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Original Article

Molecular epidemiology and antimicrobial susceptibility of carbapenemase-producing extraintestinal *Escherichia coli* in a tertiary care hospital of Dhaka, Bangladesh.

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Abstract

The emergence of carbapenemase producing *Escherichia coli*, the first hospital and community-acquired opportunistic pathogen now become a great public health concern. A total of 23 clinical isolates of carbapenemase producing *E. coli* from extraintestinal infections in a tertiary care hospital of Bangladesh were studied. Highest percentage of carbapenemase producing *E. coli* isolates were from urine samples (52.2%) followed by 21.7% from each of wound swab and pus and 4.1 % from blood samples. Among the *E. coli* isolates 69.6% were from indoor patients and 30.4% were from outdoor patients. All the isolates (100%) were positive for *NDM* of which 13% were *NDM* and *OXA*-48 co-producers by conventional PCR. Carbapenemase producing *E. coli* isolates were resistant to most of the antibiotic tested except for nitrofurantoin, colistin, polymyxin B and tigecycline with a sensitivity of 66.7%, 82.6%, 95.7% and 100% respectively.

Key wards: *Escherichia coli*, carbapenemase, *NDM*, *OXA*-48, molecular epidemiology, antimicrobial resistance, Bangladesh.

Introduction

Escherichia coli (E. coli), one of the important nosocomial pathogens of Enterobacteriaceae family is a common etiological factor of urinary tract infections, gastroenteritis, sepsis, meningitis, pneumonia, blood stream infections, intra-abdominal infections and surgical site infections[1,2]. The treatment of infections caused by *E. coli*, especially carbapenemase producing *E. coli* is challenging, because it confers resistance to most β-lactams including carbapenems and often carries additional antimicrobial resistance genes, making them resistant to most of the antibiotics[1,3]. Recently, carbapenem resistant *E. coli* isolates with limited therapeutic possibilities have been implicated in both hospital and community-acquired infections which became a major health problem all over the world[3]. Among the two types of carbapenemases (serine carbapenemase and metallo-β-lactamase), New Delhi metallo-β-lactamase (*NDM*) and carbapenem-hydrolyzing oxacillinase-48 (*OXA*-48) are the most common carbapenemases among *E. coli* worldwide[4]. Over the last decade, *NDM* producers with susceptibility to a few antibiotics including colistin have undergone rapid spread in the South-Asian continent[5]. *OXA*-48- producing *E. coli* isolates were also reported in South Asia. The first isolates of *OXA*-48 producing *E. coli* reported in Japan were isolated from a patient with a medical history in Southeast Asia[6]. In this study, molecular detection of resistant genes (*NDM*, *OXA*-48, *KPC, IMP*) along with antimicrobial susceptibility of carbapenem resistant extraintestinal *E. coli* isolates were carried out.

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Materials and Methods

Bacterial isolates: This cross-sectional study was conducted in the Department of Microbiology and Immunology of Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh from September, 2018 to August, 2019. A total of 23 strains of carbapenem resistant *E. coli* isolated from different clinical specimens such as urine, blood, wound swab and pus were included in this study. Isolates of *E. coli* were identified by standard biochemical methods. This study was ethically approved by Institutional Review Board (IRB) of Bangabandhu Sheikh Mujib Medical University.

Antimicrobial susceptibility testing: Antimicrobial susceptibility of 23 strains of *E. coli* against 21 different antibiotics were performed on Mueller-Hinton agar (MHA) plates by the modified Kirby-Bauer disc diffusion method using antibiotic discs from BioMaxima, Poland. Results were interpreted according to the criteria of the Clinical and Laboratory Standards Institute (CLSI) 2019[7] guidelines; for polymyxin B and colistin according to CLSI 2007[8] and tigecycline according to EUCAST 2016[9] guidelines.

Detection of carbapenemase genes by PCR:
The presence of carbapenemase genes were determined by conventional PCR. Bacterial DNA was extracted by boiling method of DNA extraction[10] and was stored at -20°C. All the isolates of *E. coli* were screened for the presence of *NDM*, *OXA*-48, *KPC* and *IMP* gene separately by using primers described in Table I.

Table I: Primers used for detection of carbapenemase genes.

Enzyme	Primer name	Sequence (5′ to 3′)	Amplicon size (bp)	Reference
NDM	*NDM* forward	ATG GAA TTG CCC AAT ATT ATG CAC	813	11
	NDM reverse	TCA GCG CAG CTT GTC GGC		
***OXA*-48**	*OXA*-48 forward	TTGGTGGCATCGATTATCGG	743	12
	OXA-48 reverse	GAGCACTTCTTTTGTGATGGC		
KPC	*KPC* forward	ATGTCACTGTATCGCCGTCT	887	13
	KPC reverse	TTTTCAGAGCCTTACTGCCC		
IMP	*IMP* forward	GAATAG(A/G)(A/G)TGGCTTAA(C/T)TCT	188	14
	IMP reverse	CCAAAC(C/T)ACTA(G/C)GTTATC		

Statistical Analysis: All the data were analyzed using Microsoft Excel 2019. Descriptive analysis of all relevant categorical variables was done by using frequency and percentage.

Results: All *E. coli* (n=23) isolates were found to be carbapenem (imipenem, meropenem and ertapenem) resistant by the disc diffusion method. Source of carbapenemase producing *E. coli* isolates were shown in Table II.

