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Original Research Article

Prevalence of extended spectrum beta-lactamase and metallo-beta-lactamase genes in imipenem resistant *Pseudomonas aeruginosa* from admitted patients in a tertiary level hospital, Bangladesh

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ABSTRACT

Background: *Pseudomonas aeruginosa* are known for their multiple mutations and rapid acquirement of antimicrobial resistance genes. The presence of metallo-β-lactamase (MBL) is the commonest reason for the treatment failure in carbapenem therapy. Production of extended spectrum β-lactamase (ESBL) in these isolates makes the treatment more challenging. Due to the importance of the carbapenems in resistant infection management, finding the true frequencies of such enzymes is imperative.
Methods: A total of 446 samples were collected from the admitted patients with infected burn, surgical wound, and endotracheal tube in situ. Isolation and identification of organisms and antimicrobial susceptibility testing were done by established methods. Identification of *P. aeruginosa* was confirmed by polymerase chain reaction (PCR). Production of ESBLs was detected phenotypically by double disc synergy, and MBL by double-disc synergy, combined disc, and modified Hodge test. Genes encoding ESBLs and MBLs were detected by PCR.
Results: Among the 446 samples, 84.31% yielded growth, from which 232 (61.70%) were *P. aeruginosa*. Among the *P. aeruginosa*, 72 (31.03%) were resistant to imipenem. Phenotypically, 57 (79.17%) of these strains were ESBL and all were MBL producers. blaOXA-10 was the most common ESBL encoding gene (29.83%). *bla*NDM-1 was the most prevalent MBL encoding gene (34.72%). Moreover, 27 (38%) imipenem resistant *P. aeruginosa* had concurring ESBL and MBL genes.
Conclusions: The substantial percentages of ESBL, MBL and simultaneous presence of both genes suggests routine screening of these genes which will provide an opportunity for better selection of antimicrobials in the management of resistant *P. aeruginosa*.

Keyword: Bangladesh, blaNDM-1, blaVIM, ESBL, Imipenem resistant, MBL, *P. aeruginosa*

INTRODUCTION

Diversity and adaptability are two of the characteristics of *P. aeruginosa* that help it to survive in a wide range of environments-both clinical (medical equipment and hospital environment) and non-clinical settings (soil, plant, or aquatic environment).[1] Chronic airway infections in cystic fibrosis (CF), chronic obstructive pulmonary disease (COPD), bronchiectasis or ventilator associated pneumonia are significantly associated with *P. aeruginosa*.[2,3] It is also a dominating cause of acute infections in immunocompromised individuals.[4] *P.*

aeruginosa is also related to local infections of the urinary tract, gastrointestinal tract, eyes, skin, ear and joints or central venous catheter associated bacteremia.[5]

β-lactamases are of utmost importance in the case of gram-negative bacteria (both enteric and non-fermentative pathogens), which collectively cause resistance to all β-lactam-containing antibiotics.[6] Due to the selective pressure on β-lactam antibiotics, it is now more common than ever to have multiple β-lactamases per organism: including bacteria containing three different carbapenemase genes. NDM MBLs are most common in the Indian subcontinent and Eastern Europe.[6] Resistance patterns may have geographical distinctions, but any new resistance mechanism may spread rapidly to other areas.[7] Due to the multidrug resistance pattern of *P. aeruginosa*, researchers as well as the healthcare settings are immensely concerned about them.[8]

Among the β-lactamases, ESBLs and MBLs are two of the most common clinically recognized β-lactamases in *P. aeruginosa*.[9,10] Presently, carbapenem resistance in *P. aeruginosa* has been increasing worldwide. Infection by MBL producing pathogens including NDM-1 is increasing in Bangladesh in the last few years, which poses potential therapeutic failures with current empirical treatments.[11] Although MBLs are the most important mechanisms resulting in treatment failure in carbapenem therapy of *P. aeruginosa* infections, these isolates also produce β-lactamases of class A and D.[12] blaOXA and blaPER are important β-lactamase enzymes in *P. aeruginosa* including blaSHV, blaTEM etc.

