

Occurrence of Metallo- β -lactamase Variants Among Heterogeneous Population of Carbapenem Resistant Clinical *Pseudomonas aeruginosa* from Savar, Bangladesh

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ABSTRACT

Background and Aim: Worldwide dissemination of Metallo- β -Lactamases (MBLs) has been reported as a major mechanism of resistance to carbapenems in clinical Gram-negative isolates, including *Pseudomonas aeruginosa* (*P. aeruginosa*). The present study aimed to investigate the occurrence of MBL variants in carbapenem resistant *P. aeruginosa* isolates from Dhaka, Bangladesh.

Materials and Methods: Samples from patients (n=238) with urinary tract infections, wound infections, and catheter-associated infections from two academic hospitals of Savar, Dhaka were investigated. Carbapenem susceptibility of the isolates was tested against imipenem and meropenem. MBL-mediated carbapenem resistance was investigated phenotypically and through PCR amplification of four MBL variants. Plasmid extraction was conducted to observe association with MBL carriage. ERIC-PCR was carried out to determine possible epidemiological linkages.

Results: The presence of *P. aeruginosa* was detected in 22% (53/238) of the collected clinical samples. Resistance to carbapenems was present among 30% (16/53) of these clinical isolates, which is more than 2-fold higher compared to that of previous studies. Phenotypic expression of MBL was detected in 90% (14/16) of resistant isolates. Molecular analysis revealed that 63% (10/16) of the carbapenem-resistant isolates carried at least one of the MBL variants, either *bla*-VIM or *bla*-NDM-1. All positive *bla*-NDM-1 variants carried a 0.5 MDa plasmid. ERIC-PCR revealed highly heterogeneous nature of *P. aeruginosa* isolates indicating multiple sources of infection within the hospital.

Conclusion: These findings indicate an increased MBL-mediated resistance to carbapenem in *P. aeruginosa*, which poses a serious threat to the treatment of infections among hospitalized patients.

Keywords: Carbapenem-resistance, Genetic Variants, Metallo- β -lactamase, Nosocomial Infection, *Pseudomonas aeruginosa*

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1. Introduction

Pseudomonas aeruginosa (*P. aeruginosa*) is one of the most common pathogens associated with nosocomial infections (1). This Gram-negative bacterium is a member of the infamous ESKAPE group of bacteria, known for their rapidly increasing antibiotic resistance in healthcare facilities (2, 3). The

global priority list of emerging antibiotic-resistant bacteria recognizes *P. aeruginosa* as a critical pathogen that requires an in-depth study (4). It is commonly associated with wound infections, urinary tract infections (UTIs) (particularly catheter-

associated UTIs), ventilator-associated respiratory tract infections (RTI), and cystic fibrosis (1, 5).

The carbapenem group of antibiotics (imipenem, meropenem, ertrapenem, and doripenem) is clinically effective against *P. aeruginosa* (5, 6). However, a steep rise in carbapenem resistance has been reported over the last few years (7). Susceptibility to carbapenems is dependent on their entry to bacteria through the outer membrane porin protein OprD (8). Thus, a reduced expression of OprD can lead to carbapenem resistance in *P. aeruginosa* (8). Other mechanisms of carbapenem resistance include decreased permeability of outer membrane porins, increased production of molecular efflux pumps, acquisition of chromosomal cephalosporinases, and expression of broad-spectrum beta-lactamases, especially carbapenemases like metallo- β -lactamases (MBLs) (9). Acquisition of MBLs through horizontal gene transfer has been increasingly identified in Gram-negative isolates from hospitalized patients (10). These enzymes can hydrolyze most of the β -lactams and are not repressed by serine β -lactamase repressors like tazobactam and clavulanates (11). As MBLs depend on bivalent zinc ions for the hydrolysis of β -lactam antibiotics, they are inactivated by metal chelators like EDTA (12).

