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Original Article

*bla*NDM-1, *bla*KPC, *bla*OXA-181- as the major mediators of carbapenem resistance in carbapenemase producing *Escherichia coli* and *Klebsiella* species isolated from a tertiary care hospital in Bangladesh

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Abstract

Background and objectives: Carbapenem resistance is an emerging problem worldwide. Detection of carbapenem resistance genes is important to institute appropriate therapy and to initiate preventive measures. This study was conducted to determine the presence of carbapenemase enzyme producing *Escherichia coli* and *Klebsiella* species in a tertiary care hospital of Bangladesh, as well as to observe the patterns of antibiotic resistance and carbapenem resistance genes among them.

Methodology: Total 166 *Escherichia coli, Klebsiella pneumoniae* and *Klebsiella oxytoca* were isolated from urine, wound swab, pus, sputum and blood samples of patients of Dhaka Medical College Hospital. Antibiotic susceptibility test was performed by disk-diffusion technique. Carbapenemase producers were detected phenotypically by Double-disk synergy (DDS) test, Modified Hodge test (MHT) and Combined disk (CD) assay. Minimum inhibitory concentration (MIC) of imipenem was done by agar dilution method among carbapenemase producing strains. Genotypically carbapenemase genes (*bla*NDM-1, *bla*VIM, *bla*IMP, *bla*KPC, *bla*OXA-48/*bla*OXA-181) among the imipenem resistant *Escherichia coli* and *Klebsiella* species were detected by polymerase chain reaction (PCR). Sequencing was done to differentiate *bla*OXA-181 gene from *bla*OXA-48 gene. Class 1 integron were also detected by PCR using specific primer among carbapenemase producers.

Results: Thirty seven (22.29%) imipenem resistant isolates were detected during disk-diffusion technique, among them 16 (43.24%) carbapenemase producers were detected by MHT, 20 (54.05%) by DDS test, 22 (59.46%) by CD assay and 23 (62.16%) by PCR. Out of 23 carbapenemase producing strains, MIC of imipenem ranged from 4 µg/ml up to ≥128 µg/ml. NDM-1 (43.24%) was the dominant genotype in imipenem resistant strains followed by KPC (21.62%) and OXA-181 (18.92%). Class 1 integron were present in 16 (69.57%) of the genotypically identified carbapenemase producers.

Conclusion: The results of this study showed high proportion of carbapenemase enzyme producing *Escherichia coli* and *Klebsiella* species in Bangladesh. Genes encoding carbapenemase enzymes including *bla*NDM-1, *bla*KPC, *bla*VIM, *bla*IMP and *bla*OXA-181 were responsible for imipenem resistance. *bla*NDM-1 producers are increasing and *bla*KPC and *bla*OXA-181 producers are emerging in Bangladesh. Regular surveillance of antibiotic resistance should be done in every tertiary care hospital to prevent spread of these strains.

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Introduction

New resistance mechanisms emerge and spread globally. A high percentage of hospital acquired infections are caused by multi-drug resistant gram negative bacteria[1]. Among the gram negative bacteria implicated in nosocomial infections, *Klebsiella pneumoniae* and *Escherichia coli* are the most prevalent[2]. Carbapenem resistance among *Enterobacteriaceae*, in particular among *Klebsiella pneumoniae* and *Escherichia coli*, is an emerging problem worldwide and carbapenemases are the most prominent enzymes that neutralize carbapenems[3]. The vast majority of acquired carbapenamases belong to three of the four known classes of β-lactamases, namely Ambler class A enzyme such as *Klebsiella pneumoniae* carbapenemase (KPC) types, Ambler class B enzymes or metallo-β-lactamases such as VIM, IMP, NDM-1 types and Ambler class D enzymes or oxacillanases such as OXA-23, OXA-48, OXA-181 types[4,5]. There is a lack of information on molecular characterization of carbapenemase enzyme producing organisms isolated in Bangladesh. 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 DMCH to the antimicrobial agents which are currently being used in treatment purpose, along with detection of genes encoding carbapenemases by PCR and sequencing and to find out the presence of class 1 integron among carbapenemase producing *Escherichia coli* and *Klebsiella* spp. by PCR.

Material and method

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°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[6].

Antimicrobial susceptibility testing

All isolated *Escherichia coli, Klebsiella pneumoniae* and *Klebsiella oxytoca* were tested for antimicrobial susceptibility by disc diffusion method following the guidelines of Clinical and Laboratory Standards Institute[7,] using commercially available antibiotic discs (Oxoid Ltd, Basingstoke, United Kingdom). Antibiotic discs 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[8]. *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 disc 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 37^0C, the falcon tubes were centrifuged at 4,000 rpm for 10 minutes, after which the supernatant was discarded. A small amount of sterile Trypticase soy broth 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 contained bacterial pellets[9]. PCR screening for presence of different genes were performed using primers and conditions described previously[10,11,12]. The 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 GenBank database.

Statistical analysis

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

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 (Table1).

Table 1. Antibiotic resistance pattern of isolated strains (N=166).

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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)

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

Of the 166 isolates, 22.29% (n=37) imipenem resistant strains were detected during disc-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 (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 2).

Table 2. 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)**

Note: 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.

Figure 1 shows the presence of class 1 integron among genotypically detected carbapenemase producing *Escherichia coli, Klebsiella pneumoniae* and *Klebsiella oxytoca*. Among the carbapenemase producing organisms, class 1 integron were present in 16 (69.57%) of the isolates.

Figure 1. Class 1 integron among genotypically detected carbapenemase producing *Escherichia coli, Klebsiella pneumoniae* and *Klebsiella oxytoca* (N=23).

