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Original Research Article

Retrospective study of incidence of metallo- β -lactamase-producing Non-fermenters in nosocomial infections of a tertiary care hospital, in Navi Mumbai

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ABSTRACT

Background: The growing increase in the rate of antibiotic resistance is a major cause for concern in both non-fermenting bacilli. β -lactams have been the mainstay of treatment for serious infections, and the most active of these are the carbapenems. 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. We have undertaken this investigation to ascertain the incidence of MBL-producing non-fermenting bacilli, like Pseudomonas Spp. and Acinetobacter Spp.

Aim: To determine the incidence of MBL in Nosocomial infections of non-fermenters and their clinical correlation.

Objectives : Screening of nosocomial isolates for multidrug resistance by antibiotic sensitivity tests as per CLSI guidelines as a reference.

To perform phenotypic confirmatory test of potential MBL isolates by double disc synergy test

Clinical correlation with detection of these enzymes and the patients morbidity/mortality correlation with age, immunocompromised status and hospital stay.

Materials and Methods : The study was conducted over a period of 12 months in a teaching hospital, in Navi Mumbai. Isolates included in the study were screened for imipenem resistance by conventional methods. The isolates that showed imipenem resistance were tested for MBL production by imipenem (IMP)-ethylene-diamine-tetra-acetic acid (EDTA) combined disc test. Imipenem-resistant non-MBL isolates also were tested for Modified Hodge test and AmpC β -lactamases production to detect other mechanisms of carbapenem resistance.

Results: Total number of isolates tested were 107. Out of 107, 29 (27.1%) were MBL producers and 78(72.9%) were non- MBL producers. Among both types of non-fermenters , 66(61.68%) were Pseudomonas aeruginosa and 41(32.23%) were of Acinetobacters. In the sex distribution, 65.5% were males and 34.5% were females. 18.18% were MBL positive Pseudomonas and among Acinetobacter species 41.46% were MBL positive. Mean age of MBL producers was 34 years. Mean hospital stay was 28 days. Mortality rate was 51.7% in MBL producers. Mortality was higher in Acinetobacter MBL producers when compared to Pseudomonas MBL producers.

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1. Introduction

The increase in the rates of antibiotic resistance is a major cause for concern in both non-fermenting bacilli . β -lactams have been the mainstay of treatment for serious

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infections. Most active of these are the carbapenems, which are advocated for use for the treatment of infections caused by extended-spectrum- β -lactamase (ESBL)-producing Enterobacteriaceae, particularly *Escherichia coli* and *Klebsiella pneumoniae*, and non-fermenters. Among non-fermenters, *Pseudomonas* spp. and *Acinetobacter* spp. are commonly isolated.

Five different types of MBLs whose prevalence are increasing rapidly¹ are IMP (Imipenemase), VIM (Verona Integron-encoded metallo-beta lactamase; Meyrol, SPM-1 (Sao Paulo Metallo-Beta-lactamase, GIM (German imipenemase; and AIM, Australian Imipenemase; Among these, IMP and VIM (Verona Integron-encoded Metallo Beta lactamase 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 antimicrobials. Among the simple and economic methods available for testing MBL production, the imipenem (IMP)-EDTA combined disc test is sensitive and specific. According to (IMP) 10 μ g-EDTA 750 μ g combined disc test has 95.7% sensitivity and 91.0% specificity for detection of metallo-beta-lactamase in MBL-producing *Pseudomonas* spp and *Acinetobacter* spp.

Acinetobacter baumannii is a typical nosocomial pathogen causing infections and a high mortality often among patients hospitalized in the Intensive care unit.²

Pseudomonas aeruginosa has been described as the most common and serious cause of infection.³

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. Such strains are not susceptible to therapeutic serine β -lactamase inhibitors (such as clavulanate and sulfones). Moreover, their genes are carried on highly mobile elements, allowing easy dissemination. Invasive infections with MBL-producing isolates are also associated with a higher morbidity and mortality.⁴ The occurrence of an MBL-positive isolate in a hospital environment poses not only a therapeutic problem but is also a serious concern for infection control management. *Pseudomonas aeruginosa* and *Acinetobacter baumannii* are among the most important causes of healthcare-associated infections.⁵ As a result of being difficult to detect, such organisms pose significant risks, particularly due to their role in unnoticed spread within institutions and their ability to participate in horizontal MBL gene transfer with other pathogens in the hospital. In recent years, MBL genes have spread from *P. aeruginosa*, *Acinetobacter* Spp. to the members of the Enterobacteriaceae.

