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Original Research Article

The Prevalence of Metallo-β-lactamase (MBL) in Gram Negative Bacilli and their Antimicrobial Susceptibility Pattern at Tertiary Care hospital, Vadodara

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ABSTRACT

Background: Antimicrobial resistance is the major threat due to broad spectrum antimicrobials are being used in community practice. The Metallo-β-lactamases (MBLs) in gram negative bacteria have emerged as a major cause of health care associated infections. They hydrolyse all beta-lactam antibiotics including extended spectrum cephalosporins and carbapenems at the same time it is not inhibited by serine beta-lactamase inhibitors like clavulanic acid, sulbactam, and tazobactam and are resistant to many antibiotics. The present study is aimed to determine the prevalence of Metallo-β-lactamase (MBL) in gram negative bacilli and their antimicrobial susceptibility pattern at tertiary care hospital, Vadodara.

Material and methods: Total 1350 Clinical specimens were included in the study. The isolates were identified as per standard microbiological procedures od the laboratory such as staining, colony morphology & biochemical reactions. All the isolates were subjected to antibiogram study for the antibiotics plus a phenotypic screening test for MBL was done by disc diffusion test using single Imipenem disc by modified Kirby Bauer disk diffusion method. 117 Imipenem resistant isolates were tested for MBL by Imipenem EDTA combined disc synergy test as a confirmatory test.

Results: The prevalence of MBL producing isolates were 6.39%. Maximum number of MBL producers were isolated from the Medicine ward (19, 40.42%) and ICU (11, 23.40%). Maximum number of Specimens containing MBL producers were Pus and Swab (19, 40.42%) followed by Sputum (13, 27.65%). The most common bacterial isolates were Escherichia coli (311) followed by Klebsiella spp. (224) and Pseudomonas aeruginosa (97). The prevalence of MBL production were more common in Pseudomonas spp. (20 out of 97, 20.61%) followed by Acinetobacter spp. (3 out of 19, 15.78%). Polymyxin B and Colistin were the most effective drugs against MBL producers.

Conclusion: Prevalence of MBL in our study is 6.39%, which are multidrug resistance though Polymyxin–B and Colistin is still effective treatment option. So there is a need to do surveillance to detect MBL producers, judiciously use Carbapenems along with Antimicrobial Stewardship to prevent their spread.

Keywords: MBL, Gram Negative Bacilli, Antimicrobial resistance

INTRODUCTION

Antibiotics are natural substances secreted by bacteria and fungi to kill other bacteria that are competing for limited nutrients. The antibiotics used to treat people today are typically derivatives of these natural products. Several mechanisms are there in the bacteria which confer them with antibiotic resistance. These mechanisms can chemically modify the antibiotic, render it inactive through physical removal from the cell, or modify target site so that it is not recognized by the antibiotic.

The most common mode is enzymatic inactivation of the antibiotic. An existing cellular enzyme is modified to react with the antibiotic in such a way that it no longer affects the microorganism. An alternative strategy utilized by many bacteria is the alteration of the antibiotic target site. The development of resistance is inevitable following the introduction of a new antibiotic. Antibiotic resistance in bacteria may be an inherent trait of the organism that renders it naturally resistant, or it may be acquired by means of mutation in its own DNA or acquisition of resistance-conferring DNA from another source.

The increase in the rates of antibiotic resistance is a major cause for concern in infections caused by gram negative bacilli. Carbapenems, are used for the treatment of serious infections caused by Extended Spectrum-β-lactamase (ESBL) producing gram negative bacilli particularly for the members of family Enterobacteriaceae and non-fermenters, like Pseudomonas spp. and Acinetobacter spp. [1]

MBL: Based on molecular studies, two types of carbapenem hydrolysing enzymes have been described: serine enzymes possessing a serine moiety at the active site, and Metallo-β -lactamases (MBLs), requiring divalent cations, usually zinc, as metal cofactors for enzyme activity.[2,3] This class of β-lactamase is characterized by the ability to hydrolyze carbapenems and by its resistance to the commercially available β-lactamase inhibitors but susceptibility to inhibition by metal ion chelators. The substrate spectrum is quite broad; in addition to the carbapenems, most of these enzymes hydrolyze cephalosporins and penicillins but lack the ability to hydrolyze aztreonam. The mechanism of hydrolysis is dependent on interaction of the β-lactams with zinc ions in the active site of the enzyme, resulting in the distinctive trait of their inhibition by EDTA, a chelator of Zn+2 and other divalent cations 6. MBLs, like all β-lactamases, can be divided into those that are normally chromosomally mediated and those that are encoded by transferable genes, and are often located in integrons as gene cassettes and these genes are carried on highly mobile elements, which help in easy dissemination. Transmissible MBLs were first described in Pseudomonas aeruginosa in Asia in the 1980s. [4]

