Original Article

Emergence of Carbapenemase Encoding Genes in Proteus Species in Tertiary Care Hospital of Bangladesh

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Abstract

Along with different carbapenemase encoding genes, in recent year class D OXA enzymes are documented in Proteus spp which are not common in Enterobacteriaceae. The dissemination of plasmids, transposons and integrons among bacteria and species playing roles for this dissemination. So, this study was designed to observe the emergence and distribution of different classes of carbapenemase encoding genes among imipenem resistant Proteus spp. isolated from tertiary care hospital in Bangladesh. Total 15 imipenem resistant Proteus isolates were included in this study, which were collected from wound swab, pus, urine and blood samples. Identification was done by culture and biochemical test and antibiotic susceptibility test was done by disc diffusion method. MIC of imipenem (g/ml) was done among imipenem resistant P. mirabilis by agar dilution method. blaKPC, blaNDM-1, blaVIM, blaIMP, blaOXA-48 like, blaOXA-23 like, blaOXA-51 like, blaOXA-58 like carbapenemase encoding genes were detected among imipenem resistant Proteus spp. by PCR and sequencing of blaOXA-48 like, blaOXA-51 like gene done by capillary method to compare the sequences with the same gene, available in gene bank. Among 15 imipenem resistant isolates blaNDM-1 (26.67%), blaKPC (20%), blaVIM (20%), blaOXA-484 (20%) were predominant carbapenemase encoding genes followed by blaOXA-66(6.67%).

This study finds that blaOXA-484 gene and blaOXA-66 class D carbapenemase encoding genes are emerging in Proteus spp. and may play a contributing factor in developing carbapenem resistance.

Keywords: *Proteus, blaOXA-484, blaOXA-66, carbapenemases,*

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INTRODUCTION

The genus *Proteus,* are commonly implicated pathogens in hospitals and as a cause of community acquired infections.[1] This pathogen has various mode of transmission and causes infections in different anatomical sites.[2] It ranks as 3rd cause of health care associated infections.[3] Carbapenem antibiotics have been used as a last resort to treat infections caused by multidrug resistant gram negative bacteria till date.[4] But in recent years rampant global spread of carbapenem resistant Enterobacteriaceae members becoming a threat, including *Proteus*.[5] Acquisition of carbapenemase enzymes and loss of porins are the main mechanism of resistance to this group of antibiotic.[6] The most common carbapenemases include veronica integrin metallo-beta-lactamases types (VIM), imipenemase (IMP) types, *Klebsiella pneumoniae* carbapenemase (KPC), oxacillinase-48 (OXA-48), and New Delhi metallo-beta-lactamase-1 (NDM-1), encoded by carbapenem resistance determining genes *bla*VIM, *bla*IMP,*bla*KPC, *bla*OXA-48 and *bla*NDM, respectively.[7]

This study was designed to evaluate the carbapenem resistance pattern along with distribution of genes encoding carbapenem resistance among *Proteus spp.* isolated from tertiary care hospital in Bangladesh.

MATERIALS AND METHODS

This cross sectional analytic type of study was conducted in the Department of Microbiology, Dhaka Medical College, Dhaka, from January 2016 to December 2016. Ethical clearance was taken from the ethical review committee of the institution. A total 310 wound swabs, pus, urine and blood samples were isolated from of clinically suspected infected patients in Dhaka Medical College Hospital.

IDENTIFICATION OF *PROTEUS* SPECIES

Allthe wound swab, pus, urine and blood samples, collected from the patients were inoculated on blood agar and Mac Conkey's agar media and incubated at 37ºC

aerobically for 24 hours. Primary blood culture was done in Trypticase Soy Broth followed by subculture on blood and MacConkey's agar media. Then *proteus* spp. were identified by characteristic swarming growth and fishy smell on blood agar media, non- lactose fermenting colony on MacConkey's agar media. It was glucose fermenter, oxidase negative, urease positive on biochemical reactions.