Our hospital, a tertiary care centre in Navi Mumbai Maharashtra, has a high prevalence of infections due to non-fermenting bacilli and Enterobacteriaceae. Carbapenems

and cephalosporin inhibitor combinations are being used as the “last resort” in these infections since the last few years. This research investigation is undertaken to ascertain the prevalence of MBL-producing non-fermenting bacilli in our hospital. Although a global increase in the prevalence of MBL-producing non-fermenting bacilli has been reported limited data is available from NaviMumbai.

2. Materials and Methods

The study was conducted over period of 1 year in our hospital, which is a 1250 bedded teaching hospital with ICUs, trauma centre and one neonatal intensive care unit (NICU). Clinical samples like pus, blood, urine, body fluids, tissues and respiratory secretions from patients admitted in medical, surgical intensive care unit were collected using aseptic precautions. All samples were processed using standard Microbiological procedures.

2.1. Metallo- β -lactamase screening

MBL production was detected in imipenem-resistant isolates by phenotypic tests. The Imipenem (IMP)-EDTA combined disc test was used.⁵

The Imipenem (IMP)-EDTA combined disk test was performed as described by *Pseudomonas aeruginosa* ATCC 27853 used as the control. Test organisms were inoculated on to plates of Mueller-Hinton agar as recommended by the CLSI. 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). The inhibition zones of the imipenem and Imipenem(IMP)-EDTA disks were compared after 16–18 h of incubation in air at 35°C. In the combined disc test, if the increase in inhibition zone with the imipenem and EDTA disc was ≥ 7 mm than the imipenem disc alone, it was considered as MBL positive.

2.2. Antimicrobial susceptibility

Antimicrobial susceptibility of all the isolates was performed by the Kirby-Bauer disc diffusion method according to the CLSI guidelines.⁶

Antibiotics used are amikacin (30mcg), gentamicin (10mcg), netilmicin (30mcg), cefoperazone (75mcg), cefipime (30mcg) ceftazidime (30mcg) ceftroxime (30mcg) ciprofloxacin (5mcg), lomefloxacin (10mcg)

3. Results

3.1. Bacterial isolates

A total of 107 gram negative non-fermenting bacteria were isolated from various clinical samples of admitted patients were included in the study. Clinical samples were all clinical branches and also from patients with co morbid conditions

like burns, cancer and diabetes.

All isolates were non-duplicate. The isolates were identified by conventional methods. Out of 107 samples, 51 were from pus, 18 from tracheal aspirates, 22 from urine, 8 from sputum and 10 from blood culture.

Pseudomonas aeruginosa was isolated from 66 (61.8%) samples and 41 (38.3%) are from *Acinetobacter* species. 19 (65.5%) males and 10 (34.5%) females were MBL producers, showing a male preponderance. 12 out of 66 (18.18%) were MBL producing *Pseudomonas aeruginosa*. and 17 out of 41 (41.46%) were *Acinetobacter* species. Mean age of MBL producers were 34 years

Mean patients age of MBL producers - 34 years.

3.2. Antibiotic sensitivity of MBL positive *Pseudomonas aeruginosa*

Colistin (100%), Gatifloxacin (50%), Piperacillin-tazobactam (33.3%), Chloramphenicol (33.3%) and Netilmicin (16.66%)

3.3. Antibiotic sensitivity of MBL positive *Acinetobacter spp*

Colistin (70.6%), Gatifloxacin (17.3%) and piperacillin-tazobactam (11.6%).

All 29 MBL isolates tested positive by the combined disc (Imipenem + EDTA) method

Out of 29 MBL isolates screened positive by the combined disc method, 4 (13.7%) were non-determinable by the E-test MBL

Out of the remaining 25 (86.2%) that tested positive by the E-test MBL, 6 showed deformation of the ellipse.

Mean hospital stay was 28 days in the MBL producers.

Infection associated with hospital related devices was 17 out of the 29 (58.6%) MBL producers. Of these 17, 12 (70.5%) were *Acinetobacter spp*. 5 (29.5%) were *Pseudomonas aeruginosa*.

Mortality was seen only in MBL producers, 15 (51.7%) out of 29 succumbed to death.

Mortality is higher in *Acinetobacter* MBL producers, 12 out of 17 (70.5%) as compared to *Pseudomonas aeruginosa* 3 out of 12 (20.5%).