Table II: Source of carbapenemase producing extraintestinal *E. coli* (n=23)

Samples	Number	Percentage (%)
Urine	12	52.2
Wound swab	5	21.7
Pus	5	21.7
Blood	1	4.4
Total	23	100.00

Among the isolates of carbapenemase producing *E. coli*, 69.6 % (n=16) were isolated from indoor patients and 30.4% (n=7) were isolated from outdoor patients. Of these 23 patients, 60.9% (n=14) were male and 39.1% (n=9) were females. Among tested carbapenemase encoding genes (*NDM, OXA*-48, *IMP, KPC*), only *NDM* and *OXA*-48 were detected by conventional PCR. Eighty seven percent of carbapenemase producing isolates were positive for only *NDM* and 13% were positive for both *NDM* and *OXA*-48 (Table III). No *KPC* and *IMP* genes were detected in any isolates of *E. coli*. Antimicrobial resistance pattern of carbapenemase producing *E. coli* isolates are shown in Table IV.

Table III: Target genes in carbapenem resistant *E. coli* isolates (n=23)

Target genes	Numbers of *E.coli* isolates	Percentage (%)
Only *NDM*	20	87.0
***NDM* and *OXA*-48 co-producers**	3	13.0
Total	23	100.0

Table IV: Antimicrobial resistance pattern of carbapenemase producing extraintestinal *E. coli* isolates

Antimicrobial agents	No. of resistant isolates by carbapenemase encoding genes	
	Only *NDM* (n=20) n (%)	***NDM* and *OXA*-48 co-producers (n=3); n (%)**
Amoxicillin	20 (100.0)	3 (100.0)
Amikacin	**11 (55.0)**	3 (100.0)
Aztreonam	20 (100.0)	3 (100.0)
Carbapenems	20 (100.0)	3 (100.0)
Cephalosporins	20 (100.0)	3 (100.0)
Ciprofloxacin	20 (100.0)	3 (100.0)
Cotrimoxazole	**18 (90.0)**	**2 (66.7)**
Gentamicin	15 (75.0)	3 (100.0)
Mecillinam	19 (95.0)	3 (100.0)
Nalidixic acid	20 (100.0)	3 (100.0)
Netilmicin	**11 (55.0)**	3 (100.0)
Nitrofurantoin (urinary isolates)	**4 (36.4), n=11**	**0 (0.0), n=1**
Colistin	**2 (10.0)**	**2 (66.0)**
Piperacillin+ tazobactum	20 (100.0)	3 (100.0)
Polymyxin B	**1 (5.0)**	**0 (0.0)**
Tigecycline	**0 (0.0)**	**0 (0.0)**

Note: Carbapenems include meropenem, imipenem, ertapenem and cephalosporins include cefuroxime, ceftriaxone, ceftazidime and cefotaxime.

Discussion
Carbapenems are the first-choice of treatment for *ESBL* producing *E. coli* which are resistant to third-generation cephalosporins.

Widespread use of carbapenems results in the emergence of carbapenem resistant *E. coli* which is attributed to the production of carbapenemases and /or decrease in permeability of the outer membrane[1,3]. In this study 100% (n=23) of carbapenem resistant *E. coli* were found to be positive for *NDM* of which 13.0% (n=3) were co-producers of *NDM* and *OXA*-48. A study in Bangladesh also reported *NDM*-producing uropathogenic *E. coli*[5]. Khajuria et al (2014) reported 100% of *NDM* and 55% of *NDM* and *OXA*-48 co-producers among multidrug resistant urinary *E. coli* isolates[15]. The prevalence of *NDM*-producing *E. coli* in India, China, Pakistan and Nepal were 50.3%, 21.4%, 7.4% and 6.8% respectively[16]. Easy access to broad-spectrum antibiotics without proper prescriptions, poor sanitation, increased medical tourism for health care and lack of stringent antibiotic are the risk factors for the emergence and spread of *NDM*-producing superbugs in the Indian subcontinent[16].

Highest percentage of carbapenemase producing extraintestinal *E. coli* were isolated from urine samples (52.2%), followed by wound swab and pus (21.7%). *NDM* and *OXA*-48 carbapenemase producing *E. coli* isolated from urine, blood, wound swab, cerebrospinal fluids were also reported in other studies[15,17]. All of the isolates included in this study were 100% resistant to most of the tested antibiotics (amoxicillin, aztreonam, carbapenems, cephalosporins, ciprofloxacin, nalidixic acid, piperacillin+ tazobactum). Currently polymyxins, aminoglycosides and tigecycline are regarded as the main treatment options for the treatment of invasive CRE infections[18]. In their study resistance to the most effective agents, colistin, polymyxin B and aminoglycosides (amikacin, gentamicin and netilmicin) were 17.4%, 4.3% and (60.9%, 78.3% and 60.9%) respectively. The isolates of *NDM* and *OXA*-48 co-producers were 100% resistant to amikacin, gentamicin and netilmicin. All the isolates of this study were 100% sensitive to tigecycline but the status of colistin and aminoglycoside resistance is of particular concern as they are used in empiric treatment protocols to treat invasive CRE infections. Similar findings of resistance to reserve antibiotics were also reported in previous study[18]. Among the urinary isolates of carbapenemase producing *E. coli*, 66.7% were sensitive to nitrofurantoin. 96% and 40% susceptibility of carbapenemase producing *E. coli* isolates to nitrofurantoin were also reported in other studies[19,20]. These findings are of great importance because 30.4% isolates of this study were from outdoor patients and nitrofurantoin is frequently prescribed for urinary tract infections in the community, even without microbiological documentation. Reports of *E. coli* harboring *NDM*-1 and *OXA*-48 from various parts of the world are a major concern because these genes are located in plasmids which can horizontally transfer between bacteria[5].

Conclusion

Being a nosocomial pathogen, *E. coli* are responsible for both community and hospital-acquired infections, thus raising the fear of dissemination of carbapenemase producing *E. coli* in the community. Emergence of carbapenemase producing strains of *E. coli* with a sensitivity to fewer antibiotics is a very serious concern, especially in a developing country like Bangladesh which frequently experiences antibiotic misuses.

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