Original Article

Emergence of OXA-833 in *Proteus* Species at a Tertiary Care Hospital in Dhaka, Bangladesh

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Abstract

Context: *Proteus* species are liable for multitude of infections and associated with resistance to routinely used antibiotics even to reserve drugs such as carbapenems. **Aims:** The aim of this study was to detect the presence of MBL producers, including *bla*OXA-833 gene in *Proteus* spp. along with their antibiotic resistance pattern. **Settings and Design:** This cross-sectional study was conducted in the Department of Microbiology of a tertiary care hospital of Bangladesh during July 2018 to June 2019. **Subjects and Methods:** *Proteus* spp. was isolated from a total of 500 samples. Antibiotic susceptibility was performed by disk-diffusion technique. Minimum inhibitory concentration (MIC) of imipenem was determined by agar dilution method. Carbapenemase producers were phenotypically detected by double disc synergy (DDS) test, combined disc (CD) assay, and modified Hodge test (MHT). Carbapenemase genes *(bla*KPC, *bla*VIM, *bla*IMP, *bla*NDM-1, *bla*OXA-23, *bla*OXA-48-like/*bla*OXA-833, and *bla*OXA-58) among imipenem-resistant *Proteus* spp. were detected by polymerase chain reaction (PCR). Sequencing was performed to differentiate OXA-833 from OXA-48-like gene by capillary method, and the nucleotide sequence of OXA-833 has been deposited to GenBank. **Results:** Ten (25%) imipenem-resistant isolates were detected during disk-diffusion technique, among them 60%, 70%, 50% carbapenemase producers were detected by DDS test, CD assay, MHT, respectively, and 70% by PCR. A significant increase in MIC was found between 8 and ≥128 μg/ml to imipenem. PCR revealed that 40% imipenem-resistant isolates were positive for *bla*NDM-1 and *bla*VIM followed by 20% for *bla*OXA-48-like/*bla*OXA-833 and *bla*OXA-23, respectively. Sequencing of *bla*OXA-48-like gene established the OXA-833 variant of class D carbapenemase encoding gene. **Conclusion:** The results of this study showed the presence of high proportion of carbapenemase enzyme-producing *Proteus* spp. in Bangladesh. *bla*OXA-833 is emerging in Bangladesh.

Keywords: *BlaOXA-833 gene, carbapenemase, Proteus spp., sequencing and Bangladesh*

Introduction

Carbapenems are considered to be one of the most effective drugs for the treatment of infections caused by multidrug-resistant Gram-negative bacteria.[1] Carbapenem-resistant *Enterobacteriaceae* (CRE) has emerged as a global threat.[2] *Proteus* species usually show high resistance to antibiotics that are commonly used.[3] Due to the threat of emergence of extensively drug-resistant or pandrug-resistant strains, wide dissemination of *bla*OXA-48 and other carbapenemase gene is of major concern within bacterial species such as *P. mirabilis* exhibiting intrinsic resistance to tetracyclines and polymyxins.[4] Since the first detection of OXA-48, different OXA-48-like β-lactamases have been identified worldwide (OXA-162, OXA-181, OXA-163, OXA-204, and OXA-232), differing by few amino acid substitutions or deletions.[5] To the best of our knowledge, no study has so far been carried out among *Proteus* spp. isolated from wound swab and pus, urine, and blood samples in Bangladesh regarding detection of OXA-833. Considering the public health threat of acquisition of MBL determinants in *Proteus* species, this study has been designed to obtain data on the resistance patterns of *Proteus* spp. along with the detection of genes encoding carbapenemases by polymerase chain reaction (PCR) and sequencing.

Subjects and Methods

After obtaining approval from the institutional ethical committee, this cross-sectional study was conducted in the Department of Microbiology of a tertiary

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care hospital of Bangladesh during July 2018 to June 2019. Informed written consent was taken from each patient or their legal guardian. Wound swab and pus, urine, and blood of adult patients having clinically suspected infections admitting in a tertiary care hospital of Bangladesh or attending in the Microbiology department for culture and sensitivity were included in this study irrespective of sex and antibiotic intake.