The origins of MBLs remain unclear; it is most likely transferred from environmental bacteria like *Enterobacteriaceae* (13). The genes encoding MBLs are mainly spread through mobile genetic elements like integrons residing on plasmids or bacterial genomic DNA (13, 14). The majority of MBLs belong to subclass B1 (15, 16) and the 3 most common MBLs, namely IMP (imipenemase), VIM (Verona integron-encoded Metallo- β -lactamase), and NDM (New Delhi Metallo- β -lactamase) are well-known for their epidemiological and clinical importance (12, 13, 17). The *bla*-VIM was first detected in *P. aeruginosa* in European countries during the late 1990s, and more than 20 different VIM allotypes have been identified worldwide (13, 18). However, *bla*-NDM-1, which was detected in *K. pneumoniae* in 2008, has been widely disseminated among *Enterobacteriaceae* in India (14). A significant increase in prevalence of these MBLs has been seen throughout the world, including the United Kingdom and Southeast-Asian countries like India (14), Pakistan (19), and Bangladesh (20).

In this study, we investigated the presence of carbapenem-resistant *P. aeruginosa* infection among hospitalized patients in Dhaka, Bangladesh. We also aimed to analyze the presence of different MBL-encoding genes like *bla*-IMP, *bla*-VIM, *bla*-SPM and *bla*-NDM-1 to deduce the role of MBL variants in

carbapenem-resistance of *P. aeruginosa*. The possible epidemiological association or link among the isolates was also explored by Enterobacterial Repetitive Intergenic Consensus-Polymerase Chain Reaction (ERIC-PCR) approach. We observed that resistance to carbapenems has been doubled in the past decade (6). Such a rapid increase in carbapenem resistance was mediated by MBL. We also detected the expression of MBL-variants in carbapenem resistant *P. aeruginosa* isolates and determined the heterogenic nature of the clinical isolates.

2. Materials and Methods

2.1 Isolation and Characterization of Clinical Isolates

Clinical samples were collected from patients admitted to two academic hospitals, Enam Medical College Hospital (EMCH) and Gonoshasthaya Samaj Vittik Medical College Hospital (GMCH) located at Savar, Dhaka, Bangladesh from April 2020 to January 2021. The presence of *P. aeruginosa* was studied in 238 samples consisting of 115 midstream urine, 69 pus, 21 secondary wound infection swab, 12 urinary catheter swab, 10 burn wounds, 9 blood, and 2 tracheal aspirate specimens. *Pseudomonas* Cetrimide Agar (Scharlab S. L., Spain) was used to determine the colony morphology, fluorescence, and pigment production, followed by conventional biochemical tests according to Bargey's Manual of Systemic Bacteriology for presumptive identification of *P. aeruginosa* as described previously (21, 22). *P. aeruginosa* ATCC 27853 was used as a positive control. Selected isolates were stored in glycerol broth at -80°C.

2.2 Molecular Identification

Molecular identification was carried out by sequencing 16S rDNA of 14 representative isolates. DNA templates prepared from the selected isolates were subjected to polymerase chain reaction (PCR) (23). PCR products were purified using Promega Wizard SV Gel and PCR Clean-up System (USA) and sequenced. The identity of the isolates was confirmed by BLAST analysis.

2.3. Screening of Carbapenem Resistance

To determine the susceptibility of clinical *P. aeruginosa* isolates to carbapenem antibiotics, Kirby Bauer disk diffusion test was used (24), followed by determination of minimum inhibitory concentration (MIC) against imipenem (IMP 10 μ g) and meropenem (MPM 10 μ g). Antibiotic disks were obtained from Oxoid, UK. *P. aeruginosa* PAO1 was used as control. The results were interpreted according to CLSI

standards and guidelines (25). Multidrug resistant (MDR) nature of isolates was observed against 9 additional antibiotics of 6 different groups (data not included in this study) and interpreted accordingly (26, 27).

2.4. Phenotypic Detection of MBL Production

To detect the production of MBL, imipenem-resistant isolates were subjected to IMP-EDTA double disk synergistic test. The pure cultures of selected isolates were plated on Mueller-Hinton agar (Oxoid, UK) to prepare bacterial lawn. Two imipenem (IMP 10 µg) disks were placed on prepared lawn by at least 20 mm distance. One of the disks was impregnated with 0.5M EDTA to achieve 750 µg/disk concentrations, which can act on MBL by removing zinc ions from the active site of the enzyme, effectively nullifying its activity. The plates were incubated overnight at 37°C, and zone diameters for both IMP and IMP-EDTA disks were measured. Isolates exhibiting ≥17 mm inhibition zones with IMP-

EDTA disk were considered MBL-positive, while isolates with ≤14 mm inhibition zones were considered MBL-negative (28).