For management of infection by these multi drug resistant organisms carbapenems are very important. Multidrug resistance and an increase in the prevalence of ESBL encoding genes among imipenem-resistant *P. aeruginosa* can make the treatment of multidrug-resistant isolates very challenging. Production of carbapenemase hydrolyzing metalloenzyme and aztreonam hydrolyzing blaOXA enzymes can lead to resistance to all β- lactam antibiotics.[13]

Recent studies, regarding the prevalence of these genes and their simultaneous presence among imipenem resistant *P. aeruginosa* isolated are lacking in Dhaka medical college hospital. Therefore, this study was conducted to find the true frequencies of imipenem resistance, as well as ESBL and MBL producing *P. aeruginosa* among hospital admitted patients in a tertiary level hospital of Bangladesh.

METHODS

Sample collection

This cross-sectional study was conducted on the patients admitted at Dhaka medical college hospital, Dhaka, Bangladesh (DMCH) in the period of July 2018 to December 2018. A total of 446 samples of wound swab, and endotracheal aspirate, were collected from patients with clinically diagnosed infected wounds who got admitted in burn, surgery and orthopedics unit, and clinically suspected respiratory tract infected patients in intensive care unit of DMCH respectively, irrespective of age, sex and antibiotic intake after 48 hours of admission. Wound swab and ETA samples received in the department of microbiology, DMCH were also included.

Bacterial isolation

After collecting aseptically, the samples were inoculated in the blood agar media and MacConkey agar media followed by incubation aerobically at 37°C and 42°C for 48 hours. ETA samples were homogenized with vortexing and centrifugation followed by culture in the semi-quantitative method.[14]

P. aeruginosa were isolated and identified by observing colony morphology on the blood agar media (hemolytic, smooth to mucoid, white or cream-coloured colony), MacConkey agar media (pale colour/ non-lactose fermenting colony), Gram staining (gram negative bacilli), biochemical tests (oxidase positive, catalase positive, red-butt, red-slant, no H_2S or gas in TSI agar, negative urease, negative indole, motile bacteria in MIU agar, citrate utilization test positive in Simmon's Citrate agar media), growth at 37°C and 42°C (*P. aeruginosa*).[15] Identification of *P. aeruginosa* were confirmed by PCR from culture with specific primers. Demographic distribution of patients infected by *P. aeruginosa* (n=232) is shown in Table 3.

Antimicrobial susceptibility testing

Isolates were tested for antimicrobial susceptibility using Kirby Bauer modified disc diffusion method and agar dilution method of MIC.[16] Antibiotic susceptibility was interpreted following CLSI and FDA guideline.[17,18] Antibiotic discs such as cefotaxime (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), amoxiclav (amoxicillin 20 µg and clavulanic acid 10 µg), ciprofloxacine (30 µg), amikacin (30 µg), gentamicin (10 µg), netilmicin (30 µg), piperacillin/ tazobactam (100/10 µg), aztreonam (30 µg), imipenem (10 µg), tigecycline (15 µg) were used. Distribution of imipenem resistant *P. aeruginosa* in different samples has been shown in Table 2. In Table 4, antibiotic resistance pattern among isolated imipenem resistant *P. aeruginosa* (n=72) is shown. ESBL and MBL producing *P. aeruginosa* were identified phenotypically (double disc synergy for ESBL and double disk synergy, combined disc, modified Hodge test for MBL) as well as by PCR.

