Original Article

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Detection of Carbapenemase Producing Uropathogens in a Tertiary Care Hospital, Chattogram

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ABSTRACT

Background: *Antimicrobial Resistance (AMR) posses a complex threat to global health security and universal health coverage. Gram negative bacteria have developed the broadest spectrum of resistance due to multiple structural adaptions and antibiotic degradation enzymes including Extended Spectrum Beta Lactamase, AmpC Cephalosporinase and Carbapenemase. Carbapenemases are beta-lactamases with versatile hydrolytic capacities. They have the ability to hydrolyze penicillins, cephalosporins, monobactam, and carbapenems. The classical phenotypic method cannot provide an efficient means of diagnosis of the carbapenemase producer. Multiplex PCR also helps in simultaneous detection of various genes, reducing materials, manpower and helps in determining epidemiology related to these genes and infection subsidence. This study was designed to determine the presence of carbapenemase producers among the carbapenem resistant uropathogens and to compare the phenotypic method with genotypic method along with the antimicrobial resistance patterns.*

Materials and methods: *This cross-sectional study was carried out in the Department of Microbiology Chittagong Medical College, Chattogram for quantitative culture, mCIM test, and multiplex PCR for carbapenemase producers. A total 68 Gram negative uropathogens were isolated and identified by conventional methods. Antibiotic susceptibility test was performed by disk-diffusion technique. Carbapenemase producers were detected phenotypically by modified carbapenem inactivation method among the meropenem resistant isolates. Gene encoding bla NDM and coexistence of bla NDM +bal OXA-48 were identified by multiplex PCR.*

Results: *Twenty (29.41%) meropenem resistant strains were detected among 68 Gram-negative uropathogens. The most common isolates were Escherichia coli and Pseudomonas spp. Among 20 meropenem resistant strains, 19(95%) carbapenemase producers were detected by multiplex PCR, 17(85%) by modified carbapenem inactivation method. The most prevalent gene was blaNDM (94.73%). More than one carbapenemase gene was present in one (5.6%) isolate. Overall, carbapenemase encoding genes were detected in 11.11% of the studied Gram negative uropathogenes. All of the carbapenemase producing organisms were 100% resistant to Ampicillin, Cefuroxime, Ceftriaxone, Ceftazidime, Aztreonam, 94.73% to both Cotrimoxazole and Nitrofurantoin, 68.42% to Pipercillin-Tazobactam and 63.15%, to Amikacin.*

Conclusion: *The study shows that rapid dissemination of blaNDM in Bangladesh demands the effective measures along with antibiotics policies in hospitals which combat the spread of this strains.*

Key words: Antimicrobial resistance; Carbapenemasep Uropathogens.

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Introduction

Carbapenem resistance, among Gram-negative pathogens, is an ongoing public-health problem of global dimension. This type of antimicrobial resistance, especially mediated by transferable carbapenemase-encoding genes, is spreading rapidly causing serious outbreaks and dramatically limiting treatment options.[1] The capture, accumulation, and dissemination of resistance genes are largely due to the actions of Mobile Genetic Elements (MGE), a term which is used to elements that promote intracellular DNA mobility (e.g, From the chromosome to a plasmid or between plasmids) as well as that enable intercellular DNA mobility. Moreover, resistance is mediated by production of carbapenemase [Either serine based carbapenemase or Metallo-Beta-Lactamases (MBLs)]

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by modification of membrane permeability (i.e, Porin loss) or by production of carbapenem hydrolyzing beta lactamases (i.e, Hyperproduction of AmpC beta lactamase, certain ESBLs with increased capacity to hydrolyze carbapenems) and upregulation of efflux pumps.[2] Carbapenemase are member of Ambler class A, class B and class D β-lactamases. Class A enzymes are serine carbapenemase (KPC, SME, IMI, NMS, and GES, inhibited by clavulanic acid), Class B are metallo-β-lactamases (MBLs) (IMP, VIM, NDM, SPM, GIM, and SIM, inhibited by metal chelators carbapenem) and Class D are oxacillinase-type (OXA types) carbapenemase.[3] Rapid worldwide expansion of carbapenemase producers now create a public health problem because they remain extensively resistant to nearly all antibiotics, endangering the efficacy of antibiotics, that saved millions of lives.[4] As phenotypic method is subjective, time consuming , some result might be ignored and it has a disadvantage of inability to discriminate the type of carbapenemase.[5] Contrary, multiplex PCR is a reliable assay for rapid screening and allows a rapid identification of resistance traits that helps in determining epidemiology related to genes and infection control.[6] So accurate detection of the genes related with carbapenemase production by multiplex PCR overcome the limitation of the phenotypic methods.[7]