ANTIMICROBIAL SUSCEPTIBILITY TEST

Antimicrobial susceptibility testof isolated *Proteus* spp. was performed using Kirby-Bauer disc diffusion method. Commercially available antibiotic disc (OxoidLtd, Basingstoke, United kindom)[8]such as ceftazidime (30 µg), cefuroxime (30µg), ceftriaxone (30µg), cefoxitin (30µg), cefepime (30µg), imipenem (10µg), amoxiclav (amoxicillin and clavulanic acid) (20/10 µg), ciprofloxacin (5 µg), amikacin (30 µg), gentamicin (10 µg), piperacillin-ZZZZ (25µg), ampicillin (10 µg), doxycycline hydrochloride (30 µg) were used. Zones of inhibition were interpreted according to CLSI guidelines (CLSI, 2015).[8]

Screening for carbapenemases by the disc diffusion technique

Screening for carbapenem-resistance was determined using the Kirby Bauer disc diffusion method with a 10 mg imipenem disc. Three to five well isolated colonies of test organisms were emulsified into 3 mL of sterile normal saline. The turbidity of the suspension was compared with the 0.5 McFarland turbidity standard and the suspension was incubated on Mueller Hinton agar plates at 37°C for 24 hours. An inhibition zone of 19mm diameter around the imipenem disc was considered resistant, 20 to 22 mm indicated intermediate and 23 mm was considered sensitive.[8]

Phenotypic detection of carbapenemase producers

All the isolates showing reduced susceptibility to imipenem (zone diameter <19 mm) were tested for carbapenemase production using the modified Hodge test. Briefly, a lawn culture (0.5 McFarland) of *E. coli* 25922 was streaked on a Mueller Hinton agar plate.A 10µg imipenem disc was placed in the center of the agar plate. The test isolates were streaked in a straight line from the edge of the disc to the edge of the plate and were incubated overnight. A positive test was indicated by a cloverleaf-like indentation at the intersection of the test organism and the standard strain, within the zone of inhibition of the carbapenem antibiotic.[8] The detection of MBL production was performed by the double-disc synergy test.

Minimum inhibitory concentration (MIC) of imipenem among imipenem resistant *Proteus*:[9]

Minimum inhibitory concentration (MIC) of imipenem was done among imipenem resistant *Proteus*by agar dilution method in Muller-Hinton media and CLSI guidelines was followed for the interpretation.

Molecular characterization of carbapenem resistance genes

By polymerase chain reaction (PCR), Class A serine carbapenemase (*bla*KPC), Ambler class B metallo beta lactamases (*bla*NDM-1, *bla*VIM, *bla*IMP), Ambler class D (*bla*OXA-48 like, *bla*OXA-51 like, *bla*OXA-58 like, *bla*OXA-23 like) genes detection wascarried out among imipenem resistant *Proteus*isolates.

Preparation of the bacterial pellets:

Aloopful of bacterial colony was taken into a falcon tube, containing trypticase soy broth and overnight incubated at 37ºc temperature. Then the tubes were centrifuged at 4,000g for 10 minutes and supernatant was discarded. A small amount of sterile trypticase soy broth was added into the falcon tubes with pellets and mixed evenly. In 2-3 microcentrifuge tubes, equal amount of bacterial suspension was taken and centrifuged at 4,000 rpm for 10 minutes. The supernatant was discarded and the microcentrifuge tube containing bacterial pellets were kept at -20ºc until DNA extraction.

Bacterial DNA was extracted by the boiling method.[10]. Three hundred µl of distilled water was added into microcentrifuge tube, containing bacterial pellets and vortexed until mixed. The tubes were boiled for 10 minutes in a heat block and placed immediately into ice kept for 5 minutes. Centrifugation was done at 14,500g for 6 minutes, 10µl supernatant was used for PCR.

Amplification of DNA:

The cycling parameters followed in this study was as follows: initial denaturation at 95°C for 10 minutes, then 30 cycles of denaturation at 95°C for one minute, annealing at 58°c for blaKPC, 52°C (for bla IMP and blaVIM), 58°C (for blaNDM-1) and 52°C for (blaOXA-48 like, blaOXA-51 like, blaOXA-58 like, blaOXA-23 like) for 45 seconds, extension at 72°C for iminute, and final extension at 72°C for 10 minutes.

