OPEN ACCESS
Saudi Journal of Pathology and Microbiology
Abbreviated Key Title: Saudi J Pathol Microbiol
ISSN 2518-3362 (Print) |ISSN 2518-3370 (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: https://saudijournals.com

Original Research Article

"Dissemination of Class 1 Integron among Different MBL Producing Acinetobacter Baumannii in ICU of DMCH, Dhaka, Bangladesh"

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DOI: 10.36348/sjpm.2021.v06i10.005 | **Received:** 29.08.2021 | **Accepted:** 04.10.2021 | **Published:** 09.10.2021

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Abstract

Introduction: Infection with the metallo-beta-lactamase (MBL) producing organisms is associated with higher rates of mortality, morbidity, and health care costs. MBL producing *Pseudomonas aeruginosa* was first reported in Japan in 1991 and since then has been found in various parts of the world. ***Objective:*** To Assess the Dissemination of Class 1 integron among different MBL producing Acinetobacter baumannii In ICU of DMCH, Dhaka, Bangladesh. ***Methodology:*** This Cross sectional study was conducted between July 2013 to June 2014 in the department of Microbiology, Dhaka Medical College (DMC), Dhaka, Bangladesh to determine the frequency and susceptibility patterns of MBL-producers among carbapenem-resistant Gram-negative rods (GNRs) from clinical isolates of a tertiary care hospital. All clinical samples were processed according to standard microbiological methods. Isolated GNRs were subjected to susceptibility testing against various antibiotics by disc diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. Carbapenem-resistant isolates were subjected to the detection of MBL production by the E-test MBL strip method. Hospitalized patients who did not give consent were excluded in this study. Data regarding age, sex, duration of hospitalization, antibiotic history were collected using a prescribed data collection form. ***Results:*** Out of 22 imipenem resistant Acinetobacter baumannii. 21 (95.45%) were positive for MBL production. The distribution of MBL genes among imipenem resistant Acinetobacter baumannii. Out of 22 imipenem resistant strains, 21 (95.45%) were positive for $bla_{NDM-1,}$ 20 (90.91%) for $bla_{NDM-like,}$ 16 (72.72%) for bla_{VIM} and all the 22 (100%) were negative for bla_{IMP} and distribution of class I integron among the MBL producers. Among the 21 MBL producing Acinetobacter baumannii, 17 (80.95%) had class I integron. This study presence of class I integron and conserved segment of class 1 integron among the different MBL producing Acinetobacter baumannii. Among 16 bla_{VIM} positive Acinetobacter baumannii. 13 (81.25%) carried class 1 integron and 11 (68.75%) carried conserved segment of class I integron. Out of 21 bla_{NDM-1} positive strains. 17 (80.95%) carried class I integron and 13 (61.9%) carried conserved segment of class 1 integron. Out of 20 $bla_{NDM-like}$ positive strains, 16 (80%) carried class 1 integron and 12 (60%) carried conserved segment of class I integron. ***Conclusion:*** The findings strongly suggest that there is a need to track the detection of MBL producers and that judicious use of imipenem is necessary to prevent the further spread of these organisms.

Keywords: Imipenem resistant, Class 1 integron, Acinetobacter baumannii, MBL producers.

I. INTRODUCTION

Infection with the metallo-beta-lactamase (MBL) producing organisms is associated with higher rates of mortality, morbidity, and health care costs. MBL producing *Pseudomonas aeruginosa* was first reported in Japan in 1991 and since then has been found in various parts of the world including Asia, Europe, Australia, South America, and North America [1-8]. The introduction of imipenem into clinical practice marked a great advance for the treatment of serious bacterial infections caused by beta-lactam-resistant bacteria [9]. Resistance to extended-spectrum beta-lactams has been frequently observed in non-fermenting bacilli such as *Pseudomonas aeruginosa* and *Acinetobacter* spp. The prevalence of imipenem resistant MBL producers ranges from 43% to 81.4% for Pseudomonas species and 63.2% for Acinetobacter species in Bangladesh [10]. About 3.5% NDM-1

