

Detection of Metallo Beta Lactamase Production in Imipenem Resistant Gram-negative non Fermenting Bacilli Isolated in a Tertiary Care Hospital in South India

Seema Solanki¹, K. Saileela², Dinesh³, Vasantha⁴, Sateesh Kumar⁵, Trinain⁶, Nilesh Khant⁷, Purti⁸

ABSTRACT

Introduction: Metallo-beta-Lactamases (MBLs) are class B β -lactamases that hydrolyze almost all clinically-available β -lactam antibiotics. The growing increase in the rates of antibiotic resistance is a major cause for concern in nonfermenting bacilli. MBLs are compromising the therapeutic efficacies of β -lactams, particularly carbapenems, which are last-resort antibiotics indicated for various multidrug-resistant bacterial infections. Acquired metallo- β -lactamases (MBL) have recently emerged as one of the most worrisome resistance mechanisms due to their ability to hydrolyze all β -lactams, including carbapenems. I have undertaken this project to ascertain the prevalence of MBL-producing nonfermenting bacilli.

Material and methods: This study was conducted for a period of 2 years (July 2012 to June 2014) at Kamineni Institute of Medical Sciences Narketpally, District Nalgonda, Hyderabad (A.P), India. Isolates included in the study were screened for imipenem resistance by conventional methods. The isolates that showed imipenem resistance were screened further for MBL production by imipenem (IMP)-ethylenediaminetetraacetic acid (EDTA) combined disc test.

Results: Of 354 gram negative non fermenting bacilli isolated (NFGNB), 48.6% accounted for multidrug resistant bacilli. 9.60% showed imipenem resistance and out of these imipenem resistant isolates, 5.81% were metallo- β -lactamases (MBL) producers. Most isolates were from the intensive care unit and from post-operative patients. Our findings shows that there are significant numbers of isolates having MBL production along with multidrug resistance. There is a urgent need for active surveillance to detect MBL producers.

Keywords: Non Fermenters Bacilli, Imipenem, Metallo- β -Lactamases (MBL), EDTA

INTRODUCTION

Though primarily regarded as contaminants or incidental organisms, gram negative non fermenting bacilli (NFGNB) are becoming increasingly important as opportunistic pathogens in immunocompromised patients.¹ They can also cause infection by gaining access to normally sterile body sites through trauma or a foreign body.

Non-fermenters are emerging with increasing frequency as agents of opportunistic and often serious infections as well as nosocomial infections.²

They are most commonly isolated from patients with serious underlying disease who had abuse of wide spectrum antimicrobials agents, surgical procedures, prolonged hospital stay, prolonged mechanical instrumentation or tracheostomy,

genitourinary instrumentation, in burns patients and low birth weight babies.³ They are also frequently isolated from cases such as septicaemia, meningitis, pneumonia, urinary tract infection and surgical wound infection.³ These infections have the potential to spread from patient to patient via fomites or hands of the medical personnel.⁴

Non-fermenting gram negative bacilli are innately resistant to many antibiotics and are known to produce Extended Spectrum Beta lactamases and Metallo Beta lactamases.⁵ Acquired Metallo- β -lactamases (MBL) have recently emerged as one of the most worrisome resistance mechanisms owing to their capacity to hydrolyze all β -lactams, including carbapenems. Metallo- β -lactamase-producing clinical isolates within institutions and their ability to participate in horizontal MBL gene transfer with other pathogens in the hospital is posing great risk. Five different types of MBLs whose prevalence are increasing rapidly are IMP, VIM, SPM, GIM and SIM. Among these, IMP and VIM are the most predominant. With the global increase in the occurrence and types of MBLs, early detection is crucial, the benefits of which include timely implementation of strict infection control practices and treatment with alternative

¹Assistant Professor, Department of Microbiology, Maharishi Markandeshwar Medical college & Hospital, Solan, H.P.,

²Professor and HOD, Department of Microbiology, Kamineni institute of Medical Science, Narketpally, Nalgonda, Hyderabad,

³Professor, Department of Microbiology, Kamineni institute of Medical Science, Narketpally, Nalgonda, Hyderabad, ⁴Professor,

Department of Microbiology, Kamineni institute of Medical Science, Narketpally, Nalgonda, Hyderabad, ⁵Assistant Professor,