Visualization of amplified products:

The amplified DNA were loaded into a 1.5% agarose gel, electrophoreses done at 100 volts for 35 minutes, stained with 1% ethidium bromide and visualized under UV light.

Procedure of DNA sequencing:

For sequencing of bacterial DNA, purification of amplified PCR products were done by using DNA purification kit (FAVORGEN, Biotech Corp). Purified PCR products were sent to Malayasia (1st BATCH Laboratories) and sequencing was done by capillary method on ABI PRISM 3500. Then the sequenced DNA was compared with data in Gene Bank.

Statistical analysis:

Data were analyzed by using Microsoft Excel (2007) software.

RESULTS

Table I shows the distribution of *P. mirabilis and P. vulgaris* isolated from different samples. Total of 42 (13.55%) *Proteus* spp. were isolated from 310 wound swab, blood and urine samples. Among 42 *proteus* spp. 32 (76.19%) were *P. mirabilis* and 10 (23.81%) were *P. vulgaris.*

Table I: Distribution of *P. mirabilis and P. vulgaris* isolated from different samples.

Organism	Wound swab n (%)	Pus n (%)	Urine n (%)	Blood n (%)	Total n (%)
P. mirabilis	18 (69.23)	6 (85.71)	7 (87.50)	1 (100.00)	32 (76.19)
P. vulgaris	8 (30.77)	1 (14.29)	1 (12.50)	0 (0.00)	10 (23.81)
Total	26 (100.00)	7 (100.00)	8 (100.00)	1 (100.00)	42 (100.00)

Out of 42 *proteus* spp. 7 (16.67%) were imipenem resistant. As the sample of imipenum resistant isolates is insufficient, additional 8 imipenem resistant *proteus* were collected from department of Microbiology of the Dhaka Medical College to detect the carbapenemase encoding genes among them.So total 15 imipenem resistant *proteus* spp. were included for further study (Table II). MIC of imipenem among these 15 imipenem resistant *Proteus* spp. ranged from 4 to 64g/ml and highest proportion (26.67%) had MIC 16g/ml.

Table II : Distribution of imipenem resistant Proteus spp. in different samples. (N = 15)

Organism	Wound swab n (%)	Pus n (%)	Urine n (%)	Blood n (%)	Total n (%)
P. mirabilis	8 (53.33)	3 (20.00)	2 (13.33)	1 (6.67)	14 (93.33)
P. vulgaris	0 (0.00)	1 (6.67)	0 (0.00)	0 (0.00)	1 (6.67)
Total	8 (53.33)	4 (26.67)	2 (13.33)	1 (6.67)	15 (100.00)

N = Total number of bacteria.

n = Total number of imipenem resistant bacteria.

“ * ” = 7 imipenem resistant *Proteus* spp. were isolated from different samples and 8 imipenem resistant *Proteus* spp. were included from department of microbiology of DMC, which were isolated from different samples.

Table III shows distribution of class metallo-β-lactamase encoding genes in *Proteus* spp. detected by PCR method. Among 15 imipenem resistant *Proteus* spp. 3(20%) were positive for *bla*KPC, 3 (20%) for *bla*VIM, 4 (26.67%) for *bla*NDM-1, 3 (20%) for *bla*OXA-48 like and one (6.67%) for *bla*OXA-51 like gene. No isolates were found positive for *bla*IMP, *bla*OXA-23 like and *bla*OXA-58 like gene.

Table III: Distribution of class metallo-β-lactamase encoding genes in *Proteus* spp. detected by PCR method (N= 15).