Materials and methods

Bacterial Isolates

We conducted a cross sectional study in the Department of Microbiology of Chittagong Medical college, Chattogram , Bangladesh during January 2021 to December 2021. This research protocol was approved by the research review committee and ethical review committee of Chittagong Medical College. A total of 68 consecutive nonduplicate Gram-negative isolates recovered from urine cultures of patients with clinically suspected urinary tract infection in the Department of Microbiology of Chittagong Medical College, Chattogram. Bacterial identification was performed by routine conventional microbial cultures and biochemical tests using standard recommended techniques.

Antimicrobial Susceptibility Testing

All bacterial isolates were subjected to antimicrobial susceptibility testing using the disc diffusion method as described by the Clinical and Laboratory Standards Institute (CLSI) 2021.[8] Escherichia coli ATCC 25922 was used as the quality control strain.

Phenotypic Detection of Carbapenemase

Modified Carbapenem Inactivation Method for detection of Carbapenemase (CLSI, 2021): In the mCIM, 1µL loopful of bacteria for Enterobacteriaceae or 10µL loopful of bacteria for Pseudomonas aeruginosa or Acinetobacter baumanni from an overnight blood agar plate were emulsified in 2mL Tripticase Soy Broth (TSB). A 10µg meropenem disk was immersed in the suspension and incubated at 35°C±2°C in ambient air for 4 hours ±15 minutes. Just before or immediately following completion of TSB-meropenem disk suspension incubation, a 0.5 Mc-Farland suspension of *E.coli* ATCC 25922 was prepared in nutrient broth or saline using the direct colony suspension method. A Mueller –Hinton Agar (MHA) plate was inoculated with *E.coli* ATCC 25922 using the routine disc diffusion procedure within 15 minutes. The plate was allowed to dry for 3-10 minutes before adding the meropenem disc. The meropenem disc was removed from the TSB-meropenem disc suspension and placed on an MHA plate previously inoculated with the E.coli ATCC 25922 indicator strain. Then plate was incubated at 35°C±2°C in ambient air for 18-24 hours. Following incubation, the zone of inhibition was measured. No zone of inhibition or 6-15mm of zone diameter or presence of pinpoint colonies within 16-18mm zone considered as carbapenemase positive. On the other hand, Zone dm of 19mm was carbapenemase negative or zone dm 16-18 mm or presence of pinpoint colonies within ≥ 19 mm zone was carbapenemase indeterminate.

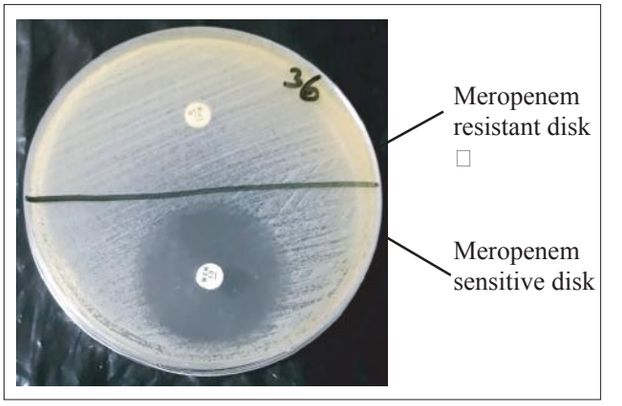

Figure 1 Modified Carbapenem Inactivation Method Shows Carbapenemase Positive Isolates

Molecular characterization of carbapenem resistance genes

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Polymerase Chain Reaction (PCR)-based detection of Ambler class B MBLs (blaIMP, blaVIM and blaNDM), Ambler class D (blaOXA-48) and class A, (blaKPC) was carried out on meropenem resistant isolates. Coexistence of carbapenemase encoding genes, namely, NDM, OXA-48 and KPC were also evaluated by PCR. Genomic DNA was extracted by the boiling method. The following pairs of previously used primers were used to yield PCR products:

Primer	Sequence	Gene	Product Size (bp)
IMP-F	GGAATAGAGTGGTCGCTTAAYTCTC	bla IMP	232
IMP-R	GGTTTAAYAAAACAACCACC		
VIM-F	GATGGTGTTTGGTCGCATA	bla VIM	390
VIM-R	CGAATGCGCAGCACCAG		
OXA-F	GCGTGGTTAAGGATGAACAC	bla OXA	438
OXA-R	CATCAAGTTCAACCCAACCG		
NDM-F	GGTTTGGCGATCTGGTTTTC	bla NDM	621
NDM-R	CGGAATGGCTCATCACGATC		
KPC-F	CGTCTAGTTCTCTGCTGTCTTG	bla KPC	798
KPC-R	CTTGTCATCCTTGTTAGGCG		