Department of Microbiology, Kamineni institute of Medical Science, Narketpally, Nalgonda, Hyderabad, ⁶Assistant Professor,

Department of Microbiology, Kamineni institute of Medical Science, Narketpally, Nalgonda, Hyderabad, ⁷Assistant Professor,

Department of Microbiology, Kamineni institute of Medical Science, Narketpally, Nalgonda, Hyderabad, ⁸Assistant Professor,

Department of Microbiology, Kamineni institute of Medical Science, Narketpally, Nalgonda, Hyderabad, India

Corresponding author: Dr Seema Solanki, Assistant Professor, Department of Microbiology, Maharishi Markandeshwar Medical college & Hospital, Solan, H.P., India

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antimicrobials.[5] Molecular techniques are available to detect MBL producers. But such techniques are not available at tertiary centers. Among the simple and cheaper methods available for testing MBL production, the imipenem (IMP)-EDTA combined disc test is sensitive and specific one.

Antimicrobial treatment of the infections caused by these agents is difficult due to its multidrug resistance (MDR) and rapid selection of high level MDR to various groups of antibiotics like Beta-lactams, Aminoglycosides and Fluoroquinolones, posing problem for both treatment and infection control.⁶⁻¹⁰

Carbapenems and cephalosporin/inhibitor combinations are being used as the "last resort" in these infections since the last few years.⁸ Therefore, this study was undertaken to identify various non-fermenters from patients admitted in our hospital and to assess their antibiotic sensitivity pattern.

MATERIAL AND METHODS

The study was conducted over period of 2 years, from September 2012 to October 2015, in our hospital, which is a 1060-bedded teaching hospital. Bacterial isolates, a total of 354 gram negative non fermenting bacilli were isolated from various clinical samples of admitted patients were included in the study. All isolates were non-duplicate. The isolates were identified by conventional methods²

The standardized Kirby Bauer disc diffusion test of Clinical and Laboratory Standard Institute (CLSI) was used for antimicrobial susceptibility testing.⁹ Inoculum was prepared and turbidity was adjusted to 0.5 McFarland turbidity tube. A lawn culture was made on the surface of the Muller-Hinton agar plate using sterile cotton swabs and antimicrobial discs were applied. That 37° C for 16-18 hours.

The diameter of each zone (including the diameter of the disc) of inhibition was measured and recorded in millimeters and the result was then compared with the zone size interpretative chart.

The quality controls for antimicrobial susceptibility was done with the standard strains of *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923).

The antimicrobials discs were obtained from HiMedia Laboratory Private Limited Mumbai. The concentration of the antibiotics employed, as per CLSI guidelines, were:

Antibiotics	Disc potency	Abbreviations
Piperacillin	100 mcg	PI
Amikacin	30 mcg	AK
Gentamicin	10 mcg	GEN
Tobramycin	10 mcg	TOB
Netilmicin	30 mcg	NET
Ciprofloxacin	5 mcg	CIP
Ofloxacin	5 mcg	OF
Norfloxacin	10 mcg	NX
Co-Trimoxazole	25 mcg	COT
Ceftazidime	30 mcg	CAZ
Ceftazidime-Clavulanic acid	30/10 mcg	CAC
Piperacillin-Tazobactam	100/10 mcg	PIT
Imipenem	10 mcg	IPM
Polymyxin-B	300 units	PB

Metallo β -lactamase detection testing

Only Imipenem resistance isolates were screened for MBL production by Imipenem-EDTA combined disc test. Metallo- β -lactamase screening for MBL production was detected in imipenem-resistant isolates by phenotypic tests

Imipenem-EDTA combined disc Test

Test organism was inoculated on to Mueller-Hinton agar as lawn culture. Two 10- μ g imipenem disks (Hi-Media Laboratories, BD Diagnostics Pvt. Ltd.) were placed on the plate and appropriate amounts of 10 μ L of EDTA solution was added to one of them to obtain the desired concentration (750 μ g). Both Imipenem discs were placed at 20 mm centre to centre on the plate. Plates were then incubated for 16-18 hours in air at 35°C. In the combined disc test, enhancement of zone of inhibition of Imipenem + EDTA disc compared to that of Imipenem disc alone by ≥ 7 mm was considered positive for MBL production.