Genes	Wound swab n (%)	Pus n (%)	Urine n (%)	Blood n (%)	Total n (%)
*bla*KPC	2 (13.333)	0 (0.00)	1 (6.67)	0 90.00)	3 (20.00)
*bla*VIM	2 (13.33)	1 (6.67)	0 (0.00)	0 (0.00)	3 (20.00)
*bla*IMP	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
*bla*NDM-1	2 (13.33)	1 (6.67)	1 (6.67)	0 (0.00)	4 (26.67)
*bla*0XA-48 like	1 (6.67)	1 (6.67)	1 (6.67)	0 (0.00)	3 (20.00)
*bla*OXA-51 like	0 (0.00)	0 (0.00)	0 (0.00)	1 (6.67)	1 (6.67)
*bla*OXA-58 like	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
*bla*OXA-23 like	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)

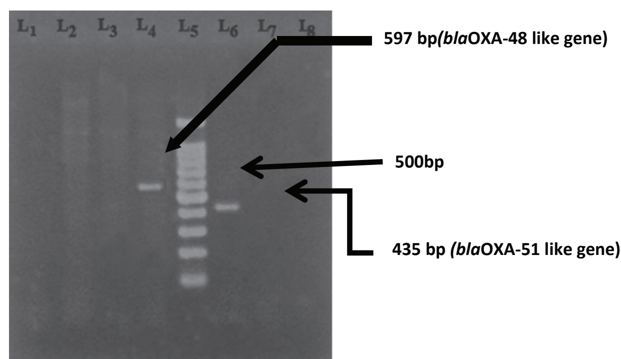

Figure 1: *Photograph of gel electrophoresis of amplified DNAof blaOXA-48 like geneand blaOXA-51 like gene.*

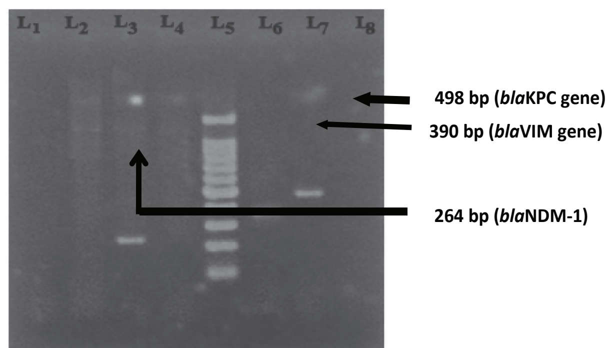

Figure 2: *Photograph of gel electrophoresis of amplified DNA of blaKPC, blaVIM and blaNDM-1 gene.*

Figure 1 is the photograph of gel electrophoresis of amplified DNAof *bla*OXA-48 like geneand *bla*OXA-51 like gene. Here lane 4 shows the amplified DNA of 597bp for *bla*OXA-48 like gene and lane 6 shows the amplified DNA of 435bp for *bla*OXA-51 like gene. Lane 1 is for negative control without DNA. Lane 2 and lane3 are for negative sample. Lane 7and lane8 are blank.

Figure 2 shows photograph of gel electrophoresis of amplified DNA of *bla*KPC, *bla*VIM and *bla*NDM-1 gene. Here lane 3 shows the amplified DNA of 264 bp for *bla*NDM-1 gene. Lane 6 shows amplified DNA of 390 bp for *bla*VIM gene and lane 7 shows amplified DNA of 498 bp for *bla*KPC gene. Lane1 is for negative control without DNA. Lane 2and lane 4 is for negative sample. Lane 8 is blank.

GGGGGGCCCAACTCCGATGTGGGCATATCCATATTCATCGCAAAAAACCACACATTATCA
TCAAGTTCAACCCAACCAACCCACCAGCCAATCTTAGGTTCGATTCTAGTCGAGTATCCC
GTTTTAGCCCGAATAATATAGTCGCCATTGGCTTCGGTCAGCATGGCTTGTTTCACGATG
CGCTGACTACGCTCAGAAACGTGCAGCTTGTTGTGATACAGCTTGCGTAAAAAGCGATT
TGCTGGGTAGCCGAAATGCGAATACCACCATCGAGCCAAAAACTGTCTACATTGCCCGAG
ATATCCTCATTGCCATAATCGAAGGCGTGCAGCATTTTACTCATACGTGCCTCACCAATT
TGGCGGGCAAATTCTTGATAAACAGGCACAACTGAGTACTTCATCGCGGTAATTAAGTCA
TGGTCACGATTCCAAGCGGCGATATCACGCGTCTGTCCATCCCACTTAAAGACTTGGTGT
TCATCCTTAACCACGCCCAAATCGAGGGCGATCAAGCTATTGGGAATTTTAAAGGTAGAT
GCGGGTAAAAATGCTTGTTCGGCCCCGTTAAAAAACAACC