2.5. PCR Detection of MBL

The presence of genes encoding 4 different variants of MBLs; *bla*-NDM-1, *bla*-SPM, *bla*-IMP, and *bla*-VIM was investigated in all isolates exhibiting phenotypic resistance against imipenem. PCR reagents were obtained from Promega, USA and the primers were designed according to the previous publications (29, 30). The primers sequences and annealing temperatures are listed in Table 1. For each PCR reaction, 2 µl of prepared bacterial DNA was added to 16 µl of prepared PCR ready-to-use mix containing (1X PCR buffer, 2.5mM MgCl₂, 0.25 mM dNTP, 1IU Taq DNA polymerase, 10 pmol of each primer), and deionized water was added to obtain a final volume of 25 µl. The PCR products were visualized using UV transilluminator after electrophoresis on 1% agarose gel.

Table 1. PCR primers specifications.

Primer	Sequence (5'-3')	Amplicon size (bp)	Annealing Temperature (°C)	Reference
<i>bla</i> -IMP	F- GGA ATA GAG TGG CTT AAT TCT C R- CCA AAC CAC TAC GTT ATC T	188	52	(29)
<i>bla</i> -VIM	F- GAT GGT GTT TGG TCG CAT A R- CGA ATG CGC AGC ACC AG	390	52	(29)
<i>bla</i> -SPM	F- AAA ATC TGG GTA CGC AAA CG R- ACA TTA TCC GCT GGA ACA GG	271	52	(29)
<i>bla</i> -NDM-1	F- CCT ACA ATC TAA CGG CGA CC R- TCG CCG TGT CCA GGT ATA AC	621	56	(30)
ERIC2	AAG TAA GTG ACT GGG GTG AGC G	----	48	(31)

2.6. ERIC-PCR for Species Diversity Analysis

To observe diversity among *P. aeruginosa* isolates, ERIC-PCR was performed using the primers mentioned elsewhere (31). PCR products were separated on 2% agarose gel and visualized under UV transilluminator. The cluster analysis was conducted using DendroUPGMA (32, 33).

2.7. Plasmid Extraction

Plasmids from carbapenem-resistant *P. aeruginosa* isolates were extracted according to the hot alkaline lysis method by Kado and Liu (34) with modification. The extracted plasmids were electrophoresed on 0.7% agarose gel with ethidium bromide (0.5 mg/mL) and visualized under UV transilluminator. Reference

strains of *E. coli* K-12 V517 and PDK9 were used as control strains for the extraction method and plasmid size marker (35, 36).

2.8. Statistical Analysis

A validated questionnaire was used for data collection. Collected data were verified and analyzed by IBM SPSS Statistics Data Editor (Version 21). STATA 15 was used for subsequent analysis. P-value of <0.05 was considered significant.

3. Results

3.1. Isolation and Identification of *P. aeruginosa*

A total of 238 clinical samples were collected from the patients admitted into two academic hospitals in Savar City, at the outskirts of Dhaka, the capital of Bangladesh. Most of clinical samples were collected from patients with UTI, followed by secondary wound infections and abscesses (Table 2). Among the samples, 53 samples (~22%) showed *P. aeruginosa* growth on selective ceftrimide agar, which were further characterized through standard biochemical tests. Identification of the clinical isolates was confirmed by PCR-based 16S rDNA sequence analysis, which showed 95 to 99% identity to *P. aeruginosa* PAO1 sequence (e-value ≤ 0). Wound infections were more frequently positive for *P. aeruginosa* compared to other infections.

