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OXA-181- an emerging threat in Bangladesh

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ABSTRACT

This study was conducted to detect the prevalence of OXA-181 gene producing Escherichia coli and Klebsiella spp. in a tertiary care hospital of Bangladesh. Total 166 Escherichia coli and Klebsiella spp. were isolated from urine, wound swab, pus, blood and sputum samples in Dhaka Medical College Hospital. Antibiotic susceptibility test was performed by disk-diffusion technique. Carbapenemase producers were detected phenotypically by Modified Hodge test. Carbapenemase genes (blaNDM-1, blaVIM, blaIMP, blaKPC, blaOXA-48/blaOXA-181) among imipenem resistant strains among the isolated Escherichia coli and Klebsiella spp. were detected by PCR. Sequencing was done to differentiate blaOXA-181 from blaOXA-48. Thirty-seven (22.29%) imipenem resistant isolates were detected during disk-diffusion technique, among them 43.24% carbapenemase producers were detected by MHT and 62.16% by PCR. NDM-1 (43.24%) was the dominant genotype followed by KPC (21.62%) and OXA-181 (18.92%). The results of this study showed presence of high proportion of carbapenemase enzyme producing Escherichia coli and Klebsiella spp. in Bangladesh. BlaOXA-181 gene is emerging in Bangladesh.

Key Words: *Escherichia coli, Klebsiella spp., Carbapenemase, BlaOXA-181 gene and Bangladesh*

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I. Introduction

Various resistance mechanisms facilitate the emergence and spread of multidrug-resistant phenotypes of *Escherichia coli* and *Klebsiella* spp. (Zhang et al., 2012). Carbapenem resistance among *Enterobacteriaceae*, in particularly among *Klebsiella pneumoniae* and *Escherichia coli*, is an emerging problem worldwide and carbapenemases are the most prominent enzymes that neutralize carbapenems (Hindiyeh et al., 2008). There is a lack of information on molecular characterization of carbapenemase enzyme producing organisms isolated in Bangladesh. To the best of knowledge, no study has so far been carried out among *Escherichia coli* and *Klebsiella* spp. isolated from urine, wound

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swab, pus, blood and sputum samples in Dhaka Medical College Hospital regarding detection of OXA-181. Therefore, this study has been designed to obtain data on the resistance patterns of *Escherichia coli* and *Klebsiella* spp. isolated from various clinical samples of patients of Dhaka Medical College Hospital to the antimicrobial agents which are currently being used in treatment purpose, along with detection of genes encoding carbapenemases by PCR and sequencing.

II. Materials and Methods

A cross-sectional study was conducted in the Department of Microbiology of Dhaka Medical College, Dhaka, Bangladesh, during January 2015 to December 2015. This research protocol was approved by the research review committee and ethical review committee of Dhaka Medical College. Written informed consent was taken from each patient. *Escherichia coli*, *Klebsiella pneumoniae* and *Klebsiella oxytoca* were isolated from 340 urine, wound swab, pus, sputum and blood samples of clinically suspected infected patients of in-patient and out-patient departments of Dhaka Medical College Hospital, irrespective of age and sex. All samples were inoculated in blood agar and MacConkey agar media and incubated at 37^{o}C aerobically for 24 hours. Incubated plates were then examined for the presence of colonies of bacteria. Primary blood culture was done in Trypticase soy broth then subculture was done on blood agar and MacConkey agar media. *Escherichia coli*, *Klebsiella pneumoniae* and *Klebsiella oxytoca* were identified by colony morphology, staining character and biochemical tests as per standard technique (Cheesbrough, 1998).

Antimicrobial susceptibility testing: All isolated *Escherichia coli*, *Klebsiella pneumoniae* and *Klebsiella oxytoca* were tested for antimicrobial susceptibility by disk diffusion method following the guidelines of Clinical and Laboratory Standards Institute (CLSI, 2014), using commercially available antibiotic disks (Oxoid Ltd, Basingstoke, United Kingdom). Antibiotic disks such as ceftazidime (30 µg), cefuroxime (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), colistin (10 µg), sulfamethoxazole-trimethoprim (1.25/23.75 µg), tigecyline (15 µg) were used. Mueller-Hinton agar media was used for antimicrobial susceptibility test. Criteria of the United States Food and Drug Administration was used for interpretation of zone of inhibition of tigecycline (Brink et al., 2010). *Escherichia coli* ATCC 25922 was used as control strain for susceptibility test. Study isolates were phenotypically characterized for the production of carbapenemase by MHT. Antimicrobial susceptibility testing of all carbapenemase enzyme producers were also performed.