Highest mortality is seen in infections due to hospital related devices, 6 out of 15 (40%), followed by multi-organ damage 4 out of 15 (26.4%), septicemia 3 out of 15 (20%) and renal failure 2 out of 15 (13.3%).

Table 1: Total number of isolates:

MBL	Non-MBL	Total
29	78	107
27.1	72.89	100%

Parameter	Value
F(2, 3)	221.6931
p-value	0.0006

Table 2: Sex wise distribution

Sex	MBL	Non MBL
Male	19	41
Female	10	35

Table 3: Patients Mortality rate and bacteria isolated

	<i>Pseudomonas</i>	<i>Acinetobacter</i>	n
n	66	41	107
Mortality	3	12	15
Percentage	2.8%	11.21%	14.01%

Table 4: Clinical reason causing mortality in MBL producers

Device related	Multi organ Damage	Renal failure	Septicemia
6	4	2	3
5.6%	3.7%	1.86%	2.8%

Table 5: Age wise distribution

Age	12-5	28-43	43-59	60-74	Above 75
	27				
MBL	2	15	3	5	4
Non MBL	5	20	2	17	24

Table 6: Other Co-morbid conditions of the MBL isolates

Burns	Cancer	Diabetes	Infections associated with Hospital Devices
2	2	8	17
1.86	1.86	7.47	15.88

4. Discussion

MBLs have been found in number of bacteria including *Pseudomonas aeruginosa* and *Acinetobacter* species. And there is no CLSI recommended methodology for their detection though simple techniques like combined disc test are available. Molecular typing (genotypic detection)⁷ would help in knowing that which alleles of the genes of MBLs are prevalent in different parts of the world. The diagnostic laboratories worldwide need in-house standardized phenotypic tests for their detection. Multi-drug resistant or pan drug resistant strains are increasingly being found in hospital settings. MBL producing *Pseudomonas aeruginosa* are responsible for nosocomial outbreaks across the world.⁸ Treatment options for these organisms are very limited which needs urgent attention by pharmaceutical companies to find new molecules.

Acinetobacter baumannii is an important cause of healthcare associated infections and are difficult to treat.

they possess antimicrobial resistance.⁹ Acquired metallo beta lactamases have recently emerged as one of the most worrisome resistant mechanisms.¹⁰ Acinetobacter Spp play a significant role in the colonization and infection of patients admitted in the hospitals.¹¹

The mainstay of curbing this dreaded menace would be strict infection control practices and restricted use of antibiotics. As in critical care units worldwide, the dictum is hit high, hit hard so start off a patient in ICU with imipenem or Meropenem. But a culture profile with an antibiotic sensitivity must be sent immediately and de-escalate the antibiotic according to the report. Cost effective technique is necessary to prevent their dissemination as well as formulation and regulation of antimicrobial stewardship policy.¹² All Microbiologists, infectious disease specialists and who ever involved in infection control committee of the every hospital should undergo such antimicrobial stewardship program to gain updated knowledge to curb such menaces. A. baumannii has been enlisted as one of the top priority pathogens by World Health Organization in 2017.¹³

We have used the combined disc method to be very sensitive for routine detection of MBL's in the laboratory. It is very easy, economical and reliable. The E-test MBL on the other hand is specifically designed to detect as many clinically significant MBL as possible. The Etest MBL was reliable for detecting the IMP-1- and VIM-2-producing Pseudomonas and Acinetobacter isolates.¹⁴ However given the cost constrains of E-test, the combined disc method is a better option for routine testing in the clinical microbiology laboratories which are incorporated in many of the laboratories.

Hospital infection control committee and antibiotic policy¹⁵ makers at hospital level, District, state, national¹⁶ and international level has to come together and device a concrete policy to treat patients as per the policy to minimize the mortality. In India we should have our own country specific policy and everyone has to follow this without any compliance.

5. Conclusion

Our findings show that there are significant numbers of isolates showing MBL production along with MDR. There is a need for active surveillance to detect MBL producers. The Imipenem(IMP)-EDTA combined disc test is a simple test that can be used in district health laboratories or where molecular diagnostic techniques are not available to detect these types of resistance. There should be judicious use of carbapenems to prevent their spread and use of effective antibiotics as per the antibiotic-sensitivity report.

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7. Conflict of Interest

The authors declare they have no conflict of interest.

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