RESULTS

Among 11,040 clinical samples, NFGNB were isolated from 348 samples. A total of 354 NFGNB were isolated from 348 samples which accounted for an isolation rate of NFGNB to be 3.20%. Monomicrobial growth was seen in 266 specimens, whereas 82 specimens showed polymicrobial growth. Out of 82 specimens, 76 were both fermenters and non-fermenters but 6 samples yielded both as non-fermenters.

MDR was observed in 68.42% of *Acinetobacter* strains followed by *Pseudomonas* spp (41.33%) and *Moraxella* spp (7.69%).

6 isolates of *Pseudomonas* spp and 26 isolates of *Acinetobacter* spp were Imipenem resistant. *Stenotrophomonas* spp and *Burkholderia* spp showed inherent resistance to Imipenem. All strains of *Moraxella* spp were sensitive to Imipenem.

2 out of 93 multidrug resistant isolates of *Pseudomonas* spp and 8 out of 78 multidrug resistant *Acinetobacter* spp were Metallo beta lactamases producers.

The majority of these MBL isolates were from patients of the surgical wards followed by ICU, where the majority of critically ill patients are admitted. Surgical wards and ICU are the places where invasive procedures and indwelling devices are most commonly done. Such procedures play important role in transmission of infective agents. Most studies have used the Imipenem (IMP)-EDTA combined disc, double disc synergy test using the Imipenem (IMP)-EDTA and modified Hodge tests. According to those studies, MBL production ranged from 7 to 65%. Most of these studies reported MBL production in non-fermenters like *Pseudomonas aeruginosa* and *Acinetobacter baumannii* (fig-1). The number of MBL producers in our institute is much less than that reported from other centers. But the fact remains that even in a teaching hospital with fewer ICUs and critical care units, MBL producers have made their presence felt. As our institute does not have a molecular set-up, we were unable to confirm these findings by the genotypic method, which is a drawback in our study. Reports from various parts of the world showing emergence of MBL enzymes in gram negative bacilli is alarming, and reflects the excessive

Wards	Number of patients	Percentage
Surgical Wards	184	53
Medical Wards	66	19
ICU	98	28
Total	348	100

Table-1: Ward wise Distribution of cases (n=348)

Clinical Isolates	Number of MDR	Percentage
<i>Pseudomonas spp</i> (225)	93	41.33 (93/225)
<i>Acinetobacter spp</i> (114)	78	68.42 (78/114)
<i>Moraxella spp</i> (13)	1	7.69 (1/13)
<i>Burkholderia</i> (1)	0	0 (0/1)
<i>Stenotrophomonas</i> (1)	0	0 (0/1)
Total (354)	172	48.6 (172/354)

*Acquired non-susceptibility to atleast 1 drug in ≥ 3 categories

Table-2: Multi Drug Resistant * NFGNB isolates (n = 354)

Clinical Isolates	Number	Percentage
<i>Pseudomonas spp</i> (225)	6	2.66 (6/225)
<i>Acinetobacter spp</i> (114)	26	22.8 (26/114)
<i>Moraxella spp</i> (13)	0	0
<i>Burkholderia</i> (1)	1	100 (1/1)
<i>Stenotrophomonas</i> (1)	1	100 (1/1)
Total (354)	32	9.03 (32/354)

Table-3: Imipenem Resistant NFGNB isolates (n=354)

Clinical MDR Isolates (n)	MBL in MDRs	MBL producers n (%)
<i>Pseudomonas spp</i> (93)	2	2.15 (2/93)
<i>Acinetobacter spp</i> (78)	8	10.25 (8/78)
<i>Moraxella spp</i> (1)	0	0
<i>Burkholderia</i> (0)	0	0
<i>Stenotrophomonas</i> (0)	0	0
Total (172)	10	5.81(10/172)

Table-4: MBL producers in MDR isolates

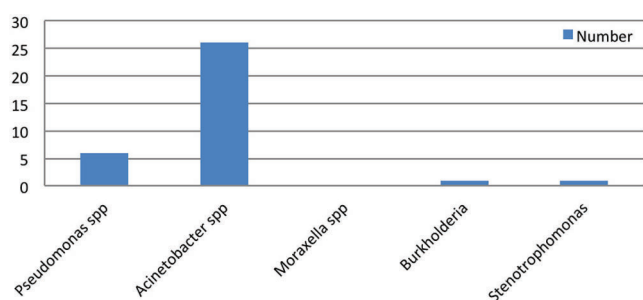


Figure-1: Imipenem Resistant NFGNB isolates

use of carbapenems. Therefore, early detection and prompt instillation of infection control measures is important to prevent further spread of MBLs to other gram-negative bacilli. Additionally, it is also important to follow antibiotic restriction policies to avoid excessive use of carbapenems and other broad-spectrum antibiotics.