producers are reported from Bangladesh among E. coli, K.pneumoniae, Acinetobacter baumannii, Providencia rettgeri and Citrobacter freundii [11]. About 22% of the imipenem resistant Acinetobacter baumannii arebla$_{NDM}$.1 positive (Farzana, 2012). The presence of NDM-1 gene in Bangladeshi strainspossess a threat to control multidrug resistant bacteria in Bangladesh [12]. The spectrum of acquisition of genes encoding MBL is particularly important, becausethese encode resistance to all beta-lactam antibiotics except aztreonam. Resistance is mediated by a lack of drug penetration for example due to porin mutations, efflux pumps or hydrolysis by β-lactamases [13]. Based on molecular studies, carbapenem hydrolyzing enzymes are classified into four groups: A, B, C and D. The MBLs, which belong to group B, are enzymes requiring divalent cations as cofactors for optimal enzyme activity, being inhibited by the action of a metal ion chelator [13]. The MBLs efficiently hydrolyze all β-lactams. Several phenotypic methods are available for the detection of MBL-producing bacteria. All these methods are based on the ability of metal chelators such as Ethylenediaminetetraacetic acid (EDTA) and thiol-based compounds to inhibit the activity of MBLs. These tests include the double disc synergy tests using various combinations such as EDTA with imipenem (IPM) or ceftazidime (CAZ) [13-15]; 2-mercaptopropionic acid with CAZ or IPM [18]; the Hodge test [13, 14]; a combined disk test using EDTA with CAZ or IPM [17-18]; the MBL E-test (AB BioDisk) [20]; and a micro dilution method using EDTA and 1,10-phenanthroline with IPM [21]. Genomic characterization of MBL genes revealed that most of the acquired bla MBLs were the part of integron and resides in large transmissible plasmids [22]. Because of the association of blaMBLs with integrans, they have theability to spread rapidly [23]. A previous study in Bangladesh revealed 74%class 1integron carrying MBL producers [24]. Class 1integrons were found in 68% of worldwide nosocomial isolates of Acinetobacter baumannii [25]. Among several classes of integrons, class 1 integron is the most commom andhave been found in isolates of Acinetobacter spp. may act as a reservoir of integron-associated antibiotic resistance gene, which could then spread to other pathogens in thehospital environment [26]. Gram-negative bacilli associated with hospital infections are often difficult to eradicate because they are resistant to drugs. Therefore, detection of MBL-producing Gram-negative bacilli is crucial to control the spread of resistance and for the optimal treatment of patients, particularly the critically ill and hospitalized patients [27]. There is not much information concerning MBL-producing isolates available in Bangladesh. Therefore, this study was conducted to detect the presence of MBL in carbapenem-resistant isolates obtained from the clinical isolates from a tertiary care hospital, which has intensive care units with a heavy patient turnover and extensive antibiotic use.

II. METHODOLOGY

This Cross sectional study was conducted between July 2013 to June 2014 in the department of Microbiology, Dhaka Medical College (DMC), and Dhaka, Bangladesh to determine the frequency and susceptibility patterns of MBL-producers among imipenem-resistant Gram-negative rods (GNRs) from clinical isolates of a tertiary care hospital. All clinical samples were processed according to standard microbiological methods. Isolated GNRs were subjected to susceptibility testing against various antibiotics by disc diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. Carbapenem-resistant isolates were subjected to the detection of MBL production by the E-test MBL strip method. Hospitalized patients who did not give consent were excluded in this study. Data regarding age, sex, duration of hospitalization, antibiotic history were collected using a prescribed data collection form.

Specimen Collection

Endotracheal tube aspirates were collected from clinically suspected respiratory tract infected patients in ICU of DMCH. With all aseptic precaution, endotracheal aspirate was collected using a 50 cm and 14Fr suction catheter. The suction catheter was gently introduced through the endotracheal tube for a distance of approximately 25-26 cm. The endotracheal aspirate was obtained by suction, without instilling saline and the catheter was withdrawn from the endotracheal tube. After the catheter was withdrawn, 2 ml of 0.9% sterile normal saline was injected into the suction catheter with a sterile syringe to flash the exudates. The exudates were collected into a sterile falcon tube and were transported immediately to the laboratory for further processing (Dey and Bairy, 2007). Smear was prepared for gram staining from ETA.