Operational definitions of ESBL and MBL

ESBL: Organism resistant to penicillin along with 1st, 2nd and 3rd generation cephalosporins, aztreonam (but not to cephamycin or carbapenem) and inhibited by β-lactamase inhibitors such as clavulanic acid.[19]

MBL: All MBLs able to hydrolyze carbapenems and not inhibited by the serine-β-lactamase inhibitors.[20]

Detection of ESBL producers by double disc synergy test: 30 µg ceftazidime disc and a disc containing amoxicillin 20 µg and clavulanic acid 10 µg, were placed 25 mm apart (center to center) in Mueller-Hinton agar media. Following incubation at 37ºC for 24 hours, a clear extension of the edge of zone of inhibition of cephalosporin disc towards amoxyclav disc was interpreted as ESBL producing organism.[21]

Phenotypic detection of carbapenemase producers

Double disc synergy (DDS) test: Imipenem disc was placed on Mueller- Hinton agar plate with test inoculum. Blank discs containing 20 µl Tris- EDTA (1.0 M Tris-HCL, 0.1 M EDTA, pH 8.0) and 20 µl of 1: 320 diluted 2- mercaptopropionic acid were placed 10mm apart and was incubated at 37ºC for 24 hours. A clear extension of the edge of the inhibition zone of imipenem disc towards Tris- EDTA- MPA disc was interpreted as MBLs production.[22]

Combined disc (CD) assay: In Mueller-Hinton agar plate two imipenem discs (one supplemented with 5 µl of 0.5 M EDTA solution containing approximately 930 µg EDTA) were placed. It was incubated at 37ºC for 24 hours. MBL producer was indicated by an increased zone of diameter of ≥6 mm around the disc containing imipenem supplemented with EDTA compared to the disc containing imipenem only.[23]

Modified Hodge test (MHT): A lawn culture of 1: 10 dilutions of 0.5 McFarland's standard *E. coli* ATCC 25922 broth was done on a Mueller- Hinton agar plate. A 10µg imipenem disc was placed in the center of the plate. Strike of three imipenem resistant strains, from the edge of the disc to the periphery of the plate in three different directions were done.

After overnight incubation, the presence of clover leaf shaped zone of inhibition was interpreted as MHT positive.[24]

Phenotypic detection of carbapenemase producers among imipenem resistant *P. aeruginosa* by DDS, CD and MHT are shown in Table 6.

Detection of anti-microbial resistance genes by PCR

DNA extraction: DNA was extracted by mixing the bacterial pellets with 300µl distilled water followed by boiling at 100°C for 10 minutes in block heater (DAIHA Scientific, Seoul, Korea), cooling by placing on ice pack, and centrifugation at 4°C at 13500 g for 10 minutes. The extracted DNAs were then kept at -20°C.

Amplification through thermal cycler: After a total 25 µl mixture of mastermix, primer and DNA template PCR were performed in a DNA thermal cycler (Eppendorf AG, Mastercycler gradient, Hamburg, Germany). Each PCR run was conducted after a preheat at 94°C for 10 minutes, followed by 36 cycles of denaturation at 94°C for 1 minutes, annealing at specified temperatures for 45 seconds, extension at 72°C for 1 minutes with final extension at 72°C for 10 minutes.

The amplified DNA was analyzed by 1.5% agarose gel-electrophoresis at 100 volts for 35 minutes, stained with 1% ethidium bromide and visualized under UV transilluminator (Gel Doc, Major science, Taiwan).

In Table 5, distribution of ESBL encoding genes in imipenem resistant *P. aeruginosa* isolated from different samples has been displayed.

The distribution of MBL encoding genes among imipenem resistant *P. aeruginosa* by PCR isolated from different samples.

Distribution of ESBL, MBL and both genes in imipenem resistant *P. aeruginosa* is displayed in Figure 1 and Table 8, distribution of different combination of both ESBL and MBL genes in imipenem resistant *P. aeruginosa* (n=72) is shown.

