# Prevalence of Metallo Beta-Lactamase in Clinical Isolates of Non-Fermenters in A Tertiary Care Hospital

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**Abstract:** Background & objectives: Metallo-beta-lactamase (MBL) producing Pseudomonas Spp. and Acinetobacter spp. have become a growing therapeutic concern worldwide. Therefore, an attempt has been made in this study to identify metallo beta-lactamases in carbapenem resistant isolates of non-fermenters. Methods: During July-September 2016, out of total 4869 clinical isolates, non-fermenters were 1353. All the non-fermenters were subjected to antibiotic susceptibility testing by Kirby-Bauer disc diffusion test as per the Clinical and Laboratory Standards Institute (CLSI) guidelines. Selection criteria for MBL detection among these isolates was resistance to imipenem. Imipenem resistant isolates were tested for the detection of MBL production by Imipenem-EDTA combined disc method, double-disc synergy test and minimum inhibitory concentration (MIC) of imipenem was determined by E-strip. Enhancement of inhibition zone around imipenem discs impregnated with EDTA as compared to those without EDTA confirmed MBL production. Results: Out of 1353 non-fermenter isolates from various clinical specimens, 920(68%) were Pseudomonas spp. and 433(32%) were Acinetobacter spp. They were identified by using standard microbiological techniques. Out of 920 Pseudomonas spp., Imipenem resistance was found in 208(22.61%) isolates. MBL detection test was performed on these 208 isolates & 120 (13.04%) were MBL positive. Out of 433 Acinetobacter spp., Imipenem resistance was found in 172 (39.72%) isolates. MBL detection test was performed on these 172 isolates & 133(30.71%) were MBL positive. Interpretation & conclusion: MBL-mediated imipenem resistance in Pseudomonas spp. and Acinetobacter spp. is a cause for concern in the therapy of patients. Therefore, a strict antibiotic policy should be followed to prevent further spread of MBLs. Therapeutic options for such isolates are Colistin and Polymyxin B for Pseudomonas spp. and Colistin, Polymyxin B and Tigecycline for Acinetobacter spp. [Rathod R NJIRM 2017; 8(2):149-152]

**Key Words:** Carbapenem resistance, MBL, Non-Fermenters

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**Introduction:** MBL resistance has been observed frequently in non-fermenters Pseudomonas spp. and Acinetobacter spp. [1]. Functional classified as Group 3 are the zinc-based metallo beta-lactamases corresponding to the molecular class B [2], which are the only enzymes acting by the metal ion zinc. Metallo B-lactamases are able to hydrolyze penicillin, cephalosporin and carbapenems. Resistance to carbapenem is due to decreased outer membrane permeability, increased efflux systems, alteration of penicillin binding proteins and carbapenem hydrolyzing enzymes. [3,4]. These carbapenemase are class B metallo beta-lactamases (IMP, VIM). Metallo beta lactamase (MBL) is a group of beta-lactamases which requires divalent cations of zinc as cofactors for enzyme activity. These have potent hydrolyzing activity not only against carbapenem but also against other b-lactam antibiotics. The IMP and VIM genes responsible for MBL production are horizontally transferable via plasmids and can rapidly spread to other bacteria. Thus, MBL-producing strains have been reported as important causes of nosocomial infections [5,6] associated with clonal spread. Non-fermenters are often difficult to eradicate due to their resistant drug profile. Therefore, detection of MBL-producing Gram negative bacilli especially non-fermenters is crucial for the optimal treatment of patients particularly in critically ill and hospitalized patients, and to control the spread of resistance.

**Methods**: Present study was carried out at Microbiology Department, B. J. Medical college, Ahmedabad. During July-September 2016. Among 4869 clinical isolates, non-fermenter isolates were 1353.All the non-fermenters were subjected to antibiotic susceptibility testing by Kirby-Bauer disc diffusion test as per the Clinical and Laboratory Standards Institute (CLSI) guidelines. For Pseudomonas spp. zone diameter of imipenem less than or equal to 15mm considered as resistance. For Acinetobacter spp. zone diameter of imipenem less than or equal to 18mm considered as resistance. MIC (minimum inhibitory concentration) testing of imipenem performed on these isolates, which were further confirmed by imipenem-EDTA combined disc

method and imipenem- EDTA double disc synergy test [7].

**MIC (minimum inhibitory concentration) testing of imipenem:** MIC (minimum inhibitory concentration) testing of imipenem were performed on these isolates, MIC interpretive criteria for Pseudomonas spp. and Acinetobacter spp. resistance is a value greater than or equal to 8µg/ml.