CD and DDS Test

Double disc -synergy tests (DDS) and combined disk (CD) assays were performed to screen MBLs producers. For the DDS test, imipenem and a blank disk containing 20 µl of Tris-EDTA (1.0M Tris HCL, O.IM EDTA, p^{H} approximately 8.0) and 20 µl of 1:320 diluted 2- mercaptopropionic acid (MPA) were placed 10 mm apart in an inoculated Mueller- Hinton agar plate and incubated at 37^{o}C for 24 hours. A clear extension of the edge of the inhibition zone of imipenem disk toward the Tris-EDTA-MPA disk was interpreted as MBLs production. For the CD assay, two imipenem disks were placed on an inoculated Mueller-Hinton agar plate. One imipenem disk was supplemented with 5 µl of 0.5 M EDTA and incubated at 37°C for 24 hours. An increased zone diameter of ≥6 mm around the disk containing imipenem supplemented with EDTA compared to the disk containing imipenem alone was interpreted as MBLs production. MBL producers were detected among the isolated imipenem

resistant strains. Initially sensitivity to imipenem was observed by disk-diffusion method. Then MIC of imipenem was done using agar dilution method. Demonstrates the phenotypic detection of MBL producers among imipenem resistant Acinetobacter baumannii by CD ussay and DDS test among the 22 imipenem resistant strains 19 (86.36%) MBL producers were detected by CD assay and 17 (72.27%) were detected by DDS test.

III. RESULTS

Out of 22 imipenem resistant Acinetobacter baumannii. 21 (95.45%) were positive for MBL production (Table-1). Demonstrates the antimicrobial resistance pattern of the MBL producing Acinetobacter baumannii. All the 21(100%) MBL positive Acinetobacter baumannii showed resistance to amoxiclav, ceftazidime, cefotaxime, ciprofloxacin, imipenem and cefoxitin, 20 (95.23%) were resistant to cefepime and piperacillin-tazobactam, 19 (90.47%) were resistant to amikacin, 8 (38.09%) were resistant to aztreonam and 13 (61.9%) were resistant to sulbactam-cefoperazone. All the 21 (100%) MBL producing Acinetobacter baumannii were sensitive to colistin (Table-2). (Table-3) shows the distribution of MBL genes among imipenem resistant Acinetobacter baumannii. Out of 22 imipenem resistant strains, 21 (95.45%) were positive for $bla_{NDM-1,}$ 20 (90.91%) for $bla_{NDM-like,}$ 16 (72.72%) for bla_{VIM} and all the 22 (100%) were negative for bla_{IMP}. (Fig-1): shows the distribution of class I integron among the MBL producers. Among the 21 MBL producing Acinetobacter baumannii, 17 (80.95%) had class I integron. (Table-4) shows the presence of class I integron and conserved segment of class 1 integron among the different MBL producing Acinetobacter baumannii. Among 16 bla_{VIM} positive Acinetobacter baumannii. 13 (81.25%) carried class 1 integron and 11 (68.75%) carried conserved segment of class I integron. Out of 21 bla_{NDM-1} positive strains. 17 (80.95%) carried class I integron and 13 (61.9%) carried conserved segment of class 1 integron. Out of 20 $bla_{NDM-like}$ positive strains, 16 (80%) carried class 1 integron and 12 (60%) carried conserved segment of class I integron.

Table-1: The results of moleculer detection of MBL producers among imipenem resistant Acinetobacter baumannii (n=22).

MBL producers	Number	Percentage
Positive	21	95.45
Negative	1	4.55
Total	22	100.00

Table-2: Antimicrobial drug susceptibility among MBL producing Acinetobacter baumannii (n=21).

Antimicrobial drugs	Sensitive n (%)	Resistant n (%)
Amoxiclav	0 (0.00)	21 (100.00)
Ceftazidime	0 (0.00)	21 (100.00)
Cefotaxime	0 (0.00)	21 (100.00)
Cefepime	1 (4.77)	20 (95.23)
Amikacin	2 (9.53)	19 (90.47)
Ciprofloxacin	0 (0.00)	20 (95.23)
Imipenem	0 (0.00)	21 (100.00)
Aztreonam	13 (61.91)	8 (38.09)
Sulbactam-cefoperazone	8 (38.10)	13 (61.90)
colistin	21 (100.00)	0 (0.00)
Piperacillin-tazobactam	1 (4.77)	20 (95.23)
Cefoxitin	0 (0.00)	21 (100.00)

Table-3: Distribution of MBL encoding genes in the imipenem resistant strains of Acinetobacter baumannii (n=22).