Table 1 (A): Primers for identification of *P. aeruginosa*.[25]

Gene	Sequence (5'- 3')	Size (bp)
***Pseudomonas* species: PA-GS-F**	GACGGGTGAGTAATGCCTA	618
PA-GS-R	CACTGGTGTTCCTTCCTATA	
***P. aeruginosa*: PA-SS-F**	GGGGGATCTTCGGACCTCA	956
PA-SS-R	TCCTTAGAGTGCCCACCCG	

Table 1 (B): Primers for ESBL genes.[12]

Gene	Sequence (5'- 3')	Size (bp)
blaPER- 1- F	ATGAATGTCATTATAAAAGCT	927
blaPER- 1- R	TAATTTGGGCTTAGG	
blaOXA- 10- F	TCAACAAATCGCCAGAGAAG	276
blaOXA- 10- R	TCCCACACCAGAAAAACCAG	
blaSHV- F	AAGATCCACTATCGCCAGCAG	231
blaSHV- R	ATTCAGTTCCGTTTCCCAGCGG	
blaTEM- F	ATGAGT	858
blaTEM- R	ATTCAACATTTCCGCCAATGCTTAATCAGTGAGG	

Table 1 (C): Primers for MBL genes.[26,27]

Gene	Sequence (5'-3')	Size (bp)
blaIMP- F **blaIMP- R**	GGAATAGAGTGGTTAAYTCTC CCAAACYACTASGTTATCT	188
blaVIM- F **blaVIM-R**	GATGGTGTTTGGTCGCATA CGAATGCGCAGCACCAG	390
blaNDM-1- F **blaNDM-1- R**	ACCGCCTGGACCGATGACCA GCCAAAGTTGGGCGCGGTTG	264

Data collection method

Data regarding age, sex and duration of hospital stay were collected using the predesigned data collection sheet.

Data analysis

Data analysis was done by using 'Microsoft office excel 2010' program and according to the objectives of the study. The test of significant will be calculated by using X^2 test. $P<0.05$ was taken as minimal level of significance.

Ethics

This study was approved by the research review committee (RRC) of the department of microbiology and ethical review committee (ERC) of DMC. Informed written consent was taken from each patient or authorized legal guardian before sample collection. Participants were informed about the purpose of the study and about their right to withdraw themselves from the study whenever they want. Anonymity of the patients and confidentiality of information was maintained strictly.

RESULTS

Among the total 446 collected samples, 376 (84.31%) yielded growth, of which 232 (61.70%) were *P. aeruginosa*. They were mostly prevalent in burn wounds (85.03%) followed by ETA and other wounds. Out of all 232 *P. aeruginosa*, 72 (31.03%) were imipenem resistant (Table 2). Out of all 232 positive strains, 41.81% belonged to the age group of 0-20 years and 54.31% and 45.69% were male and female patients respectively (Table 3).

Among the 72 imipenem-resistant *P. aeruginosa*, all were resistant to ceftriaxone and 69 (95.83%) were resistant to ciprofloxacin and gentamicin, 67 (93.06%) to ceftazidime and cefotaxime, 63 (87.5%) to aztreonam and 62 (86.11%) to amikacin. Only 4 (5.56%) imipenem resistant isolates were resistant to colistin (Table 4).

Out of 72 imipenem resistant *P. aeruginosa*, 57 (79.17%) were phenotypically detected as ESBL producers.

Among 57 phenotypically ESBL producing imipenem resistant *P. aeruginosa*, 17 (29.83%) were blaOXA-10 positive, 10 (17.55%) were positive for blaSHV and blaTEM, 8 (14.04%) for both blaSHV and blaOXA-10. Twelve of them possessed at least two, whereas, the other four contained three ESBL genes in different combinations. One imipenem resistant *P. aeruginosa* isolated from burn wound sample had all four ESBL encoding genes (Table 5).

All the 72-imipenem resistant *P. aeruginosa* were phenotypically carbapenemase producers by CD, DDS and MHT. Among them, 30 (41.67%) were positive in all three methods, followed by 36.11% by CD test, 16.67% by DDS, and 5.55% by both DDS and CD. Fifty five percent of the imipenem resistant *P. aeruginosa* isolated from the burn wounds, and 75% from that of the ETA samples produce carbapenemase phenotypically (Table 6).