3.2. Carbapenem Resistance in Clinical *P. aeruginosa* Isolates

All 53 positive *P. aeruginosa* isolates were tested for susceptibility to carbapenem (imipenem and meropenem) using disk diffusion method followed by MIC assay. Out of 53 isolates, 16 (30%) were resistant to both imipenem and meropenem. However, the antibiotic tolerance levels varied widely in MIC assay. While 50% (8/16) of carbapenem-resistant isolates showed moderate resistance (8 $\mu\text{g/ml}$), the other 50% isolates had much higher resistance level (≥ 16 to 128 $\mu\text{g/ml}$). Among 16 carbapenem-resistant *P. aeruginosa* isolates, 6 were from Gonoshasthaya Samaj Vittik Medical College & Hospital (GMCH) (38%), while the rest 10 (62%) were from Enam Medical College & Hospital (EMCH) (Table 2). Antibiogram profile identified 12 isolates as MDR, and another 2 isolates as extensively drug resistant (XDR). Moreover, uniform resistance against third generation cephalosporins like ceftriaxone and cefotaxime and significantly lower susceptibility to important antibiotics like azithromycin and tetracycline was observed, which is alarmingly higher than previous regional reports (37).

Table 2. Collection of samples and culture results.

Sample type	Samples collected from EMCH		Samples collected from GMCH		Total samples	Proportion of positive samples
	Number of samples	Positive for <i>P. aeruginosa</i>	Number of samples	Positive for <i>P. aeruginosa</i>		
Urine	72	16	43	7	115	23 (20%)
Urinary Catheter	7	4	5	1	12	5 (42%)
Sec. wound infection	12	7	9	4	21	11 (52%)
Pus	37	5	32	5	69	10 (14%)
Burn wound	8	2	2	0	10	2 (20%)
Blood	9	1	0	0	9	1 (11%)
Tracheal aspirate	2	1	0	0	2	1 (50%)
Total	147	36	91	17	238	53 (22%)

GMCH: Gonoshasthaya Samaj Vittik Medical College & Hospital; EMCH: Enam Medical College & Hospital.

3.3. Detection of MBL in *P. aeruginosa* isolates

All of the 16 imipenem-resistant *P. aeruginosa* isolates were subjected to enzymatic activity and PCR-based genotypic detection of MBL variants. Of 16 isolates, 14 (~88%) were positive for MBL activity in imipenem-EDTA double-disk synergistic test. PCR-based detection of MBL variants, *bla*-VIM, *bla*-NDM1, *bla*-IMP, and *bla*-SPM, showed that 10 out of the 16 (~63%) resistant isolates carried at least one variant. While *bla*-VIM was detected in 7 isolates (44%) and *bla*-NDM-1 was detected in 3 isolates (19.75%) (Fig. 1). No MBL variant was detected in other 6 isolates. Moreover, none of the isolates were multiple MBL genes carrier (Table 3).

3.4. Detection of Plasmids in MBL-Positive Isolates

Plasmid extraction and gel electrophoresis showed the presence of plasmids in 7 out of 16 (43%) MBL-positive isolates. Among 7 *bla*-VIM positive isolates, 4 carried a plasmid of 1.2 MDa, while the other 3 isolates did not carry any plasmid (Fig. 2). In contrast all 3 of the *bla*-NDM1 positive *P. aeruginosa* isolates carried a plasmid of 0.5 MDa (Table 3).

3.5. ERIC-PCR and Cluster Analysis

ERIC-PCR was performed to determine possible epidemiological relationships among the clinical *P. aeruginosa* isolates (21). Our results revealed distinct patterns of DNA, indicating species diversity among carbapenem-resistant *P. aeruginosa* isolates. Highly

heterogeneous results indicated that isolates did not share any significant genetic relatedness. Phylogenetic cluster analysis based on unweighted pair group method with arithmetic mean (UPGMA) exhibited the

presence of three distinct clusters I-III (Fig. 3). Majority of XDR isolates belonged to cluster III, while clusters I and II were composed of MDR isolates.

Table 3. Antibiotic susceptibility and resistance determinants in imipenem-resistant clinical *P. aeruginosa*.