Genes	Present	Absent
	N (%)	N (%)
NDM-1	21 (95.45)	1 (4.55)
NDM-like	20 (90.91)	2 (9.09)
VIM	16 (72.72)	6 (27.28)
IMP	0 (0.00)	22 (100.00)

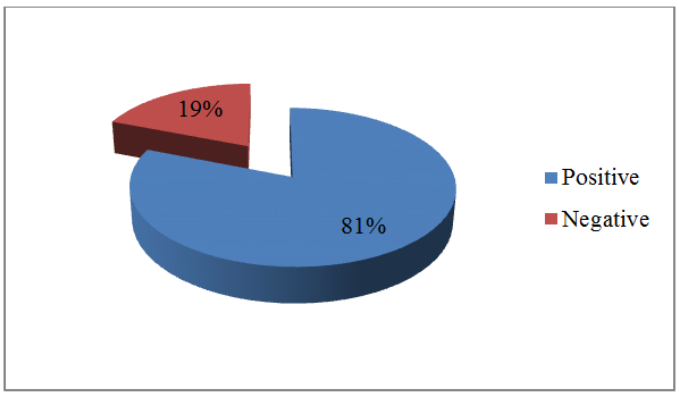

Fig-1: The distribution of class I integron among the MBL producers.

Table-4: Class 1 integron and Conserved segment of class 1 integron carriers among the different blaMBLs encoding gene in imipenem resistant Acinetobacter baumannii.

MBLs encoding genes	Class 1 integrons		Conserved segment of class 1 integrons	
	Present N (%)	Absent N (%)	Present N (%)	Absent N (%)
bla_{VIM} (n=16)	13 (81.25)	3 (18.75)	11 (68.75)	5 (31.25)
bla_{NDM-1} (n=21)	17 (80.95)	4 (19.05)	13 (61.90)	8 (38.10)
$Bla_{NDM-like}$ (n=20)	16 (80.00)	4 (20.00)	12 (60.00)	8 (40.00)

IV. DISCUSSION

Japan reported the first plasmid-mediated MBL in *Pseudomonas aeruginosa* in 1991. This was followed by a report of transferable metallo enzyme in *Bacterioides fragilis* [1]. In addition to *Pseudomonas aeruginosa*, other Gram-negative bacteria including *Klebsiella pneumoniae, Escherichia coli, Enterobacter aerogenes, Enterobacter cloacae, Citrobacter freundii, Proteus vulgaris, Acinetobacter* spp. and *Alcaligenes xylosoxidans* have also been shown to produce MBL [19]. There are frequent reports of MBL production in *Pseudomonas aeruginosa* and *Acinetobacter* species from different parts of the world, particularly in military settings [28]. MBLs have been identified from clinical isolates worldwide with increasing frequency over the past few years, and strains producing these enzymes have been responsible for prolonged nosocomial outbreaks that were accompanied by serious infections [29]. Reports have also described the significant prevalence of MBLs in *Pseudomonas aeruginosa* and *Acinetobacter spp.* from India in a study conducted in 2008 [30]. *In vitro* antibiotic susceptibility of the MBL-positive organisms was investigated by the E-test method. Of the ten drugs tested, isepamicin was the most active agent against the MBL-producing strains. Overall, the rank order of activity of the ten antibiotics, in terms of the percentages of susceptible strains was as follows: isepamicin: 73%; ciprofloxacin: 64%; amikacin: 59%; aztreonam: 18%; tobramycin: 18%; meropenem: 14%; cefoperazone-sulbactam: 5% [31]. In the present study demonstrates the antimicrobial resistance pattern of the MBL producing Acinetobacter baumannii. All the 21(100%) MBL positive Acinetobacter baumannii showed resistance to amoxiclav, ceftazidime, cefotaxime, ciprofloxacin, imipenem and cefoxitin, 20 (95.23%) were resistant to cefepime and piperacillin-tazobactam, 19 (90.47%) were resistant to amikacin, 8 (38.09%) were resistant to aztreonam and 13 (61.9%) were resistant to sulbactam-cefoperazone. All the 21 (100%) MBL producing Acinetobacter baumannii were sensitive to colistin. No data regarding presence of more than one MBL genes in a single strain was available to compare this result. In the present study, 100% sulbactam-cetoperazone resistant isolates were the class 1 integron harboring isolntes, followed by cefotaxime und piperacillin-tazo bactam with 78.26%. 77.27% amikacin, celepime and imipenem resistant isolates were the intl harboring isolales. 75% amoxiclav, ciprolloxacin, ceftazidime and cefoxitin resistant isolates were the inti harboring isolates. Previous studies have reported a high frequency of multidrug-resistant gram-negative isolates containing integrons [26]. Class 1 integrons were found in 68% of worldwide nosocomial isolates of Acinetobacter baunannii [25]. These studies showed the occurrence of MBL genes simultaneously undindependently in different parts of these respective countries. The findings of thepresent study have confirmed the presence of bla_{VIM} and bla_{IMP} containing organismsin Bangladesh. From Italy and Greece, 87.5% and 100% VIM-I producers wereidentified, respectively which were not in accordance with the present findings [33]. The proportion of MBL producer's fromdifferent studies including the one suggests that the prevalence of MBL producers varies with geographical areas and time. Co-resistance to non-B-lactams among the MBLL producers in this study might be due to cassette-associated antimicrobial resistance genes within integron conter resistance simultaneously to P-lactams and non-lactams, as described previously [34]. However, in this study, class1 integron negative MBL producers were 50% resistant to amikacin and 75%