Identification of *Proteus* spp.

All samples were inoculated in MacConkey agar and blood agar media and incubated overnight aerobically at 37°C. Trypticase soya broth was used for primary blood culture then subculture was done on blood agar and MacConkey agar media. *Proteus mirabilis* and *Proteus vulgaris* were identified by colony morphology, staining character, characteristic "fishy smell," swarming growth on blood agar media, and biochemical tests as per standard technique.[6]

Antimicrobial susceptibility testing

Kirby–Bauer modified disc diffusion technique was used for antimicrobial susceptibility using Mueller-Hinton agar plates and the zone of inhibition was interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines and criteria of the European Committee on Antimicrobial susceptibility testing were used for fosfomycin (Oxoid Ltd., UK).[7-9] Antibiotic disks such as ceftazidime (30 µg), ceftriaxone (30 µg), cefoxitin (30 µg), cefepime (30 µg), imipenem (10 µg), amoxiclav (amoxicillin and clavulanic acid) (20/10 µg), ciprofloxacin (5 µg), amikacin (30 µg), gentamicin (10 µg), aztreonam (30 µg), trimethoprim-sulfamethoxazole (25 µg), piperacillin-tazobactam (100/10 µg), and fosfomycin (200 µg) were used. *Escherichia coli* ATCC 25922 was used as control strain for susceptibility test.

Determination of minimum inhibitory concentration of imipenem

MIC of imipenem was determined by agar dilution method following CLSI guideline 2018.[8]

Phenotypic detection of carbapenemase producers

Carbapenemase-producing *Proteus* spp. were phenotypically detected by DDS test, CD assay, MHT on Mueller-Hinton agar media by Kim *et al*., Qu *et al.,* and Amjad *et al.,* respectively.[10-12]

Molecular characterization of carbapenemase producers

PCR was done to detect carbapenemase genes (*bla*KPC, *bla*IMP, *bla*VIM, *bla*NDM-1, *bla*OXA-23, *bla*OXA-48-like/OXA-833, and *bla*OXA-58) among the imipenem-resistant *Proteus* spp. To prepare bacterial pellets, a loop full of bacterial colonies was introduced into a Falcon tube containing Trypticase soya broth. After overnight incubation at 37°C, the Falcon tubes were centrifuged at 4,000 rpm for 10 min at 4°C. The supernatant was discarded and the deposit was kept at –20°C until DNA extraction. Boiling method was used for DNA extraction.[13] Genes were detected by PCR using the primers shown in table 1.[14-17]

Table 1: Primers used in this study

Target gene	Primer sequence (5′-3′)	Size (bp)
*bla*KPC	F-CGTCTAGTTCTGCTGTCTTG	498
	R-CTTGTCATCCTTGTTAGGCG	
*bla*IMP	F-GGAATAGAGTGGTTAAYTCTC	188
	R-CCAAACYACTASGTTATCT	
*bla*VIM	F-GATGGTGTTTGGTCGCATA	390
	R-CGAATGCGCAGCACCAG	
*bla*NDM-1	F-ACCGCCTGGACCGATGACCA	264
	R-GCCAAAGTTGGGCGCGGTTG	
*bla*OXA-23	F-GATCGGATTGGAGAACCAGA	501
	R-ATTTCTGACCGCATTTCCAT	
*bla*OXA-48 like	F-ATGCGTGTATTAGCCTTATCG	888
	R-AACTACAAGCGCATCGAGCA	
*bla*OXA-58	F-GCCATTCCCCAGCCACTTTTA	599
	R-CACGCATTTAGACCGAGCA	

PCR condition included initial denaturation at 94°C for 10 min, then 35 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 40 s, extension at 72°C for 1 min followed by a single final extension step at 72°C for 10 min. The PCR product was loaded into a 1.5% agarose gel, electrophoresed at 100 volts for 35 min, stained with 1% ethidium bromide, and visualized under UV light [Figure 1].