Isolate ID	Specimen (Hospital)	MIC value ($\mu\text{g/ml}$)	Phenotypic detection of MBL production			Presence of MBL gene				Plasmid (MDa)
			IMP (Zone, mm)	IMP+ EDTA (Zone, mm)	Interpretation	<i>bla</i> -IMP	<i>bla</i> -VIM	<i>bla</i> -SPM	<i>bla</i> -NDM1	
PA01 ^a	Reference	0.5	19	20	Negative	-	-	-	-	-
PU1	Urine (A)	8	0	0	Negative	-	-	-	-	-
PU5	Urine (A)	8	0	14	Negative	-	-	-	-	-
PU6	Urine (A)	8	0	15	Intermediate	-	-	-	-	-
PUS1	Cath. Swb (A)	8	0	15	Intermediate	-	-	-	-	-
PS1	Wound Swb (A)	16	0	16	Intermediate	-	-	-	-	-
PS2	Wound Swb (A)	8	0	15	Intermediate	-	-	-	-	-
PU17	Urine (B)	128	0	22	Positive	-	-	-	+	0.5
PU26	Urine (B)	8	0	20	Positive	-	+	-	-	1.2
PWS12	Wound Swb (B)	32	0	20	Positive	-	+	-	-	-
PWS17	Cath. Swb (B)	128	0	24	Positive	-	-	-	+	0.5, 1.2
PWS18	Cath. Swb (B)	128	0	21	Positive	-	-	-	+	0.5, 1.2
PWS21	Wound Swb (B)	128	0	24	Positive	-	+	-	-	0.2, 1.2
PWS22	Wound Swb (B)	16	0	22	Positive	-	+	-	-	0.2, 1.2
PWS23	Wound Swb (B)	16	0	16	Intermediate	-	+	-	-	-
PWS25	Cath. Swb (B)	8	0	16	Intermediate	-	+	-	-	0.2, 1.2
PWS26	Tracheal Asp (B)	128	0	23	Positive	-	+	-	-	-

'+' denotes positive; '-' denotes negative; IMP: imipenem; A: Gonoshasthaya Samaj Vittik Medical College & Hospital (GMCH); B: Enam Medical College & Hospital (EMCH). Cath. Swb: Catheter Swab; Wound Swb: Secondary Wound Swab; Tracheal Asp: Tracheal Aspirate; aPAO1: Reference strain in susceptibility testing and β -lactamase assays (NCBI: txid208964).

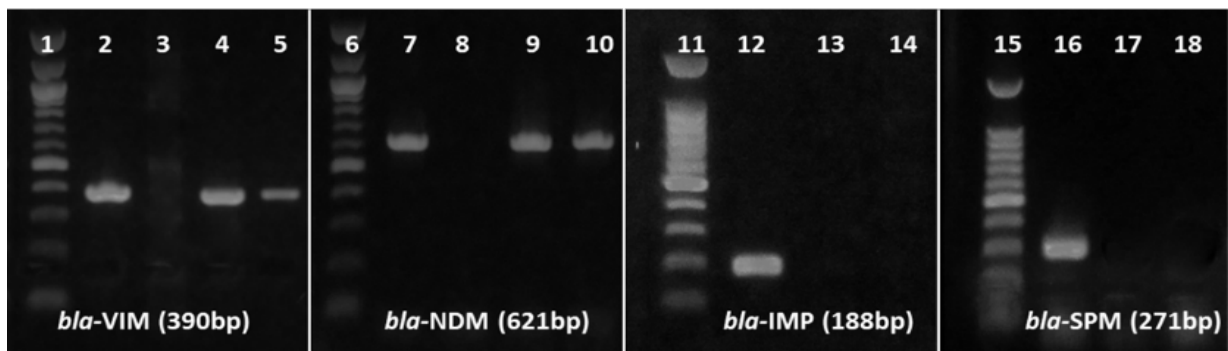


Figure 1. PCR-based detection of MBL variants. MBL gene sequences were PCR amplified using variant-specific primers. PCR products were electrophoresed through 1% agarose gel, stained with ethidium bromide, and visualized with UV light. The 100 bp DNA ladder indicates the size of PCR products. Lanes 2, 7, 12 and 16 represent variant-specific positive controls, and lanes 3, 8, 13 and 17 represent negative controls. Lanes 4, 5, 9, 10, 14 and 18 represent test isolates. No isolates were positive in *bla-IMP* and *bla-SPM* specific PCR. No isolates were positive in *bla-IMP* and *bla-SPM* specific PCR.

