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

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 blaOXA-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 (blaKPC, blaVIM, blaIMP, blaNDM-1, blaOXA-23, blaOXA-48-like/blaOXA-833, and blaOXA-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 blaNDM-1 and blaVIM followed by 20% for blaOXA-48-like/blaOXA-833 and blaOXA-23, respectively. Sequencing of blaOXA-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. blaOXA-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 blaOXA-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
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), trimethoprim-sulfamethoxazole (25 μg), and fosfomycin (100 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.[10]

**Phenotypic detection of carbapenemase producers**

Carbapenemase-producing *Proteus* spp. were phenotypically detected by DDS test, CD assay, MHT on Mueller-Hinton 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 carbapenemase genes.[10-12] 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 (FA VORGEN, 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 was the most sensitive drug followed by imipenem. MIC of 10 imipenem-resistant isolates ranged from 8 μg/ml to ≥128 μg/ml [Table 3].

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**Table 1: Primers used in this study**

| Target gene | Primer sequence (5'-3') | Size (bp) |
|-------------|-------------------------|-----------|
| blaKPC      | F-CGTCATGTTTGGCTGCATTTG  | 498       |
|             | R-CTTGTACACTTGTGTAGCCG   |           |
| blaIMP      | F-GGAATAGGTGATTAATTCCTC  | 188       |
|             | R-CCAAAYACTAAGTTATCTT    |           |
| blaVIM      | F-GATGGTGTTTGGTGCCGATA   | 390       |
|             | R-CGAATGGCCAGCACCG       |           |
| blaNDM-1    | F-ACGGCTTGAGGCTGACCCA    | 264       |
|             | R-GCCAAATGTTCGGCGATG     |           |
| blaOXA-23   | F-GATCGGATTGGAGAACCAGA   | 501       |
|             | R-ATTTCGACGCCATTCAT      |           |
| blaOXA-48 like | F-ATGGTGTTTGGTGCCGATA    | 888       |
|             | R-AACTACAAGGCCATCG       |           |
| blaOXA-58   | F-GCCATTCCCAAGACCAATTTA  | 599       |
|             | R-CACGATTGAGACCGAGA      |           |
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 blaNDM-1 and blaVIM gene followed by 2 (20%) positive for blaOXA-23 and blaOXA-48-like/blaOXA-833 gene, respectively. The combinations of different genes in single strains were detected. Table 5 shows that co-carriage of blaNDM-1 and blaVIM was found in two (20%) isolates detected in wound swab and pus sample and co-carriage of blaNDM-1 and blaOXA-48-like were found in one (10%) isolate detected in wound swab and pus sample and co-carriage of blaVIM, blaOXA-23, blaOXA-48-like was found in one (10%) isolate detected in urine sample. None of the isolates were positive for blaKPC, blaIMP, and blaOXA-58 genes [Table 5].

Sequencing of 2 blaOXA-48-like gene had 99% and 95% identity with the blaOXA-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.

### Discussion

In this study, 7 (70%) carbapenemase producers were detected by PCR. BlaNDM-1, blaVIM, blaOXA-23, and blaOXA-833 were found to be responsible for imipenem resistance. BlaNDM-1 (40%) and blaVIM (40%) were the most prevalent carbapenemase encoding genes. In Bangladesh, Farzana et al. revealed (22.86%) blaNDM-1 and (37.15%) blaVIM gene among Gram-negative bacteria.[18] In India, Naim et al. reported 50% blaNDM-1 producing Proteus spp. among carbapenem-resistant isolates, which was nearly in agreement with the present study.[19] This increase in the proportion of blaNDM-1 and blaVIM might be due to the fact that, in the recent past, the use of carbapenem had increased due to emergence
of resistance against cephalosporin and penicillin. In the present study, 20% blaOXA-23-positive _Proteus_ spp. were detected by PCR. Study by Österblad et al. reported _Acinetobacter_ type class D carbapenemase _blaOXA-23_ gene in _P. mirabilis_. In the present study, _blaOXA-48-like/-833_ gene was detected in _P. mirabilis_ isolate. 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% _blaOXA-48_ gene in _Proteus_ spp. which was close to the present finding. In the present study, co-carriage of _blaNDM-1_ and _blaVIM_ was found in 20% isolates detected in wound swab and pus sample and co-carriage of _blaNDM-1_ and _blaOXA-48-like_ were found in 10% isolate detected in wound swab and pus sample and co-carriage of _blaVIM, blaOXA-23, blaOXA-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. 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. 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**

_BlaOXA-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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**Table 5: Distribution of blaKPC, blaVIM, blaIMP, blaNDM-1, blaOXA-23, blaOXA-48-like/833, and blaOXA-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)   |
| 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.
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