Figure 3. *DNA sequence of amplified PCR product of blaOXA-48 like gene using specific primer.*

Score	**Expect**	**Identities**	**Gaps**	**Strand**
984 bits (1090)	**0.0**	**556/561 (99%)**	**3/561 (0%)**	**Plus/Minus**

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Sbjct  734  TCCGATGTGGGCATATCCATATTCATCGCAAAAAACCACACATTATCATCAAGTTCAACC  675

Query  73   CAACCAACCCACCAGCCAATCTTAGGTTCGATTCTAGTCGAGTATCCCGTTTTAGCCCGA  132
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Sbjct  674  CAACCAACCCACCAGCCAATCTTAGGTTCGATTCCAGACGAGTATCCCGTTTTAGCCCGA  615

Query  133  ATAATATAGTCGCCATTGGCTTCGGTCAGCATGGCTTGTTTCACGATGCGCTGACTACGC  192
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  614  ATAATATAGTCGCCATTGGCTTCGGTCAGCATGGCTTGTTTCACGATGCGCTGACTACGC  555

Query  193  TCAGAAACGTGCAGCTTGTTGTGATACAGCTTGCGTAAAAAAGCGATTTGCTGGGTAGCC  252
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Sbjct  554  TCAGAAACGTGCAGCTTGTTGTGATACAGCTTGCGTAAAAAAGCGATTTGCTGGGTAGCC  495

Query  253  GAAATGCGAATACCACCATCGAGCCAAAAACTGTCTACATTGCCCGAGATATCCTCATTG  312
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Sbjct  494  GAAATGCGAATACCACCATCGAGCCAAAAACTGTCTACATTGCCCGAGATATCCTCATTG  435

Query  313  CCATAATCGAAGGCGTGCAGCATTTTACTCATACGTGCCTCACCAATTTGGCGGGCAAAT  372
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Sbjct  434  CCATAATCGAAGGCGTGCAGCATTTTACTCATACGTGCCTCACCAATTTGGCGGGCAAAT  375

Query  373  TCTTGATAAACAGGCACAACTGAGTACTTCATCGCGGTAATTAAGTCATGGTCACGATTC  432
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Sbjct  374  TCTTGATAAACAGGCACAACTGAGTACTTCATCGCGGTAATTAAGTCATGGTCACGATTC  315

Query  433  CAAGCGGCGATATCACGCGTCTGTCCATCCCACTTAAAGACTTGGTGTTCATCCTTAACC  492
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Sbjct  314  CAAGCGGCGATATCACGCGTCTGTCCATCCCACTTAAAGACTTGGTGTTCATCCTTAACC  255

Query  493  ACGCCCAAATCGAGGGCGATCAAGCTATTGGGAATTTTAAAGGTAGATGCGGGTAAAAAT  552
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Sbjct  254  ACGCCCAAATCGAGGGCGATCAAGCTATTGGGAATTTTAAAGGTAGATGCGGGTAAAAAT  195

Query  553  GCTT-GTTCGGCCCCGTTAAA  572
            |||| |||||  |||||| ||
Sbjct  194  GCTTGGTTCG--CCCGTTTAA  176
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Figure 4: *Comparison of DNA sequence of the amplified PCR product of blaOXA-48 like gene and Klebsiella pneumoniae H141920513 blaOXA gene for OXA-48 family class D beta-lactamase OXA-484.*