Among 72 imipenem resistant *P. aeruginosa*, 36 (50%) were MBL positive and 25 (34.72%) were positive for blaNDM-1. blaVIM alone was present in 9.72% of all the MBL positive strains. None of the MBL producers had blaIMP gene (Table 7).

Forty-two percent of the imipenem resistant *P. aeruginosa* were positive for only ESBL. Nine (13%) of the imipenem resistant *P. aeruginosa* possessed only MBL (Figure 1). Twenty-seven imipenem resistant *P. aeruginosa* (38%) which were positive for both ESBL and MBL genes, had different combination of these genes (Table 8).

Table 2: Distribution of imipenem resistant *P. aeruginosa* in different samples.

Samples	Total	Growth, N (%)	*P. aeruginosa*, N (%)	Imipenem resistant *P. aeruginosa*, N (%)
Surgical/ traumatic wounds	274	207 (75.55)	96 (46.38)	32 (33.33)
Burn wounds	148	147 (99.32)	125 (85.03)	36 (28.80)
ETA	24	22 (91.67)	11 (50.00)	4 (36.36)
Total	446	376 (84.31)	232 (61.70)	72 (31.03)

Table 3: Demographic distribution of patients infected by *P. aeruginosa* (N=232).

Age group (in) years	Number of patients infected by *P. aeruginosa*, N (%)	Male	Female
0-20	97 (41.81)	47	50
0-10	58	24	34
11-20	39	23	16
21-40	81 (34.91)	49	32
21-30	58	37	21
31-40	23	12	11
41-60	38 (16.38)	19	19
41-50	24	14	10
51-60	14	5	9
>60	16 (6.90)	11	5
Total	232 (100)	126 (54.31)	106 (45.69)

Table 4: Antibiotic resistance pattern among isolated imipenem resistant *P. aeruginosa,* (n=72).

Antimicrobial agents	N (%)
Amoxicillin/ clavulnic acid	68 (94.44)
Piperacillin/ Tazobactam	51 (70.83)
Amikacin	62 (86.11)
Gentamicin	69 (95.83)
Netilmicin	54 (75)
Aztreonam	63 (87.5)
Ciprofloxacin	69 (95.83)
Ceftriaxone	72 (100)
Cefotaxime	67 (93.06)
Ceftazidime	67 (93.06)
Colistin	4 (5.56)

Table 5: Distribution of ESBL encoding genes in imipenem resistant *P. aeruginosa* isolated from different samples.

ESBL encoding genes	Burn wounds, n=35 (%)	Surgical/ traumatic wounds, n=20 (%)	ETA, n=2 (%)	Total, n=57 (%)
blaPER-1	1 (2.86)	2 (1)	0 (0)	3 (5.26)
blaSHV	3 (8.57)	7 (35)	0 (0)	10 (17.55)
blaTEM	10 (28.57)	0 (0)	0 (0)	10 (17.55)
blaOXA-10	9 (25.71)	7 (35)	1 (50)	17 (29.83)
blaPER-1+ blaSHV	0 (0)	1 (5)	0 (0)	1 (1.75)
blaPER-1+ blaOXA-10	1 (2.86)	0 (0)	0 (0)	1 (1.75)
blaTEM+ blaSHV	1 (2.86)	0 (0)	0 (0)	1 (1.75)
blaTEM+ blaOXA-10	0 (0)	0 (0)	1 (50)	1 (1.75)
blaOXA-10+ blaSHV	5 (14.29)	3 (15)	0 (0)	8 (14.04)
blaPER-1+ blaSHV+ blaOXA-10	2 (5.71)	0 (0)	0 (0)	2 (3.51)
blaTEM+ blaSHV+ blaOXA-10	2 (5.71)	0 (0)	0 (0)	2 (3.51)
blaPER-1+ blaSHV+ blaTEM+ blaOXA-10	1 (2.86)	0 (0)	0 (0)	1 (1.75)

Table 6: Phenotypic detection of carbapenemase producers among imipenem resistant *P. aeruginosa* by DDS, CD and MHT.