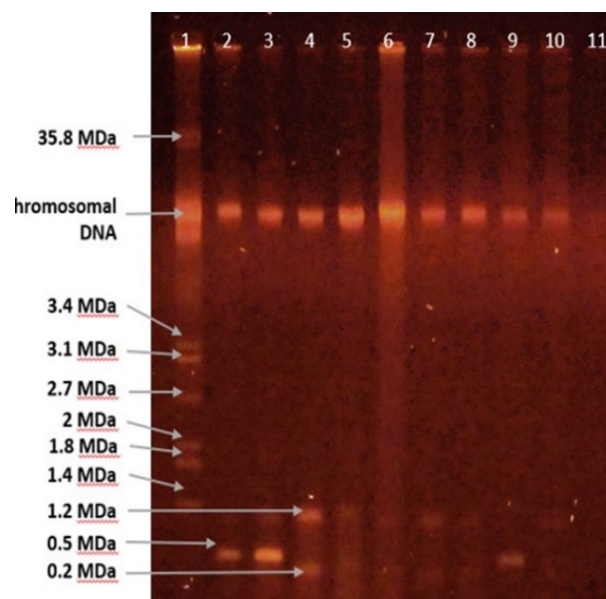


Figure 2. Plasmids in MBL-positive isolates. Plasmid extraction was conducted using modified hot alkaline lysis method. Plasmids were electrophoresed on 0.7% agarose gel stained with ethidium bromide, and visualized with UV light. Lane 1 represents the reference strain of *E. coli* K-12 V517 as visual marker; Lanes 2-11 represent extracted products from MBL-positive *P. aeruginosa* isolates.

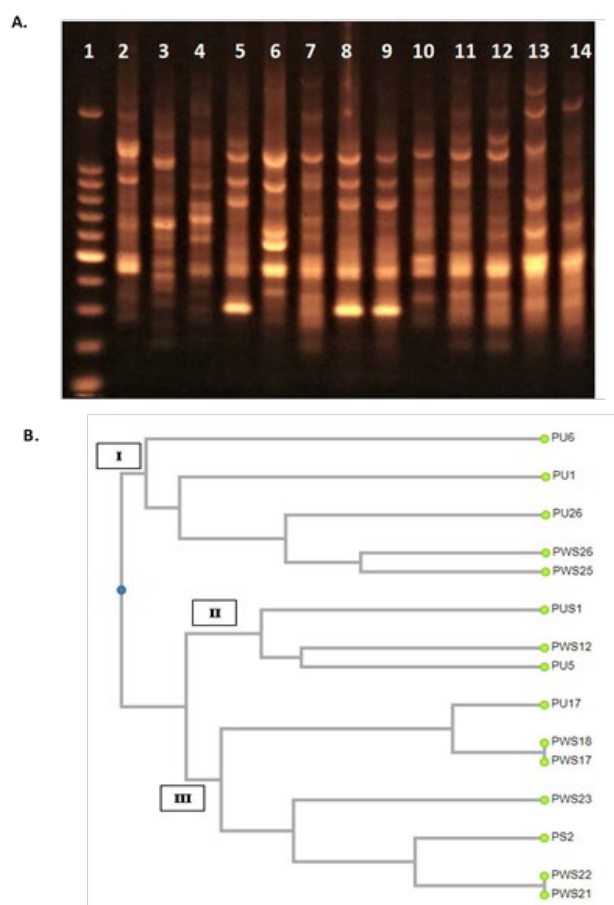


Figure 3. UPGMA cluster analysis of ERIC-PCR results. A). ERIC-PCR products were electrophoresed on 1% agarose gel, stained with ethidium bromide, and visualized with UV light. Lane 1 represents 100 bp DNA ladder; Lanes 8 and 9 represent isolates PWS18 and PWS17, respectively showing identical amplification pattern. Similarly, Lanes 10 and 11 represent isolates PWS22 and PWS21, respectively showing identical amplification. The rest of the lanes represent unique PCR amplification profiles. B). Dendrogram based on UPGMA cluster analysis of ERIC-PCR results; the names of the analyzed strains are mentioned with their phylogenetic groups denominated as Cluster I, II and III.