DNA sequence analysis

Sequencing was performed to differentiate OXA-833 gene from OXA-48-like gene. After PCR, purification of amplicons was done by using DNA purification kit (FAVORGEN, Biotech Corp.) and subjected to automated DNA sequencing (ABI PRISM 3500). BLAST (Basic Local Alignment Search Tool) analysis was performed to search for homologous sequences into the GenBank database.

Statistical analysis

Data were analyzed by using Microsoft Office Excel (2013) software (Microsoft, Redmond, WA, USA).

Results

Among the 500 samples, forty *Proteus* spp. were identified. Of them, 32 (80%) were *Proteus mirabilis* and 8 (20%) were *Proteus vulgaris*. Out of 40 isolates, 10 (25%) were resistant to imipenem during disk-diffusion technique, of which 8 (80%) were *P. mirabilis* and 2 (20%) were *P. vulgaris*. A significant proportion of *Proteus* spp. showed high resistance to commonly used antibiotics whereas fosfomycin was found the most sensitive drug followed by imipenem [Table 2]. MIC of 10 imipenem-resistant isolates ranged from 8 µg/ml to ≥128 µg/ml [Table 3].

Table 2: Resistance pattern of imipenem-resistant *Proteus* spp. (*n*=10)

Antimicrobial agent	*Proteus mirabilis* (*N*=8), *n* (%)	*Proteus vulgaris* (*N*=2), *n* (%)	Total (*N*=10), *n* (%)
Fosfomycin	0 (0.00)	0 (0.00)	0 (0.00)
Piperacillin-tazobactam	7 (87.50)	1 (50.00)	8 (80.00)
Aztreonam	7 (87.50)	2 (100.00)	9 (90.00)
Cefoxitin	7 (87.50)	2 (100.00)	9 (90.00)
Ceftriaxone	8 (100.00)	2 (100.00)	10 (100.00)
Ceftazidime	5 (62.50)	2 (100.00)	7 (70.00)
Cefepime	6 (75.00)	2 (100.00)	8 (80.00)
Amikacin	7 (87.50)	1 (50.00)	8 (80.00)
Gentamicin	8 (100.00)	2 (100.00)	10 (100.00)
Ciprofloxacin	8 (100.00)	2 (100.00)	10 (100.00)
Amoxiclav	8 (100.00)	2 (100.00)	10 (100.00)
Trimethoprim-sulfamethoxazole	7 (87.50)	2 (100.00)	9 (90.00)

N=Total number of bacteria; *n*=Number of resistant bacteria

Table 3: Minimum inhibitory concentration of imipenem among metallo-beta-lactamases producers

MBL producers	MIC of imipenem (µg/ml)					
	≥256	128	64	32	16	≤8
Proteus mirabilis (*n*=8)	-	2 (25.00)	2 (25.00)	3 (37.50)	1 (12.50)	-
Proteus vulgaris (*n*=2)	-	-	-	-	1 (50.00)	1 (50.00)
Total (*n*=10)	-	2 (20.00)	2 (20.00)	3 (30.00)	2 (20.00)	1 (10.00)

Figure within parenthesis indicates percentage. MIC: Minimum inhibitory concentration; MBL: Metallo-beta-lactamases

Among 10 imipenem-resistant isolates, 8 (80%) were isolated from wound swab and pus, 2 (20%) from urine samples and 6 (60%), 7 (70%), 5 (50%) carbapenemase producers were detected by DDS test, CD assay, MHT, respectively, and 7 (70%) by PCR. Out of 8 imipenem-resistant *P. mirabilis,* 7 (87.5%) carbapenemase producers were detected by PCR. No *P. vulgaris* isolates were detected positive for any carbapenemase encoding genes by PCR [Table 4]. Four (40%) of the imipenem-resistant strains were found positive for *bla*NDM-1 and *bla*VIM gene followed by 2 (20%) positive for *bla*OXA-23 and *bla*OXA-48-like/*bla*OXA-833 gene, respectively. The combinations of different genes in single strains were detected. Table 5 shows that co-carriage of *bla*NDM-1 and *bla*VIM was found in two (20%) isolates detected in wound swab and pus sample and co-carriage of *bla*NDM-1 and *bla*OXA-48-like were found in one (10%) isolate detected in wound swab and pus sample and co-carriage of *bla*VIM, *bla*OXA-23, *bla*OXA-48-like was found in one (10%) isolate detected in urine sample. None of the isolates were positive for *bla*KPC, *bla*IMP, and *bla*OXA-58 genes [Table 5].