**Imipenem-EDTA combined disc method (CDT):** Imipenem-EDTA combined disc method (CDT) was performed as described by Yong et al. A lawn culture of test isolates was prepared. After allowing it to dry for five minutes, two imipenem discs, one with 0.5 M EDTA and the other a plain imipenem disc, were placed on the surface of agar plates approximately 30mm apart. The plates were incubated overnight at 37°C. An increase in zone diameter of ≥ 7mm around imipenem + EDTA disc in comparison to imipenem disc alone indicated production of MBL.

**Imipenem-EDTA double disc synergy test (DDST):** Imipenem-EDTA double disc synergy test (DDST) was performed as described by Lee et al. Test organisms were inoculated on to plates with Mueller Hinton agar as recommended by CLSI. An imipenem (10µg) disc was placed 20mm Centre to Centre from a blank disc containing 10µL of 0.5 M EDTA (750µg). Enhancement of the zone of inhibition in the area between imipenem and EDTA disc in comparison with the zone of inhibition on the far side of the drug was interpreted as a positive result for MBL production.

Results: Out of 1353 non-fermenter isolates from various clinical specimens. 920(68%) were Pseudomonas spp. and 433(32%) were Acinetobacter spp. They were identified by using standard microbiological techniques.

**Table 1 showing total non-fermenters from various clinical isolates and imipenem resistant isolates among them.**

Table 1: P-value: 0.0053(p-value is <0.05 which is significant)

| Month | Total Non-Fermenters Isolates | Imipenem Resistant Non-Fermenters Isolates |
|---|---|---|
| July | 391 | 117 |
| August | 518 | 127 |
| September | 444 | 136 |
| Total | 1353 | 380(28.08%) |

**Table 2 showing total imipenem resistant non-fermenters isolates and MBL producing strains among them.**

Table 2: P-value: 0.00052(p-value is <0.05 which is significant)

| Month | Imipenem Resistance Non-Fermenters Isolates | MBL producing strains among non-fermenters |
|---|---|---|
| July | 117 | 94 |
| August | 127 | 101 |
| September | 136 | 108 |
| Total | 380 | 303(79.73%) |

In table 3 imipenem resistant Pseudomonas spp. and Acinetobacter spp. are shown among Pseudomonas spp. and Acinetobacter spp. isolated

**Table 3: P-value for Pseudomonas spp.: 0.0073 (p-value is <0.05 which is significant)**

**P-value for Acinetobacter spp.: 0.0054(p-value is <0.05 which is significant)**

| Month | Pseudo-monas spp. | Imipenem Resistant Pseudo-monas spp. | Acinetobacter spp. | Imipenem Resistant Acinetobacter spp. |
|---|---|---|---|---|
| July | 268 | 69 | 123 | 48 |
| Aug | 352 | 68 | 166 | 59 |
| Sep | 300 | 71 | 144 | 65 |
| total | 920 | 208(22.60%) | 433 | 172(39.72 %) |

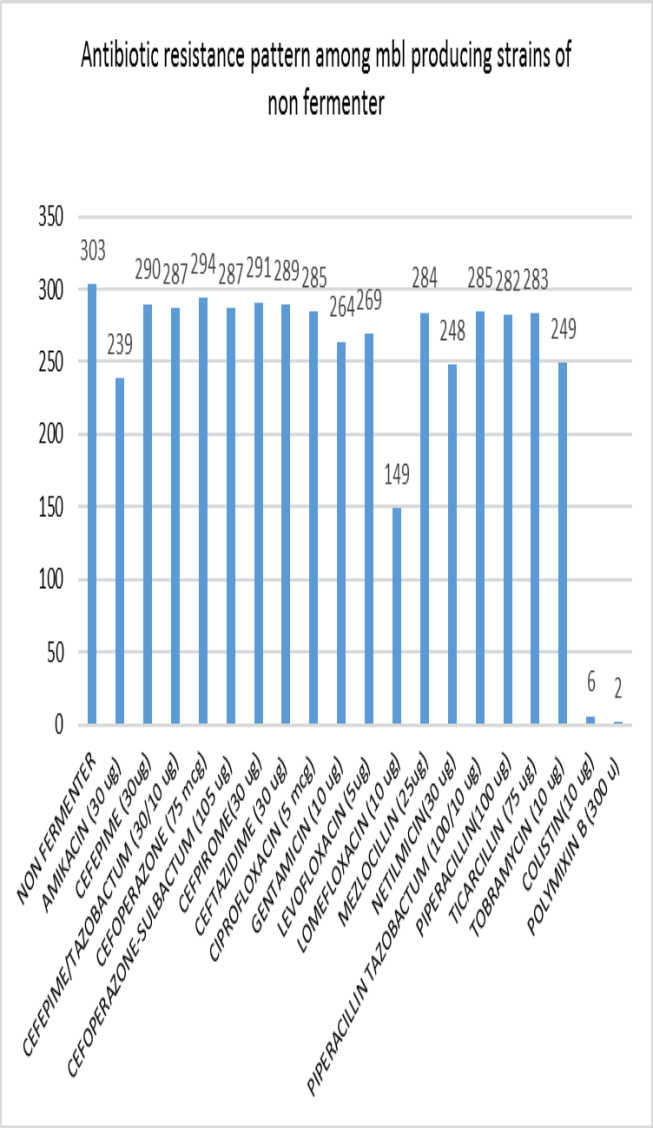
**Table 4: P-value: 0.027 (p-value is <0.05 which is significant)**