4. Discussion

Rapid development of antibiotic-resistance in Gram-negative bacteria including *P. aeruginosa* has become a major public health concern all over the world (2, 3). There is an alarming increase in MDR, XDR, and pan drug resistance (PDR) isolates of *P. aeruginosa* (37, 38). In this study, we also observed frequent occurrences of MDR among *P. aeruginosa* isolates in Bangladesh. Remarkably, one in every three isolates was resistant to carbapenems. While the interquartile range (IQR) of imipenem resistance reported in a systematic review covering studies during 2004 to 2018 was 13.5%, we detected a huge increase to 30% (6). Worldwide studies also state increasing occurrence of carbapenem-resistance in *P. aeruginosa*, including reports from China (77%), Serbia (43%), India (20%), and Japan (16%) (39-42).

Our results indicate that rapid increase in imipenem resistance among *P. aeruginosa* clinical isolates in Bangladesh is mediated by MBLs. About 88% of the resistant isolates were positive for MBL

production. The MBL-positive isolates also showed an elevated level of MIC values of imipenem and meropenem. Resistance to carbapenems is often associated with acquisition and expression of the MBLs, which includes *bla*-IMP, *bla*-VIM, *bla*-GIM, *bla*-SPM, *bla*-NDM, and *bla*-SIM genes (29).

In this study 71% of carbapenem-resistant isolates were found to carry variants of MBL genes. While the majority (7/16; 44%) of the isolates carried *bla*-VIM, only 19% (3/16) carried *bla*-NDM-1. The current investigation shows much higher prevalence of MBL genes among *P. aeruginosa* isolates compared to similar studies conducted in India (12%), Pakistan (18%), China (55%), Iran (40%), and Nigeria (17%) (43-46). Moreover, it needs to be noted that we investigated the presence of only four variants (*bla*-IMP, *bla*-VIM, *bla*-SPM, and *bla*-NDM-1). Therefore, we cannot exclude the presence of other MBL variants in remaining 29% of the isolates.

In this study, *bla*-VIM variant was found to be the most prevalent among the MBL genes tested (7/16, 44%). Dissemination of novel variants of *bla*-VIM is commonly mediated by class I integrons like *In58* and *In59* gene cassettes (47, 48). Plasmids carrying class I integrons possess strong dissemination potential (49, 50). However, several of the *bla*-VIM carrier isolates (3/7, 42%) in our study did not carry any plasmid, implying that these isolates may have integrons in their chromosomal DNA. Although *bla*-IMP is globally the most predominant variant of MBL (51), none of the isolates harbored it. Rather, *bla*-VIM was found as the most prevalent variant in this study. This reflects the recent trend of frequent finding of carbapenem-resistant *P. aeruginosa* harboring *bla*-VIM, as reported by several international studies (39, 52).

In this study, the presence of *bla*-NDM-1 was detected among 3 of 16 carbapenem-resistant isolates. Classically *bla*-NDM-1 encoding gene is also plasmid-borne, which has been found to be disseminated through the members of the *Enterobacteriaceae* and *Pseudomonaceae* families (50, 53). Transmission of *bla*-NDM-1 has been also reported in *Acinetobacter baumannii* by transposon Tn125 (54, 55). It has been shown that *bla*-NDM-1 gene can be carried on plasmids of diverse sizes with incompatibility types that allow interspecies, intergenus, and interfamily transfer (56). In this study, we observed the presence of a 0.5 MDa plasmid in *P. aeruginosa* isolates that carried *bla*-NDM-1 gene. Remarkably, all *bla*-NDM-1 carriers were isolated from UTI and urinary catheter-associated infections.