Sequencing of 2 *bla*OXA-48-like gene had 99% and 95% identity with the *bla*OXA-833 gene detected in *Klebsiella pneumoniae* (strain: B2354) (GenBank accession: NG065443.1) isolated from urine and wound swab and pus sample, respectively.

Nucleotide sequence accession number

The nucleotide sequence of OXA-833 gene of *P. mirabilis* strain F-18 obtained from urine sample and *P. mirabilis* strain D-19 obtained from wound swab and pus sample has been deposited in the GenBank database under accession no. MW048624 and MW122948, respectively.

Table 4: Detection of carbapenemase producers by polymerase chain reaction among imipenem-resistant *Proteus* spp. (*n*=10)

Organism	Carbapenemase producers		Total, *n* (%)
	Positive, *n* (%)	Negative, *n* (%)	
Proteus mirabilis	7 (87.50)	1 (12.50)	8 (100.00)
Proteus vulgaris	0 (0.00)	2 (100.00)	2 (100.00)
Total	7 (70.00)	3 (30.00)	10 (100.00)

N=Total number of imipenem-resistant *Proteus* spp.; *n*=Number of positive or negative cases of carbapenemase producers by PCR; PCR: Polymerase chain reaction

Discussion

In this study, 7 (70%) carbapenemase producers were detected by PCR. *Bla*NDM-1, *bla*VIM, *bla*OXA-23, and *bla*OXA-833 were found to be responsible for imipenem resistance. *Bla*NDM-1 (40%) and *bla*VIM (40%) were the most prevalent carbapenemase encoding genes. In Bangladesh, Farzana *et al.* revealed (22.86%) *bla*NDM-1 and (37.15%) *bla*VIM gene among Gram-negative bacteria.[18] In India, Naim *et al*. reported 50% *bla*NDM-1 producing *Proteus* spp. among carbapenem-resistant isolates, which was nearly in agreement with the present study.[19] This increase in the proportion of *bla*NDM-1 and *bla*VIM might be due to the fact that, in the recent past, the use of carbapenem had increased due to emergence

Table 5: Distribution of blaKPC, *bla*VIM, *bla*IMP, *bla*NDM-1, *bla*OXA-23, *bla*OXA-48-like/833, and *bla*OXA-58 genes among imipenem-resistant *Proteus mirabilis* in different samples (*n*=10)

Samples	KPC, *n* (%)	VIM, *n* (%)	IMP, *n* (%)	NDM-1, *n* (%)	OXA-23, *n* (%)	OXA-48-like, *n* (%)	OXA-58, *n* (%)	Total, *n* (%)
Wound swab and pus	-	+	-	-	-	-	-	2 (20.00)
Wound swab and pus	-	-	-	-	+	-	-	1 (10.00)
Wound swab and pus	-	-	-	-	-	-	-	3 (30.00)
Wound swab and pus	-	+	-	-	-	-	-	1 (10.00)
Wound swab and pus	-	-	-	-	-	+	-	1 (10.00)
Urine	-	-	-	-	-	-	-	1 (10.00)
Urine	-	+	-	-	+	+	-	1 (10.00)
Total*	0 (0.00)	4 (40.00)	0 (0.00)	4 (40.00)	2 (20.00)	2 (20.00)	0 (0.00)	10 (100.00)*