resistant to ciprofloxacin. In this study, all the MBL producers were resistant to amoxielav, ceftazidime, cefotaxime, celoxitin and imipenem. This resistance might be due to the ability of MBLs to degrade the respective classes of B-lactams and not inhibited by lactamase inhibitors [23]. These findings of the present study coincide with the results of previous studies where MBL producers were also resistant to amoxicillin, amoxiclav, ceftazidime and cefotuxime [24] Pournaras *et al*., [39] In this study, 95.23% were resistant to piperacillin-tazobactam and 38.09% were to aztreonam. A study by Farzana *et al.*, [24] revealed that 80.64% of the MBL producers were resistant to piperacillin-tazobuctum. Anothar study by Safari *et al*., [40] also revealed that 95% of the MBL producers were resistant to piperacillin-tazobactam which has similarity with the findings of the present study. In contrast to present study, Anwar *et al*. [10], found that 7.94% of the MBL producers were resistant to Piperacillin-tazobactam. Kabbaj *et al*., [38] also observed 100% of MBL producing Acinetobacter baumannii were resistant to piperacillin-tazobactam. The results are similar to those of a 2008 study conducted at Aga Khan University, Karachi, by Irfan *et al.*, in which 96.6% of the carbapenem resistant *Acinetobacter baumannii* were MBL producers and all the carbapenem-resistant *Pseudomonas spp.* were found to be MBL producers [32]. Current study demonstrated that 9047% of the MBL producing organisms were resistant to amikacin and 95.23% were resistant to ciprofloxacin. Previous studies suggest that MBL producers showed resistance to aminoglycosides and fluoroquinolones along with B-lactam antibiotics [20]. Farzana *et al*., [24] reported that 96.77% of the MBL producers were resistant to amikacin and 93.55% of the MBL producers were resistant to ciprofloxacin. Anwar *et al*. [10] also observed 39.68% amikacin resistant and 84.13% ciprofloxacin resistant MBL producers. In contrast to present findings, the authors also showed relatively less percentage of MBL producers that were resistant to non-p-lactam antibiotics. The reason for coresistance of MBL producers to non-ß-lactam antibiotics might be due to Simultaneous presence of other drug resistance mechanisms in addition with MBL genes [35, 36]. Emergence of MBL-mediated resistance in Bangladesh is of serious concern. Imipenem are effective therapeutic agents against highly resistant pathogens such as *Pseudomonas spp*. and *Acinetobacter spp*. Spread of this resistance among these pathogens and transfer to other Gram-negative bacteria would seriously restrict therapeutic options. This challenging situation is difficult to manage in a resource-limited country. Conversely, the situation continues to become more complicated by the indiscriminate use of antibiotics in the population. In the present study, all the MBL producers were sensitive to colistin. Kabbaj *et al.* [38] and a previous study in Bangladesh by Farzana *et al*. [24] also reported the similar result. In contrast to the lindings of the present study, Anwar *et al*., [10] and Franco *et al*. [37] observed 12.7% and 4.8% of the MBL producers were resistant to colistin, respectively. The occurrence of an MBL-positive isolate in a hospital setting poses a therapeutic problem, as well as serious concern for infection control management. The accurate identification and reporting of MBL-producing bacteria will aid infection control practitioners in preventing the spread of these multidrug-resistant isolates.

V. CONCLUSION

The study results demonstrate the serious therapeutic and epidemiological threat of the spread of metallo-beta-lactamase producers. Early detection and infection control practices are the best defense against these organisms; therefore systematic surveillance to detect MBL producers is necessary. Judicious use of imipenem is essential to prevent the spread of these organisms. Regarding amicrobial susceptibility, all of the isolated acinetobacter baummannii were sensitive colisun and most of them were resistant to imipenem in conclsion, it can be sad that overexpression of blabc gene or presence of NDM-1 gene is an important contnbutory actor tor acinelobacter bumannii to become multidrug-resistant. So detecton of any of these genes will be helpful in selection of agpropriate antibioties to treat the case and to prevent spresd of drug resistance.

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