Phenotypic detection of carbapenemase producers: Initially sensitivity to imipenem was observed by disk-diffusion method. Carbapenemase producing *Escherichia coli*, *Klebsiella pneumoniae* and *Klebsiella oxytoca* were phenotypically detected by MHT. MHT has been originally described by the Centers for Disease Control and Prevention (CDC) for carbapenemases detection in *Enterobacteriaceae*. In MHT, a lawn culture of 1:10 dilution of 0.5 McFarland's standard *Escherichia coli* ATCC 25922 broth was done on a Mueller-Hinton agar plate. A 10-µg imipenem disk was placed in the center of the plate. Then, imipenem resistant test strains were streaked from the edge of the disk to the periphery of the plate in three different directions. After overnight incubation, the plates were observed for the presence of a clover leaf shaped zone of inhibition and the plates with such zones were interpreted as MHT positive.

Detection of carbapenemase enzyme encoding genes: Carbapenemase genes (*bla*NDM-1, *bla*IMP, *bla*VIM, *bla*OXA-181/OXA-48 and *bla*KPC) among the imipenem resistant *Escherichia coli*, *Klebsiella pneumoniae* and *Klebsiella oxytoca* were detected by PCR. To prepare bacterial pellets, a loop full of bacterial colonies was inoculated into a Falcon tube containing Trypticase soy broth. After incubation overnight at 37^{0}C, the Falcon tubes were centrifuged at 4,000 rpm for 10 minutes, after which the supernatant was discarded. A small amount of sterile Trypticasesoybroth was added into the Falcon tubes with pellets and mixed evenly. Then an equal amount of bacterial suspension was placed into 2 to 3 to micro centrifuge tubes. The micro centrifuge tubes were then centrifuged at 4,000 rpm for 10 minutes and the supernatant was discarded. The micro centrifuge tubes containing bacterial pellets (Franco et al., 2010). PCR screening for presence of different genes were performed using primers and conditions described previously (Castanheira et al., 2011; Poirel et al. 2011; Farzana et al., 2013). The

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

DNA sequence analysis: Sequencing was performed to differentiate OXA-181 gene from OXA-48 gene. After PCR, the amplicons were purified with the DNA purification kit (FAVORGEN, Biotech Corp.), and subjected to automated DNA sequencing (ABI 3500). Nucleotide sequence similarity searches were performed using the National Center for Biotechnology Information (NCBI, National Institutes of Health) BLAST (Basic Local Alignment Search Tool) server on Gen Bank database.

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

III. Results

One hundred and sixty six *Escherichia coli*, *Klebsiella pneumoniae* and *Klebsiella oxytoca* were isolated from 340 samples. Of them, 57.35% (78/136) isolates were recovered from urine, whilst other sources included, wound swab, pus, sputum and blood. Out of 166 isolates, 106 (40.77%) were *Escherichia coli*, 42 (16.15%) were *Klebsiella pneumoniae* and 18 (6.92%) were *Klebsiella oxytoca*. Most of the isolates showed high resistance rate to several antimicrobial classes whereas imipenem, colistin and tigecycline were found to be the most effective drugs (Table 01).

Table 01. Antibiotic resistance pattern of isolated strains (N=166)

Antimicrobial Drugs	*Escherichia coli* (N=106) n (%)	*Klebsiella pneumoniae* (N=42) n (%)	*Klebsiella oxytoca* (N=18) n (%)
Amoxiclav	82 (77.36)	42 (100.00)	18 (100.00)
Cefuroxime	73 (68.87)	38 (90.48)	15 (83.33)
Cefoxitin	40 (37.74)	21 (50.00)	6 (33.33)
Ceftriaxone	86 (81.13)	42 (100.00)	18 (100.00)
Ceftazidime	78 (73.58)	42 (100.00)	18 (100.00)
Cefepime	71 (66.98)	38 (90.48)	17 (94.44)
Gentamicin	88 (83.02)	32 (76.19)	14 (77.78)
Amikacin	75 (70.75)	31 (73.81)	11 (61.11)
Ciprofloxacin	89 (83.96)	35 (83.33)	14 (77.78)
Sulfamethoxazozole-trimethoprim	96 (90.57)	40 (95.24)	17 (94.44)
Imipenem	21 (19.81)	12 (28.57)	4 (22.22)
Colistin	4 (3.77)	0 (0.00)	0 (0.00)
Tigecycline	0 (0.00)	2 (4.76)	0 (0.00)