The following cycling parameters were used: Initial Denaturation: at 94° C for 10 minutes followed by 36 cycles of amplication consisting of denaturation: at 94°C for 30 s, primer annealing: at 52°C for 40 s, extension: at 72°C for 50s and final extension: at 72°C for 5 minutes. The amplified DNA was analyzed by 1.5% agarose gel-electrophoresis at 120 volts for 20 minutes, stained with 1% ethidium bromide andvisualized under UV light.[9]

Data were analyzed by using IBM-SPSS statistics v.20.0 for windows. Comparison were performed using chi-square test by chi-square calculator. Results were presented in the form of tables and figures.

Results

A total of 20 meropenem-resistant strains by disk diffusion technique were included in this study. Seven Escherichia coli, seven Pseudomonas spp. five Klebsiella spp. and one Acinetobacter spp. were isolated from the meropenem-resistant organisms. The rate of resistance to different classes of antibiotics ranged from 60% to 100% and low resistance was observed in amikacin and pipercillin-tazobactam which was 60% and 70% respectively. Table I shows, mCIM detected 17 carbapenemase producing uropathogens where the most prevalent was Pseudomonas spp. 7(41.17) followed by Escherichia coli 6(35.29%) and Klebsiella spp. 4(23.52%).

Table I Distribution of Carbapenemase Producing uropathogens (n=17)

Uropathogens	Carbapenemase producers
Escherichia coli	6 (35.29%)
Klebsiella spp.	4 (23.52%)
Pseudomonas spp.	7 (41.17%)
Acinetobacter spp.	0 (0.00%)
Total	17(100%)

Among 68 Gram negative uropathgens, 20(29.41%) meropenem resistant isolates were subjected to multiplex PCR and carbapenemase genes were found in 19 (27.94%). Out of 20 meropenem resistant isolates, 18 (90%) carried a single carbapnemase gene, 1(5%) isolate carried more than one carbapenemase gene, and 1(5%) had no carbapenemase gene. Table 2 shows all (100%) meropenem resistant Escherichia coli and Pseudomonas spp., three (15%) of the Klebsiella spp and the only Acinetobacter spp carried blaNDM and the combination of different genes in Klebsiella spp.were observed by multiplex PCR. Table 3 shows mCIM tests detected 17 (85%) carbapenemase producers among the 20 meropenem-resistant isolates whereas PCR detected 19 carbapenemase positive isolates which was statiscally significant. Out of the one negative amplified PCR products was negative by mCIM. Sensitivity of the phenotypic methods considering PCR as gold standard was 89.5% for mCIM.

Table II Detection of Carbapenemase genes among the uropathogens

Carbapenemase genes	Uropathogens
blaNDM	18 (94.73%)
blaVIM	0
blaIMP	0
blaKPC	0
blaOXA 48	0
blaKPC + blaNDM + blaOXA 48	0
blaKPC + blaOXA 48	0
blaNDM + blaOXA 48	1 (5.26%)
Total	19 (100%)

Table III Association between mCIM and Multiplex PCR test results among the meropenem resistant isolates (n=20)

mCIM	Multiplex PCR Test			χ^2 test Significance
	Positive	Negative	Total	
Positive	17	00	17	$\chi^2 = 5.965$
Negative	02	01	03	p = 0.015
Total	19	01	20	S

- Figures within parentheses indicate percentages
- S = Significant ($p < 0.05$)

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Discussion

The present study identified 19(95%) Carbapenemase producers out of the 20 meropenem-resistant isolates. All meropenem resistant Escherichia coli, Pseudomonas spp. and Acinetobacter spp. and 60% Klebsiella spp. had carbapenemase genes and coharbouring of bla NDM and bla OXa-48 in 20% Klebsiella spp. Although acquired carbapenemase-encoding genes are frequently found in Pseudomonas spp. and Acinetobacter spp., the existence of carbapenemase-encoding genes in the species of E. coli and Klebsiella pneumonia in this study suggests that plasmid-mediated horizontal transfer of carbapenemase-encoding genes occurs continuously among Gram-negative bacilli, as reported previously. Co-harboring of multiple genes creat a new challenge for the treatment of infections caused by carbapenem resistant strains because carbapenem-resistant genes could co-exist with beta-lactamases and other resistant genes on plasmid.[10]