Table 4 is showing MDRO out of MBL which is very high and alarming.

| Month | Total MBL | MDRO Out Of MBL |
|---|---|---|
| July | 94 | 28 |
| August | 101 | 42 |
| September | 108 | 60 |
| Total | 303 | 130(42.90%) |

**In chart 1 resistance pattern among MBL non-fermenters showing resistance to aminoglycosides, fluoroquinolones, cephalosporin, and penicillin group is shown.**

**Chart 1:**




**Discussion:** The emergence of MBL in non-fermenter is becoming a therapeutic challenge. Only few drugs such as polymyxin-B, Colistin and tigecycline are suggested as possible effective treatment choices against carbapenem resistant isolates. Moreover, the treatment alternatives are expensive and most of the times unavailable. Therefore, rapid detection of MBL production is necessary to modify therapy & to initiate effective infection control to prevent their dissemination. Study conducted at Microbiology Department, B. J. Medical College, Ahmedabad during July-September 2016. Methods used for detecting MBL were Double disc synergy test(DDST), Imipenem-EDTA combined disc Method(CDT), MIC of imipenem.

In the present study, the use of EDTA impregnated imipenem disc resulted in a significant increase in the zone size for the MBL producers when compared to the non-producer. In our study, out of 1353 non-fermenter isolates from various clinical specimens, 920(68%) were Pseudomonas spp. and 433(32%) were Acinetobacter spp. They were identified by using standard microbiological techniques. Out of 920 Pseudomonas spp., Imipenem resistance was found in 208(22.61%) isolates. MBL detection test was performed on these 208 isolates & 120 (13.04%) were MBL positive. Out of 433 Acinetobacter spp., Imipenem resistance was found in 172 (39.72%) isolates. MBL detection test was performed on these 172 isolates & 133(30.71%) were MBL positive. MBL producing strains out of total non-fermenters is 303(22.39%). Out of total MBL producing strain isolated from non-fermenters 130(42.90%) MDRO. There are several other mechanisms also for imipenem resistance because of that all imipenem resistant isolates were not MBL positive.

Other studies conducted in Delhi by Shyamasree Nandy, Ayan Kumar Das, Mridu Dudeja shows the percentage of MBL production in Pseudomonas aeruginosa to be 19.76%. and conducted in north india by Pooja Singla, Rama Sikka, Antariksh Deep and Uma Chaudhary shows the percentage of MBL production in Acinetobacter Spp. to be 39%.

Therapeutic options remain for such isolates are very few viz. polymyxin-B and colistin for Pseudomonas and polymyxin-B, colistin and tigecycline for Acinetobacter. Even these drugs have started showing resistance in few cases which makes the problem bigger.

Recently, New Delhi metallo beta-lactamase(NDM) has emerged as a novel carbapenemase 8.NDM-1 is an enzyme that can hydrolyze and inactivate carbapenems. Escherichia coli and Klebsiella pneumoniae commonly expresses the gene for NDM-1. Besides NDM-1, there are other NDMs likeNDM-2 in Acinetobacter, NDM-4, NDM-5, NDM- 6, NDM-7 and NDM-8 from E. coli and other Enterobacteriaceae. new classes of antibiotic to handle these threats have been reported. Research is on to develop. This alarming rise of MBL and MDR among MBL isolates warrants strict measures for control of this spread. Steps needed include awareness & education of

clinicians regarding the problem. Judicious use of antibiotics will help curb the menace.

**Conclusion:** Carbapenem resistance is a major public health concern. Emergence of MBL producing Non-fermenters is alarming and reflects excessive use of carbapenems. The World Health Organization is emphasizing this and the need for new antibiotics to be developed, and for countries to take action to combat antimicrobial resistance. There is urgent requirement of strict statuary guidelines implanting intervention for limiting inappropriate uses of antibiotics. Therapeutic options for such isolates are colistin (an old and rather toxic antibiotic) and Polymyxin B for Pseudomonas spp. and colistin, Polymyxin B and Tigecycline (a newer antibiotic than can only be used in some, not all types of infection) for Acinetobacter as a last resort for the treatment of multi-resistant bacterial infection.

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