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

Of the 166 isolates, 22.29% (n=37) imipenem resistant strains were detected during disk-diffusion technique, of which 8.43% (14/166) were isolated from wound swab, 3.61% (6/166) from urine, 3.61% (6/166) from sputum, 3.61% (6/166) from blood and 3.01% (5/166) from pus samples. Twenty-one

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(19.81%) of the 106 *Escherichia coli*, 12 (28.57%) of the 42 *Klebsiella pneumoniae* and 4 (22.22%) of the 18 *Klebsiella* oxytoca, were imipenem resistant. Among 37 imipenem resistant strains, 16 (43.24%) carbapenemase producers were detected by MHT and 23 (62.16%) by PCR. Sixteen (43.24%), 6 (16.22%), 5 (13.51%), 8 (21.62%) and 7 (18.92%) of the imipenem resistant strains were positive for *bla*NDM-1, *bla*VIM, *bla*IMP, *bla*KPC and *bla*OXA-48/ *bla*OXA-181 genes, respectively (Table 02).

Table 02. Distribution of carbapenemase encoding genes among imipenem resistant *Escherichia coli*, *Klebsiella pneumoniae* and *Klebsiella oxytoca* (N=37)

Imipenem resistant organisms	Carbapenemase encoding genes				
	NDM-1 n (%)	VIM n (%)	IMP n (%)	KPC n (%)	OXA-48/OXA-181 n (%)
Escherichia coli (N=21)	12 (57.12)	4 (19.05)	5 (23.81)	5 (23.81)	0 (0.00)
Klebsiella pneumoniae (N=12)	4 (33.33)	2 (16.67)	0 (0.00)	3 (25.00)	5 (41.67)
Klebsiella oxytoca (N=4)	0 (0.00)	0 (0.00)	0 (0.00)	0 (00.00)	2 (50.00)
Total (N=37)	16 (43.24)	6 (16.22)	5 (13.51)	8 (21.62)	7 (18.92)

N= Total number of bacteria; n= Number of carbapenemase gene carrying bacteria;
The total of last row is more as most of the isolates had two or more genes.

Table 03. Distribution of *bla*OXA-181 gene among imipenem resistant *Escherichia coli*, *Klebsiella pneumoniae* and *Klebsiella oxytoca* detected by PCR (N=37)

Organisms	Urine n (%)	Wound n(%)	Pus n(%)	Blood n(%)	Sputum n(%)	Total n(%)
Esch. coli (N=21)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Klebsiella pneumoniae (N=12)	0 (0.00)	3 (25.00)	1 (8.33)	1 (8.33)	0 (0.00)	5 (41.67)
Klebsiella oxytoca (N=4)	0 (0.00)	2 (50.00)	0 (0.00)	0 (0.00)	0 (0.00)	2 (50.00)
Total	0 (0.00)	5 (13.51)	1 (2.70)	1 (2.70)	0 (0.00)	7 (18.92)

N = Total number of imipenem resistant bacteria;
n = Number of *bla*OXA-181 gene among imipenem resistant bacteria

Out of 37 imipenem resistant *Escherichia coli*, *Klebsiella pneumoniae* and *Klebsiella oxytoca*, 7 (18.92%) were positive for *bla*OXA-181 detected by PCR. Of them, 5 (13.51%) were from wound swab, one (2.70%) was from pus sample and one (2.70%) was from blood sample. Among 12 imipenem resistant *Klebsiella pneumoniae*, 5 (41.67%) were positive for *bla*OXA-181 gene. Two (50%) of the 4-imipenem resistant *Klebsiella oxytoca* had OXA-181 encoding gene. Among 21 imipenem resistant *Escherichia coli*, no OXA-181 encoding gene was found (Table 03).

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Sequencing of the 7 *bla*OXA-48/*bla*OXA-181 genes revealed that all had 99% identity with the *bla*OXA-181 gene detected in *Klebsiella pneumoniae* (strain: MS5166) (Genbank accession: AB972272.1). All (100%) the carbapenemase producers were resistant to cefuroxime, cefoxitin, ceftriaxone, ceftazidime, cefepime, amikacin, gentamicin, ciprofloxacin and sulfamethoxazole-trimethoprim, 17.39% to colistin and 8.70% to tigecycline (Table 04).