*Denotes the column total, +=Present, −=Absent. The total of last row is more as some of the isolates had more than one gene.
KPC: Klebsiella pneumoniae carbapenemase; VIM: Verona-Integron-encoded; IMP: Imipenem; NDM-1: New Delhi metallo-beta lactamase; OXA-23: Oxacillin hydrolyzing-23; OXA-48: Oxacillin hydrolyzing-48; OXA-58: Oxacillin hydrolyzing-58

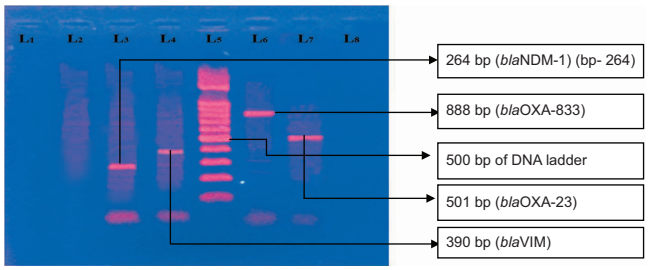

Figure 1: Photograph of gel electrophoresis of negative control without DNA (TE buffer) (lane 1), negative control *Escherichia coli* ATCC 25922 (lane 2), amplified DNA of 264 bp for *bla*NDM-1 gene (lane 3), amplified DNA of 390 bp for *bla*VIM gene (lane 4), hundred bp DNA ladder (lane 5), amplified DNA of 888 bp for *bla*OXA-833 gene (lane 6), amplified DNA of 501 bp of blaOXA-23 gene (lane 7), and negative sample (lane 8)

of resistance against cephalosporin and penicillin. In the present study, 20% *bla*OXA-23-positive *Proteus* spp. were detected by PCR. Study by Österblad *et al*. reported *Acinetobacter* type class D carbapenemase *bla*OXA-23 gene in *P. mirabilis*.[20] In the present study, *bla*OXA-48-like-positive *Proteus* spp. detected by PCR was validated by sequencing as *bla*OXA-833 gene. The identified OXA-833 in this study was the first detected class D carbapenemase encoding gene in *P. mirabilis* isolates and become an emerging threat in Bangladesh. Study by Fursova *et al*. reported 23.3% *bla*OXA-48 gene in *Proteus* spp. which was close to the present finding.[21] In the present study, co-carriage of *bla*NDM-1 and *bla*VIM was found in two (20%) isolates detected in wound swab and pus sample and co-carriage of *bla*NDM-1 and *bla*OXA-48-like were found in one (10%) isolate detected in wound swab and pus sample and co-carriage of *bla*VIM, *bla*OXA-23, *bla*OXA-48 like was found in one (10%) isolate detected in urine sample. A study by Khatun and Shamsuzzaman also reported 31.6% imipenem-resistant isolates contained two or more carbapenemase genes which is in accordance to the present study.[22] In this study, MIC of imipenem among 10 imipenem-resistant *Proteus* isolates ranged from 8 to ≥128 μg/ml. The type and expression of carbapenemase enzymes, other resistance mechanisms (ESBL and AmpC β-lactamase), reduced permeability and efflux mechanisms may be the cause of these variations in MIC value.[23] The present study reported that *Proteus* species were resistant to most of the commonly used antibiotics in Bangladesh with increased resistance to imipenem except fosfomycin which was 100% sensitive. Although colistin and tigecycline are considered to be the most effective drugs against CRE but *Proteus* spp. shows intrinsic resistance to these drugs. The high antibiotic resistance in the present study might be due to indiscriminate use of antibiotics that provide selective pressure.

Conclusion

*Bla*OXA-833 producers are emerging in Bangladesh which were detected in *Proteus mirabilis*. The acquisition of carbapenemase genes in *Proteus* species may be of major concern for physicians because this organism is intrinsically resistant to colistin and tigecycline thereby limiting treatment options. In such cases, combination therapy may be the best option for the treatment of infections caused by *Proteus* species. And also, early detection of drug-resistant bacterial strains with their resistance mechanism and application of strict antimicrobial policies may help to prevent the rapid spread of these organisms.

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Ethical clearance

Ethical permission for this study was obtained from the institutional review board.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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