Figure 4 shows DNA sequencing of *bla*OXA-48 like and *bla*OXA-51 like gene was done. The DNA sequence of the amplified PCR product of OXA-48 like gene (Figure3) which was found 99% identical to the *Klebseilla pneumoniae* H141920513*bla*OXA gene for OXA-48 family class D beta-lactamase OXA-484, which is available in the gene bank (accession number NG_049766.1). *bla*OXA-48 like gene had mutation at 108 position

CGTNCTTGAGCACCGTAAGGCAACCACCACAGAAGTATTGTAAGATGGGATGGTAAAAAAA
GGTTATTCCCAGAATGGGAAAAGGACATGACCCTAGGCGATGCCATGAAAGCTTCCGCTATT
CCAGTTTATCAAGATTTAGCTCGTCGTATTGGACTTGAGCTCATGTCTAAGGAAGTGAAGCG
TGTTGGTTATGGCAATGCAGATATCGGTACCCAAGTCGATAATTTTTGGCTGGTGGGTCCTTT
AAAAATTA CTCCTCAGCAAGAGGCACAGTTTGCTTACAAGCTAGCTAATAAAACGCTTCCAT
TTAGCCAAAAAGTCCAAGATGAAGTGCAATCCATGCTATTCATAGAAGAAAAGAATGGAAA
CAAAATATACGCAAAAAGGGGGTTGGGGAAA

Figure 5: *DNA sequence of amplified PCR product of blaOXA-51 like gene using specific primer.*

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Score                 Expect Identities      Gaps
655 bits(726)           0.0   391/396(99%)      3/396(0%)

Query  18CTTGAGCACCGTAAGGCAACCACCACAGAAGTATTGTAAGATGGGATGGTaaaaaaaGGT  77
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Sbjct  308  CTTGAGCACCATAAGGCAACCACCACAGAAGTATT-TAAG-TGGGATGGTAAAAAAAGGT  365

Query  78   TATTCCCAGAATGGGAAAAGGACATGACCCTAGGCGATGCCATGAAAGCTTCCGCTATTC  137
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Sbjct  366  TATTCCCAGAATGGGAAAAGGACATGACCCTAGGCGATGCCATGAAAGCTTCCGCTATTC  425

Query  138  CAGTTTATCAAGATTTAGCTCGTCGTATTGGACTTGAGCTCATGTCTAAGGAAGTGAAGC  197
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Sbjct  426  CAGTTTATCAAGATTTAGCTCGTCGTATTGGACTTGAGCTCATGTCTAAGGAAGTGAAGC  485

Query  198  GTGTTGGTTATGGCAATGCAGATATCGGTACCCAAGTCGATAATTTTTGGCTGGTGGGTC  257
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  486  GTGTTGGTTATGGCAATGCAGATATCGGTACCCAAGTCGATAATTTTTGGCTGGTGGGTC  545

Query  258  CTTTAAAAATTACTCCTCAGCAAGAGGCACAGTTTGCTTACAAGCTAGCTAATAAAACGC  317
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Query  318  TTCCATTTAGCCAAAAAGTCCAAGATGAAGTGCAATCCATGCTATTCATAGAAGAAAAGA  377
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
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Query  378  ATGGAAACAAAATATACGCAAAAAGGGGGTTGGGGA  413
            |||||||||||||||||||||||| | |||||||||
Sbjct  666  ATGGAAACAAAATATACGCAAAAA-GTGGTTGGGGA  700
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Figure 6: *Comparison of DNA sequence of the amplified PCR product of blaOXA-51 like gene and Acinetobacter baumannii strain AM8 blaOXA-66 (blaOxa-66) gene.*

The DNA sequence of the amplified PCR product of *bla*OXA-51 like gene (Figure 5) was 99% identical with class D beta-lactamase OXA-66, found in *Acinetobacter baumannii*strain AM8, which is available in the gene bank (accession number KY923052). *bla*OXA- 51 like gene had mutation at 28, 404 position (Figure 6)**.**

DISCUSSION

Very limited studies have been documented on *proteus* mediated infections, demographics of related patients and over all antibiotic resistance pattern of *Proteus* spp. Though the availability of a wide range antimicrobials of different categories, *Proteus* spp. mediated increased resistance to antimicrobials are documented. In recent years, researchers are giving attention to *Proteus* spp. because of high occurrence in nosocomial infections and expanding profile of antibiotic resistance.