Table 04. Antimicrobial drug resistance pattern among carbapenemase producing *Escherichia coli*, *Klebsiella pneumoniae* and *Klebsiella oxytoca* (N=23).

Antimicrobial drugs	*Escherichia coli* (N=13) n (%)	*Klebsiella pneumoniae* (N=8) n (%)	*Klebsiella oxytoca* (N=2) n (%)	Total (N=23) n (%)
Amoxiclav	13 (100.00)	8 (100.00)	2 (100.00)	23 (100.00)
Ceftriaxone	13 (100.00)	8 (100.00)	2 (100.00)	23 (100.00)
Ceftazidime	13 (100.00)	8 (100.00)	2 (100.00)	23 (100.00)
Cefuroxime	13 (100.00)	8 (100.00)	2 (100.00)	23 (100.00)
Cefepime	13 (100.00)	8 (100.00)	2 (100.00)	23 (100.00)
Cefoxitin	13 (100.00)	8 (100.00)	2 (100.00)	23 (100.00)
Imipenem	13 (100.00)	8 (100.00)	2 (100.00)	23 (100.00)
Amikacin	13 (100.00)	8 (100.00)	2 (100.00)	23 (100.00)
Gentamicin	13 (100.00)	8 (100.00)	2 (100.00)	23 (100.00)
Ciprofloxacin	13 (100.00)	8 (100.00)	2 (100.00)	23 (100.00)
SXT	13 (100.00)	8 (100.00)	2 (100.00)	23 (100.00)
Colistin	4 (30.77)	0 (0.00)	0 (0.00)	4 (17.39)
Tigecycline	0 (0.00)	2 (25.00)	0 (0.00)	2 (8.70)

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

Discussion

In the current study, 23 (62.16%) carbapenemase producers were detected by PCR. *Bla*NDM-1, *bla*KPC, *bla*VIM, *bla*IMP and *bla*OXA-181 were found to be responsible for imipenem resistance The most prevalent carbapenemase encoding genotype found were NDM-1 (43.24%). A previous study in Bangladesh revealed 22.86% *bla*NDM-1 gene among gram negative bacteria (Farzana et al., 2013). Rapid dissemination of *bla*NDM-1 producing organisms might be facilitated by the conditions like overcrowding, over-the-counter availability of antibiotics, low level of hygiene, and weak hospital antibiotic policies. OXA-181, a point mutant analog of OXA-48, with similar carbapenemase activity, had been identified in strains from India or of Indian origin (Castanheira et al., 2011; Kalpoe et al., 2011). The present study observed *bla*OXA-181 (18.92%) as emerging threat in Bangladesh. Current study demonstrated that all the carbapenemase producers were resistant to cefepime, amikacin, gentamicin, ciprofloxacin and sulfamethoxazole-trimethoprim. Selective pressure of antibiotics might have contributed to the high antimicrobial resistance in the present study. Colistin and tigecycline were found to be the most effective drugs against carbapenemase producers, still 17.39% were resistant to colistin and 8.70% to tigecycline. Therefore, colistin and tigecycline are not adequate empirical antibiotics to

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treat infections caused by carbapenemases producing bacteria. The mechanisms of tigecycline and/or colistin resistance in *Escherichia coli* and *Klebsiella* spp. warrant further investigation.

IV. Conclusion

Genes encoding carbapenemase enzymes including *bla*NDM-1, *bla*KPC, *bla*VIM, *bla*IMP and *bla*OXA-181 were responsible for imipenem resistance. *Bla*OXA-181 producers are emerging in Bangladesh which were mainly found in *Klebsiella* spp. High prevalence of carbapenemase enzyme genes in *Escherichia coli* and *Klebsiella* spp. possibly reflects the overuse and misuse of antibiotics in Bangladesh and severely limits the therapeutic options in Bangladesh. Prompt and accurate detection of drug resistant bacterial strains will prevent their spread and in vitro resistance patterns of these strains will guide the clinicians to the use of appropriate antibiotics.

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Competing interests

The authors declare that they have no competing interests. Authors are responsible for necessary human ethics, study and clinical procedures and results of this article.

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