 21(6/15), *E.coli* 11(2/9), *Acinetobacter spp.*5(2/3) respectively.

The MBLs have recently emerged as one of the resistant organism having the capacity to hydrolyze beta lactam agents. The concerned genes such as (IMP-1, IMP-2, VIM, KPC, NDM & OXA-48) which are responsible for carbapenem resistance which leads organism to be multi drug resistant. Very limited options are there for this multi drug resistant organisms and very difficult to treat.

#### Conclusion:

Present study showed a current scenario of carbapenem resistant Gram-negative bacilli. in the hospital. Multi drug resistant microorganisms are now becoming major challenge in hospital ICUs & Surgical sites. In order to control MBL producing microorganisms in a hospital, strategies such as strict infection control measures, antibiotics resistance surveillance programs & restrict to clinicians, prescribe last resort drugs only where primary & secondary drugs are resistant and antibiotic cycling must be followed. Regular monitoring and documentation of carbapenem resistance should be done.

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