We employed ERIC-PCR for molecular typing of genetic relatedness among *P. aeruginosa* isolates. ERIC-PCR revealed that the carbapenem-resistant *P. aeruginosa* isolates were distributed in three distinct clusters, with the majority belonging to cluster III. Overall analysis of our ERIC-PCR data indicated a heterogeneous nature of *P. aeruginosa* isolates and possible independent sources of dissemination. Several previous studies also reported similar heterogeneous nature of *P. aeruginosa* isolates (44, 57). However, XDR isolates found in this study were all grouped in cluster III in ERIC-PCR analysis.

Our test samples were collected from two academic hospitals. Interestingly, both carbapenem resistance and presence of MBL pattern was different between two hospitals. Although 35% (6/17) of the *P. aeruginosa* from GMCH were carbapenem resistant, 2 were phenotypically MBL-negative and none were positive for the presence of MBL variants tested. In contrast, 28% (10/36) of the *P. aeruginosa* from EMCH were carbapenem resistant, but all the isolates carried MBL genes. Compared to GMCH, EMCH is a

larger, tertiary care hospital that admits more patients for a longer period, which might establish a favorable niche for dissemination of MBL genes among nosocomial agents like *P. aeruginosa*. Previous studies also suggested that larger number of patients may harbor frequent source of drug resistant bacterial infections and prolonged hospitalization may increase the risk of acquisition of drug resistance elements through horizontal gene transfer (58, 59). A recent study in Bangladesh also demonstrated that hospital environment is a major reservoir for carbapenem-resistant *P. aeruginosa* with MBL carriage (60).

The major challenge of combating MBL-mediated carbapenem resistance lies with the rapid global dissemination of MBL through mobile genetic elements, their structural diversity, and discovering a broad-spectrum MBL-inhibitor without toxicity (61). Although the combination of ceftazidime-avibactam and aztreonam was found effective against MBL-producing Enterobacterales, they are less effective against *P. aeruginosa* for active intrinsic resistance (62). Newer antibiotics like cefiderocol also struggle from rapidly emerging resistance, thus, alternative treatment strategies to carbapenem-resistant *P. aeruginosa* infection now depends mostly on discovery of MBL inhibitors (61, 62). Inhibitors like taniborbactam show some promising aspect in neutralizing clinically relevant MBLs, but development of an inhibitor with broad-spectrum activity, metalloenzyme selectivity, metal-binding pharmacophore, and appropriate interaction *in vivo* is still an obstacle yet to overcome (63).

Indiscriminate use of therapeutically important antibiotics is presumed to be the major cause of antibiotic resistance and transmission of such resistant pathogens is more likely among hospitalized patients, especially those who need prolonged stay. Horizontal transfer of antibiotic resistance determinants further complicates the control strategies. Our results suggest that rapid screening by molecular techniques like ERIC-PCR may help track multidrug-resistant *P. aeruginosa* outbreaks in hospitals and develop point-of-care surveillance.

5. Conclusion

The emerging threat of ever-increasing antibiotic resistance among *P. aeruginosa* of clinical origin is a major concern for the treatment of nosocomial infections caused by the pathogens. The magnitude of MDR and the presence of MBL variants in clinical settings reported in this study require immediate attention to improve infection control and prevention programs. This study also suspects the hospital environment to facilitate the horizontal transfer of

resistance elements like MBL genes. Implementation of antibiotic stewardship policy alongside proper infection control now has become a public health emergency to combat the emerging threat of superbugs in healthcare settings of developing countries like Bangladesh.

6. Declarations

Acknowledgment

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Ethical Considerations

This study was approved by the Ethics and Research Review Committee of the Jahangirnagar University, Faculty of Biological Sciences [Ref No: BBEC, JU/M 2020 (1)4]. Written informed consent was obtained from patients and their personal identities along with other information were anonymized.

Authors' Contributions

All authors made a significant contribution to the work reported. M.H.R. conceptualized and supervised the study. H.A., S.Y.M., M.S.A, and M.J.M. collected the clinical samples and conducted laboratory experiments. H.A. and S.Y.M. also analyzed the data and prepared the manuscript. M.S.H. and S.I. made significant contributions to the study design and M.A.K.R. edited the manuscript. The authors agree and approve the content of the final version of the manuscript.

Conflict of Interests

The authors declare that they have no conflict of interest.

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