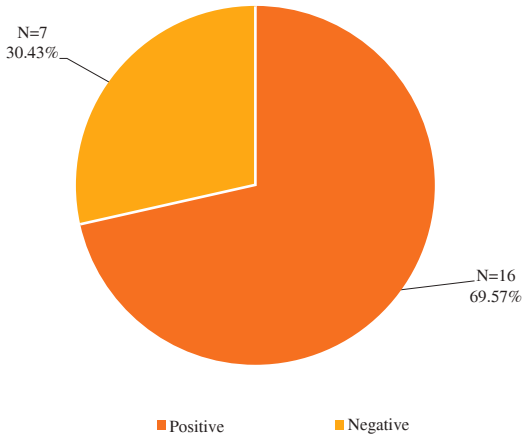

Table 3. Distribution of class 1 integron among carbapenemase gene encoding *Escherichia coli, Klebsiella pneumoniae* and *Klebsiella oxytoca* detected by PCR.

Carbapenemase encoding genes	Class 1 integron		Total n (%)
	Present n (%)	Absent n (%)	
*bla*NDM-1	12 (75.00)	4 (25.00)	16 (100.00)
*bla*VIM	4 (66.67)	2 (33.33)	6 (100.00)
*bla*IMP	4 (80.00)	1 (20.00)	5 (100.00)
*bla*KPC	5 (62.50)	3 (37.50)	8 (100.00)
*bla*OXA-181	4 (57.14)	3 (42.86)	7 (100.00)

Table 3 demonstrates the distribution of class 1 integron among carbapenemase gene encoding *Escherichia coli, Klebsiella pneumoniae* and *Klebsiella oxytoca*. Class 1 integron was found in 12 (75%) of *bla*NDM-1, 4 (66.67%) of *bla*VIM, 4 (80%) of *bla*IMP, 5 (62.50%) of *bla*KPC and 4 (57.14%) of *bla*OXA-181 gene encoding *Escherichia coli, Klebsiella pneumoniae* and *Klebsiella oxytoca.*

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[12]. 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. The present study revealed 16 (69.57%) class 1 integron carrying carbapenemase producing *Escherichia coli, Klebsiella pneumoniae* and *Klebsiella oxytoca* (Figure 1). A previous study in Bangladesh by Farzana et al. (2013) reported 74% class 1 integron carrying MBL producers[12].

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

Conclusion

Genes encoding carbapenemase enzymes including *bla*NDM-1, *bla*KPC, *bla*VIM, *bla*IMP and *bla*OXA-181 were responsible for imipenem resistance. 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. The antibiotic resistance in Bangladesh and developing countries commonly occurs due to inappropriate antibiotic use, over-prescribing and inappropriate prescribing and unethical practices of health professionals. In addition, antibiotics are available over the counter in all over Bangladesh which contribute to the misuse and over use of antibiotics by the common people. Indiscriminate use of antibiotics might be the reason of high frequency of antibiotic resistance in the present study. 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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References

1. World Health Organization (WHO). Antimicrobial resistance report: global report on surveillance. 2015; Web page at http://www.who.int/mediacentre/factsheets/fs194/en/ (accessed on 20/10/2015).
2. Podschun R, Ullmann U. Klebsiella spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. Clinical microbiology reviews. 1998 Oct 1;11(4):589-603.
3. Hindiyeh M, Smollen G, Grossman Z, Ram D, Davidson Y, Mileguir F, et al. Rapid detection of bla KPC carbapene mase genes by real-time PCR. Journal of clinical microbiology. 2008 Sep;46(9):2879-83.
4. Nordmann P, Poirel L, Toleman MA, Walsh TR. Does broad-spectrum β-lactam resistance due to NDM-1 herald the end of the antibiotic era for treatment of infections caused by Gram-negative bacteria. Journal of antimicrobial chemotherapy. 2011Apr 1;66(4):689-92.
5. Miriagou V, Cornaglia G, Edelstein M, Galani I, Giske CG, Gniadkowski M, et al. Acquired carbapenemases in Gram-negative bacterial pathogens: detection and surveillance issues. Clinical microbiology and infection. 2010 Feb 1;16(2): 112-22.
6. Cheesbrough M. Microscopical techniques used in Microbiology, culturing bacterial pathogens, biochemical tests to identify bacteria. District Laboratory Practice in Topical Countries, Part. 1998;2:35-70.
7. Clinical and Laboratory Standard Institute (CLSI); 2014. Performance standards for antimicrobial susceptibility testing: Twenty-Fouth Informational Supplement. CLSI document M100-S24. Wayne, PA: CLSI.
8. Brink AJ, Bizos D, Boffard KD, Feldman C, Grolman DC, Pretorius J, et al. Guideline: appropriate use of tigecycline. SAMJ: South African medical journal. 2010 Jun;100(6):388-94.
9. Franco MR, Caiaffa-Filho HH, Burattini MN, Rossi F. Metallo-beta-lactamases among imipenem-resistant Pseudomonas aeruginosa in a Brazilian university hospital. Clinics. 2010;65(9):825-9.
10. Castanheira M, Deshpande LM, Mathai D, Bell JM, Jones RN, Mendes RE. Early dissemination of NDM-1-and OXA-181-producing Enterobacteriaceae in Indian hospitals: report from the SENTRY Antimicrobial Surveillance Program, 2006-2007. Antimicrobial agents and chemotherapy. 2011 Mar;55(3):1274-8.
11. Poirel L, Walsh TR, Cuvillier V, Nordmann P. Multiplex PCR for detection of acquired carbapenemase genes. Diagnostic microbiology and infectious disease. 2011 May 1;70(1):119-23.
12. Farzana R, Shamsuzzaman SM, Mamun KZ. Isolation and molecular characterization of New Delhi metallo- beta-lactamase-1 producing superbug in Bangladesh. The Journal of infection in developing countries. 2013 Mar 14;7(03):161-8.