Khan et al India, reported that the Asian continent, especially China and India were a reservoir of NDM in which about 58.20% abundance of the blaNDM-1 varients.[11] The result of the current study reflects that the emergence of carbapenemase gene mainly blaNDM is alarming for therapeutic options and its rising in Bangladesh like India, Pakistan and Nepal indicating inappropriate and nonprescription antibiotic use as a probable cause of development of resistance in this subcontinent. Furthermore, rapid dissemination of blaNDM producing organisms might be facilitated by conditions such as overcrowding, over the counter availability of antibiotics, low level of hygiene and weak hospital antibiotic policies.[12]

Copresence of multiple resistance genes is a big concern in the spread of antibiotic resistance. It has been observed that majority of Gram negative bacteria had more than one Carbapenemase gene, where co-existence genes were mostly found in Escherichia coli and Klebsiella spp.[13] In China, Han et al. found that the coharbouring of bla-NDM+bla-OXA-48 was present in 1 Klebsiella spp. which is supported to our study.[14] Plasmids carrying the blaNDM-1 gene are diverse and can harbor a high number of resistance genes associated with other carbapenemase genes (OXA-48 types, VIM types), plasmid-mediated cephalosporinase genes, ESBL genes, aminoglycoside resistance genes (16S RNA methylases), macrolide resistance genes (Esterase), and rifampin (Rifampin-modifying enzymes) and sulfamethoxazole resistance genes as sources of multidrug resistance and pandrug resistance.[15] The prevalence of carbapenemase producers still remain obscure because of the lack of proper detection method in many countries, especially those with limited resources and poor laboratory setting.[16] In the present study, 29.23% (19/68) of Gram negative uropathogens showed the occurrence of carbapenemase encoding genes, which is higher than other data reported in Bangladesh.

The discrepancy in the findings might be due to the increased use of carbapenem in Chattogram, Bangladesh. However, it is observed One meropenem-resistant strain showed negative amplification by PCR. Gram-negative bacteria have the capacity to elude the action of carbapenems through modification of outer membrane permeability (i.e. porin loss) upregulation of efflux systems, production of carbapenem-Hydrolyzing β-lactamases (i.e. hyperproduction of AmpC β-lactamases, certain ESBLs with increased capacity to hydrolyze carbapenems) and production of carbapenemases (Either serine based carbapenemases or MBLs). The plethora of meropenem resistance among the noncarbapenemase producers in this study might be due to resistance mechanisms other than carbapenemase production. In addition to showing resistance to β-lactams, all the carbapenemase-producing organisms were highly resistant to most other antibiotic classes. The higher antibiotic resistance in the present study might be due to the fact that common antibiotics are sold over the counter in Bangladesh and anybody can buy them without a doctor's advice.[17] By contrast, selective pressure and/or the simultaneous presence of other drug resistance genes such as gene cassettes or other resistance mechanisms might be the reason for the co-resistance.

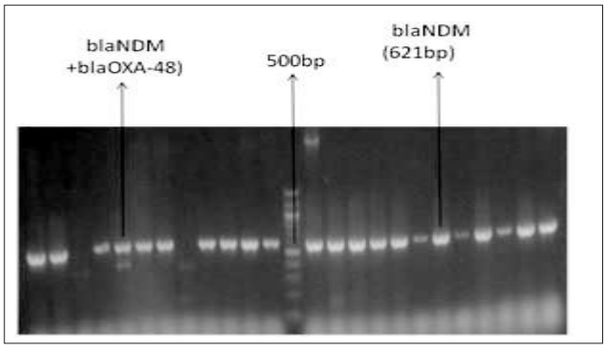

Figure 2 Multiplex PCR shows Carbapenemase genes among uropathogens

Conclusion

Carbapenems are highly effective against many bacterial species and less vulnerable to most beta-lactam resistance determinants, they are considered to be the most reliable last-resort treatment for multidrug resistsant bacterial infections. For these reasons, the emergence and rapid spread through all continents of carbapenem resistance, mainly among Gram-negative

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bacteria, constitutes a global public-healthcare problem of major importance. This study shows a significant rate of carbapenemase producing Gram-negative uropathogens. This is extremely worrisome, as dissemination of plasmids carrying resistant determinant genes from one species to another makes organisms refractory to the common antibiotics used in clinical practice. The need of the hour is a strong antimicrobial stewardship program, which is followed by all concerned doctors, with further emphasis on better, cost-effective, logical infection control measures to prevent the dissemination of these multidrug resistant bacteria.

Disclosure

All the author declared no competing interest.

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