In las few years, MBL genes have been spread from Ps. aeruginosa to members of the family Enterobacteriaceae. Infections with MBL producing isolates are associated with a high morbidity and mortality.[5] The presence of an MBL positive isolate in a hospital environment is a therapeutic problem as well as a serious concern for infection control management. Treatment of these infections is worrisome as the carbapenems are often agents of the last resort for resistant Gram negative infections.[6,7] Techniques available to detect MBL producers are Molecular and the simple and cheaper one is the imipenem (IMP)-EDTA combined disc test.[8]

MATERIAL AND METHODS

The present study was conducted at tertiary care hospital, Vadodara from January 2024 to August 2024. Total 1350 Clinical specimens were included in the study. 735 gram negative bacilli isolated from the 1350 clinical specimens. The isolates were identified as per laboratory standard protocol like staining, colony morphology & biochemical reactions. All the clinical specimens were inoculated on Nutrient agar, Blood agar and McConkey's agar. All the inoculated plates were incubated aerobically at 37°C for 24 hours. On the next day, identified the isolates by performing Gram staining and colony characteristics. Isolated colonies were processed for various biochemical tests including Motility test, Pigmentation, Oxidase test, Indole test, MR test, VP test, Citrate test, TSI test, PPA test and Sugar fermentation tests. All the isolates were subjected to antibiogram study for the antibiotics plus

a phenotypic screening test for MBL was done by disc diffusion test using single Imipenem disc by modified Kirby Bauer disk diffusion method. If the zone diameter is ≤ 19mm, considered resistant. Isolates resistant to Imipenem (10 µg) were considered screening positive. A phenotypic confirmatory test for MBL was done by Imipenem EDTA combined disc synergy test. 57 Imipenem resistant isolates were tested for MBL.

The test organisms were inoculated on to plates with Mueller Hinton agar as recommended by the CLSI. A 0.5 McFarland standard suspension of the test organism was prepared and lawn culture was done on Muller Hinton Agar (MHA) plate and Imipenem (10 µg) and Imipenem EDTA (10 µg/500 µg) discs were placed at a distance of 30 mm apart. After that the plates were incubated at 37°C for 18 – 24 hours. In the combined disc test, if the zone of inhibition of Imipenem EDTA disc was ≥ 7 mm than that of Imipenem alone, it was considered MBL Positive.[9] Escherichia coli ATCC 25922 and P. aeruginosa ATCC 27853 were used as control strains.

RESULTS

Total 1350 clinical specimens were received from January 2024 to August 2024, out of 440 clinical specimens, 735 did shown growth of different gram negative bacilli. Out of 735 gram negative bacilli, 117 gram negative bacilli were Imipenem resistant. From 117 Imipenem resistant gram negative bacilli only 47 isolates were MBL producers, so the prevalence rate of MBL in our study is 6.39%.

Maximum number of MBL producers isolated from IPD (44, 93.61%) than OPD (3, 6.38%) (Table 1). Maximum number of specimens were received from Medicine ward (557) followed by ICU (269), Orthopedic ward (241) and Surgery ward (236) and maximum number of gram negative bacilli were isolated from medicine (271) followed by ICU (175), Surgery (132) and orthopedic (128) (Table 2).

Maximum number of MBL producers were isolated from the Medicine ward (19, 40.42%), ICU (11, 23.40%), Surgery ward (8, 17.02%), Orthopedic ward (6, 12.76%) and ENT ward (3, 6.38%) (Table 3). Maximum number of Specimens containing MBL producers were Pus and Swab (19, 40.42%) followed by Sputum (13, 27.65%), Urine (10, 21.27%), Blood (3, 6.38%) and Body fluids (2, 4.25%) (Table 4). The most common bacterial isolates were Escherichia coli (311) followed by Klebsiella spp. (224), Pseudomonas aeruginosa (97), Proteus spp. (73), Acinetobacter spp. (19) and Citrobacter (11). The prevalence of MBL production were more common in Pseudomonas spp. (20 out of 97, 20.61%) followed by Acinetobacter spp. (3 out of 19, 15.78%), Proteus spp. (4 out of 73, 5.47%), Klebsiella spp. (9 out of 224, 4.01%0 and E. coli (11 out of 311, 3.53%) (Table 5).

Polymyxin B and Colistin were the most effective drugs against MBL producers. Both were 100% sensitive to all MBL producers. (Table 6).