DISCUSSION

Our study showed 48.6% of MDR NFGNB isolates which was in concordance to the Amutha R¹⁶ study showing 45.2%

of MDR isolates but Mathai AS et al¹⁷ study showed higher MDR isolates of 70%.

This can be explained on the basis as their study was done on the ICU patients who were mostly on ventilators and had more chances of hospital acquired infection with multidrug resistant strains. Imipenem showed maximum sensitivity in both *Pseudomonas* and *Acinetobacter* spp.^{10,11} This was in concordance to other studies as shown in the given table:

In our study an overall Imipenem resistance among NFGNB was 9.46% and this corroborates well with the study by Gladstone P et al¹⁴ and Nautiyal S et al²¹ who had 12.2% and 11.6% respectively.

Previous studies by other authors have also reported Imipenem resistance among NFGNB. In the present study, 2.66% of *Pseudomonas* species and 23% of *Acinetobacter* species were Imipenem resistant and this is in contrast to the findings of Gladstone P et al¹⁴ who have reported the same to be 42.8% and 14.2% respectively. This can be explained on the basis that Gladstone study was done on ICU admitted patients, who were more exposed to the hospital acquired infection especially with the multidrug resistant strains.

We believe that documenting resistance among NFGNB is very important especially the Carbapenem resistance, as these strains may often cause outbreaks in the ICU setting and can limit therapeutic option due to the high degree of multi drug resistance.²⁴ These organisms can also spread resistance to other susceptible bacteria by horizontal gene transfer.^{12,13}

In our study, the Imipenem resistant isolates also show resistance to other groups of antibiotics, which is a unique problem with MBLs that show a broad-spectrum resistance profile. The genes encoding MBLs are often procured by class 1 (sometimes class 3) integrons.^{22,23} Other gene cassettes within the integrons confer resistance to other antibiotics such as Fluoroquinolones, Aminoglycosides and Co-trimoxazole. Integrons are, in turn, embedded in Transposons, resulting in a highly transmissible genetic apparatus that can be transferred between bacteria.^{15,20}

In the present study 5.81% of NFGNB were MBL producers, which are on a lower side as compared to Agarwal et al¹⁸ study who had reported 8.50% of MBL producers Jayanthi S et al²⁰ had reported 31% of MBL producers respectively which are on higher side as compared to our study.

Metallo beta lactamase production in our study was lower as compared to other studies. This may be due to the following reasons-

- Other studies were done in higher tertiary care centers (urban centers), whereas our study was done in a rural medical college hospital.
- Sample size and duration in other studies done were of smaller sample size and done over a short duration of time

The screening of the MBL production in our study was done by the Imipenem-EDTA combined disk method which was cost effective and comfortable method especially in a tertiary set up.^{18,19}

CONCLUSION

Non-fermenting Gram-negative bacilli isolated from various clinical specimens should not be overlooked and should be identified by using standard methods. There is an alarmingly increase in emergence of Metallo beta lactamase in non-fermenters which are common etiological agents in nosocomial infections worldwide. There is also high rate of resistance to many antimicrobials by the non-fermenter Gram-negative bacilli especially carbapenems which are used in treatment of MDR infections.

Regular detection and monitoring of Carbapenamase production by NFGNB in hospitals is very much needed to prevent the nosocomial spread of these drug resistant strains in the hospital.

Double disc synergy test is useful for detection of MBL producers among NFGNB. It can be established in our routine clinical microbiology laboratories, for the MBL recognition especially in imipenem resistant isolates due to its cost efficiency.

Finally, clinicians should apply logic in selection and use of drugs in treating severely ill patients. Working in close liaison with a microbiologist will greatly facilitate the decision-making for selection of right antibiotics.

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