Types of samples	DDS, N (%)	CD, N (%)	DDS+CD, N (%)	DDS+ CD+ MHT, N (%)
Burn wounds, (N=40)	12 (30)	4 (10)	2 (5)	22 (55)
Surgery/ traumatic wounds, (N=24)	0 (0.00)	22 (91.67)	0 (0.00)	2 (8.33)
ETA, (N=8)	0 (0.00)	0 (0.00)	2 (25)	6 (75)
Total, (N=72)	12 (16.67)	26 (36.11)	4 (5.55)	30 (41.67)

Table 7: Distribution of MBL encoding genes among imipenem resistant *P. aeruginosa* by PCR isolated from different samples.

MBL encoding genes	Burn wounds, n=40 (%)	Surgical/ traumatic wounds, n=24 (%)	ETA, n=8 (%)	Total, n=72 (%)
blaNDM-1	13 (32.5)	7 (29.17)	5 (62.5)	25 (34.72)
blaVIM	4 (10)	3 (12.5)	0 (0)	7 (9.72)
blaNDM-1+blaVIM	4 (10)	0 (0)	0 (0)	4 (5.56)
blaIMP	0 (0)	0 (0)	0 (0)	0 (0)

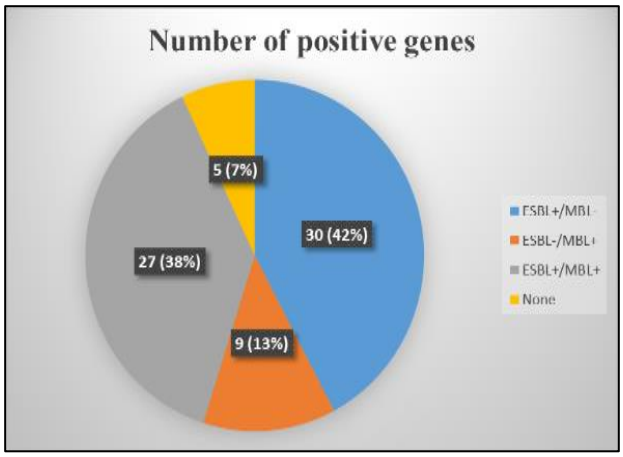

Figure 1: Distribution of ESBL, MBL and both genes in imipenem resistant *P. aeruginosa*.

Table 8: Distribution of different combination of both ESBL and MBL genes in imipenem resistant *P. aeruginosa* (N=72).

ESBL$^+$ / MBL$^+$	Number of positive strains
NDM+ VIM+ PER-1+ OXA-10+ SHV	1
NDM+ VIM+ OXA-10+ SHV	1
NDM+ VIM+ PER-1+ OXA-10+ SHV+ TEM	1
NDM+ VIM+ OXA-10+ SHV+ TEM	1
NDM+ TEM	4
NDM+ SHV	4
NDM+ OXA-10+ SHV	3
NDM+ PER-1+ SHV	1
NDM+ PER-1+ OXA-10	1
NDM+ OXA-10+ SHV+ TEM	2
NDM+ OXA-10+ TEM	1
NDM+ OXA-10	2
VIM+OXA-10	3
VIM+ TEM	1
VIM+ PER-1+ OXA-10+ SHV	1
Total (N=72)	27 (38%)

DISCUSSION

In recent years, *P. aeruginosa* became an acute problem in hospitals due to its capacity to acquire resistance to all effective antibiotics as well as intrinsic resistance to important antibiotic classes. It is now considered as a major microorganism to be monitored for antibiotic resistance.[28] This study overviewed the prevalence of imipenem resistance, prevalence of ESBL and MBL encoding genes and co-presence of multiple antibiotic resistance genes among *P. aeruginosa*. There is a limited data in Bangladesh regarding this aspect. Moreover, in Bangladesh, empirical treatments are widely practiced without sensitivity reports. Therefore, this study will help to provide an insight to the clinicians to select appropriate antibiotics which in turn will help them to control the spread of antibiotic resistant organisms.