Table-1: OPD & IPD wise distribution of MBL

OPD	3 (6.38%)
IPD	44 (93.617%)

Table-2: Ward wise distribution of Specimens and Gram negative bacilli

Ward	No of Specimens	No of Isolates	No of Imipenem Resistant
Medicine	557	271	46
ICU	269	175	22
Surgery	236	132	17
Orthopedic	241	128	20
ENT	47	29	12
Total	1350	735	117

Table-3: Ward wise distribution of MBL

Ward	No of MBL	% of MBL out of Total No of Isolates	% of MBL of total No of MBL Producers
Medicine	19	7.01%	40.42%
ICU	11	6.28%	23.40%
Surgery	8	6.06%	17.02%
Orthopedic	6	4.68%	12.76%
ENT	3	10.34%	6.38%
Total	47	6.39%	100%

Table-4: Specimen wise distribution of MBL

Specimen	Number	%
Pus and Swab	19	40.42
Sputum	13	27.65
Urine	10	21.27
Blood	3	6.38
Body fluid	2	4.25
Total	47	100

Table-5: Prevalence of MBL in different bacteria

Bacteria	Number of Isolates	Imipenem Resistant Isolates	Number of MBL Producers	Prevalence of MBL out of Total No of Isolates
Pseudomonas aeruginosa	97	28	20	20.61%
E. coli	311	33	11	3.53%
Klebsiella spp.	224	21	9	4.01%
Proteus ssp.	73	9	4	5.47%
Acinetobacter spp.	19	8	3	15.78%
Citrobacter spp.	11	0	0	0%
Total	735	117	47	6.39

Table-6: Antibiotic Sensitivity Pattern of MBL

Antibiotic	Pseudomonas (n=20)	E. coli (n=11)	Klebsiella spp. (n=9)	Proteus ssp. (n=4)	Acinetobacter spp. (n=3)
Amoxicillin Clavulanic acid	0	0	0	0	0
Gentamycin	3	4	2	1	1
Ciprofloxacin	0	0	0	0	0
Amikacin	4	2	1	2	1
Piperacillin Tazobactam	2	2	1	3	1
Co-trimoxazole	0	0	0	0	0
Polymyxin B	20	11	9	4	3
Colistin	20	11	9	4	3
Imipenem	0	0	0	0	0

DISCUSSION

In the present study the prevalence rate of MBL producing bacteria was 6.39%. Prevalence rate of MBL were found 0.22% in Korea, 0.5% in Japan, 6.5% in Italy and 19.67% in South America.[10] The data suggest that there is a wide variation in the occurrence of MBL producing bacteria throughout the world. Probable reason may be the studies were carried out in different areas, age groups, clinical samples and clinical setups with different methods. Other studies in India reported the prevalence rate of MBL producing bacteria ranging from 7.48% - 43.6%. (Table 7) The highest prevalence rate of MBL producing bacteria in the study of Shah S et al [11] (43.6%) followed by Vamki KS et al [12] (19.8%), Mahendra Prasad Shrestha et al [13] (18.79%), A Radhika et al [14] (15%), Bakshi R et al [15] (10.8%), Madhavi RB et al [16] (10.6%) and Pathak P et al [17] (7.48%). The maximum number of MBL isolates were found from the Medicine ward (19) followed by ICU (11), Surgery (8), Orthopedic (6) and ENT (3). The most common bacterial isolate from various clinical specimens was Pseudomonas aeruginosa (20) similar to study of Bakshi R et al [15] and Shah S et al. [11].

In present study, MBL producing isolates were most sensitive to Polymyxin B (100%) and Colistin (100%), while showed very low sensitivity to Gentamycin, Amikacin and Piperacillin Tazobactam. Similar findings can be seen in the study of Bakshi R et al [15], Rastogi M et al [18] and A Radhika et al [14]. While Colistin showed some resistant in the study of Vamki KS et al. [12] (3.68%). Amoxicillin Clavulanic acid, Ciprofloxacin and Co – trimoxazole showed 100% resistance. Similar findings can be seen in the study of Bakshi R et al [15] except Ciprofloxacin.

Table-7: Prevalence of MBL in different studies

Author	Prevalence
Bakshi R et al [15] (2018)	10.8%
Mahendra Prasad Shrestha et al [13] (2021)	18.79%
Madhavi RB et al [16] (2023)	10.6%
Pathak P et al [17] (2017)	7.48%
A Radhika et al [14] (2022)	15%
Shah S et al. [11] (2019)	43.6%
Vamki KS et al. [12] (2021)	19.8%
Present Study	6.39%

CONCLUSIONS

The worldwide epidemic of antibiotic resistance is touching all patients and medical practitioners. It is an ecological disaster of unknown consequence and, unlike global warming, has no obvious solution. The prevalence of MBL isolates in our study is low as compared to other study, but all are multidrug resistance though Polymyxin–B and Colistin are still effective treatment option. So, there is a need to do surveillance to detect MBL producers, judiciously use carbapenems to prevent their spread. The high degree of bacterial resistance to common antibiotic in the community indicates a grave situation that needs to be tackled urgently by Complete eradication of infectious agents before affected patients are discharged and proper hand hygiene of patient as well as health care workers. Nosocomial infection with multi drug resistant bugs should be prevented and controlled by Continuous surveillance of hospital, especially ICUs, OT's and wards housing high risk patients and Antimicrobial Stewardship Policy.

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