In this study, highest proportion of imipenem resistant *Proteus* isolates were multidrug resistant and showed increased resistance to ciprofloxacin, ceftriaxone, cefepime, gentamicin, amoxiclav and sensitive to piperacillin-tazobactam (40%) in narrow range. Out of 15 imipenem resistant *Proteus* spp., 20% isolates were positive for *bla*KPC gene. Single *bla*KPC-2 positive *P. mirabilis* was reported in the studies of Pilato *et al.* (2016) and Cabral *et al.* (2014), respectively.11,12 Possibility of this gene transmission may be due to horizontal transmission by transposons, the mobile genetic elements which can transfer from one bacterium to another.[13]

Till now only a few numbers of NDM-1 producing *P. mirabilis* has been detected in different studies).**[14,15]** In the present study, 26.67% *bla*NDM-1 producing *P. mirabilis* were detected by PCR. Qin *et al.* (2015) and Girlich*et al.* (2015) reported single XDR *P. mirabilis* harboring *bla*NDM-1 gene, respectively.[14,15] Asian continent serves as the major reservoir of NDM-1 producers, with around 58.15% abundance of NDM-1 variant distributed mostly in China and India.

In this study, 20% *P. mirabilis* were positive for *bla*VIM gene. On the other hand, Vourli*et al.* (2006) reported, 100% isolated *proteus* were *bla*VIM positive. The proportion of MBL producers from different studies including the present one suggests that the prevalence of MBL producers varies on geographical distribution and time.[16]

One fifth of *P. mirabilis* were positive for *bla*OXA-48 like gene and validated by sequencing as *bla*OXA-484 gene. Inconsistent to present findings 23.3% OXA-48 like positive *Proteus* spp., were reported by Fursova*et al.,* (2015) which was close to the present finding.[17]Since most clinical microbiology laboratories do not test for the presence of OXA-48 like enzymes and the associated phenotype (i.e. low-level carbapenem resistance) may be difficult to recognize, the incidence of OXA-48-like gene positive carbapenem resistant *Enterobacteriaceae* is likely underestimated.

In this study, base sequence of the PCR product of OXA-48 like gene which was 99% identical to the *Klebseilla pneumoniae* H141920513 (*bla*OXA-484) gene which is available in the gene bank (accession number NG_ 049766.1). The OXA-484 gene had mutation at 108 position (Figure 4). In the present study, single *bla*OXA-51 like positive *P. mirabilis* was detected by PCR and the result was validated by sequencing. Sequencing result confirmed *bla*0XA-66 variant (Table 6). It is to be noted that in a recent study by Osterblad*et al.* (2016) reported, *Acinatobactor* type class D carbapenemase*bla*OXA-23 gene in *P. mirabilis.*[18]

The base sequence of PCR product of OXA-51 like gene was 99% identical to the *Acinatobactorbaumannii* strain AM8 *bla*OXA-66 (*bla*OXA-66) gene which is available in the gene bank (Accession number KY923052.1). *bla*OXA-66 gene had mutation at 28 and 404 position (Figure 6).

CONCLUSIONS

In this study *bla*KPC, *bla*VIM, *bla*NDM-1, *bla*OXA-484 and *bla*OXA-66 were predominant carbapenemase encoding genes among imipenem resistant *proteus.* Both *bla*OXA-484 and *bla*OXA-66 were the new variant of class D carbapenemase encoding genes among imipenem resistant *Proteus* spp. This study reflects that *bla*NDM-1 positive *proteus* are increasing and *bla*OXA-484 and *bla*OXA-66 are emerging in Bangladesh. Further study should be carried out to detect the resistant genes and proper implementation of antimicrobial policies infection control programs will surely limit the rapid dissemination of this type of infection.

Competing interests:

There is no conflict of interest.

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