A lot of patients with wound infection and burn are managed in DMCH. There is an ICU, where endotracheal tubes are often used. Despite the technological advances in patient management and as well as in hygiene maintenance, wound infection is regarded as a common nosocomial infection. In this study, we observed that the highest percentage of *P. aeruginosa* (85.03%) were isolated from the infected burn wounds.

In consistent with the present findings, 35.2% carbapenem resistant *P. aeruginosa* were reported.[29] Besides, a study on hospital environment of tertiary care hospital, showed 39% carbapenem resistant *P. aeruginosa*.[30] The higher proportion of carbapenem resistant *P. aeruginosa* might be due to the increased use of carbapenem as well as heavy burden of carbapenem resistant *P. aeruginosa* in the hospital environment.

In this study, ESBL encoding genes were found in substantial number in imipenem resistant *P. aeruginosa* (79.17%). blaOXA most prevalent ESBL encoding gene followed by blaSHV, blaTEM and blaPER in different combination in this study. A similar prevalence was reported in a previous study in 2015.[12] Jobayer et al also observed high rate of ESBL producing *P. aeruginosa*.[31] However, there was an increasing trend of ESBL producers up to 2015 among the gram-negative bacteria isolated from the patients of DMCH. It might be due to the fact that, these strains are now treated invariably by carbapenems. As a result, MBL producers are increasing now due to overuse and misuse of this valuable resource.

In the present study, although all the imipenem resistant *P. aeruginosa* were phenotypically positive for MBL, it was observed that half of these strains had MBL genes

detected by PCR. This incidence might be due to the presence of other carbapenemase genes among these isolates. Contradicting this study, a previous study in DMCH demonstrated 55% *bla*NDM-1, 15% blaVIM, and 10% blaIMP positive uropathogens in 2016.[32] The reasons might be the less sample size, and different organisms in that respective study.

In this study, 38% of the imipenem resistant *P. aeruginosa* harbored both ESBL and MBL genes in different combinations, which is triple than that of a previous study.[33] However, multiple studies conducted in neighboring countries have also reported co-existence of NDM-1 and other ESBL encoding genes in clinical isolates.[34,35] According to Mirza et al coproduction of these enzymes is organism independent.[35] These observations are alarming on regard of spreading hazardous infections by means of these organisms that are simultaneously possessing multiple antibiotic resistance genes. Moreover, monobactam is sensitive and used to treat MBL producing organisms in many cases, whereas, ESBL producing organisms are resistant to monobactams.

In this study, a majority (87.5%) of imipenem resistant isolates were resistant to aztreonam, which makes the treatment of infections caused by this organism more challenging.[36]

Due to resource and time constraints, all the pseudomonads, and other antibiotic resistance genes were not detected and DNA sequencing of the genes in this study were not done.

Due to resource and time constraints, all the ESBL and MBL genes could not be detected and DNA sequencing of the genes in this study were not done.

CONCLUSION

The occurrence of ESBL, MBL and concurring both genes in a single strain of *P. aeruginosa* highlights the emerging therapeutic challenge in Bangladesh. In this study, imipenem resistant *P. aeruginosa* showed simultaneous presence of multiple genes as well as high prevalence of ESBL encoding gene blaOXA-10 and MBL encoding gene blaNDM-1. It signifies a great possibility of widespread dissemination of these genes in other bacteria through *P. aeruginosa* in hospital settings. Thus, early screening, implementation of infection prevention control programs and strict antimicrobial policies should be encouraged.

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