

## Original Article

# Antimicrobial Resistance Pattern of Extensively Drug Resistant Carbapenemase Producing *Enterobacteriaceae*

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### Abstract

Carbapenems, often agents of last resort for multidrug resistant bacterial infections are now threatened by widespread dissemination of carbapenem-resistant *Enterobacteriaceae* (CRE). Production of carbapenemases remain the most clinically important mechanism of carbapenem resistance in *Enterobacteriaceae*. The objective of this study was to determine the antibiogram pattern of carbapenemase producing *Enterobacteriaceae*. A cross sectional study was conducted at department of Microbiology and Immunology, BSMMU from September 2018 to August 2019. A total of 145 CRE isolates from different clinical samples were studied. Antimicrobial susceptibility was examined by disk diffusion method and MIC of colistin by broth microdilution method. Resistant carbapenemase genes NDM and OXA-48 were identified by polymerase chain reaction. Out of 145 CRE isolates, 104 were NDM, 73 were OXA-48 and 34 isolates were both NDM and OXA-48 co-producers. All the NDM and OXA-48 carbapenemase producing isolates were 100% resistant to meropenem, imipenem, ertapenem, ceftriaxone, ceftazidime, cefotaxime, cefuroxime, amoxicillin + clavulanic acid and piperacillin + tazobactam. Resistance rates of reserved antimicrobials to treat CRE isolates were also alarming. Thirty seven percent, 9.6% and 5.5 % of OXA-48 carbapenemase producers and 26.0%, 10.6% and 2.9% of NDM carbapenemase producers were resistant to colistin, polymyxin B and tigecycline respectively. Among the carbapenemase producing isolates, 16.6% (24) were multidrug resistant (MDR), 82.1% (119) were extensively drug resistant (XDR) and 1.3% (2) isolates were pan drug resistant which highlights the emerging therapeutic challenge for these superbugs.

**Key words:** Carbapenem resistant *Enterobacteriaceae*, carbapenemase, MDR, XDR.

### Introduction

Over years, there is an increased use of carbapenem to treat infections with multidrug resistant *Enterobacteriaceae* due to extended spectrum  $\beta$ -lactamases and its variants.<sup>1</sup> The emergence of carbapenem resistant *Enterobacteriaceae* (CRE) due to the production of carbapenemase, a group of enzyme that hydrolyzes all  $\beta$ -lactams antibiotics including carbapenems have become a major global concern because of extremely limited therapeutic options and their association with high (56.7%) case-fatality rates in the current antibiotic pipeline.<sup>1,2,3</sup> Class A *K.pneumoniae* carbapenemase (KPC), Class B metallo New Delhi metallo- $\beta$ -lactamase (NDM), Verona integron-encoded metallo- $\beta$ -lactamase (VIM), Imipenemase (IMP) and Class D oxacillinase

(OXA)-48 and its variants are the most important carbapenemases in *Enterobacteriaceae*. However, NDM-1, OXA-48 are the frequently reported carbapenemases among *Enterobacteriaceae*, particularly in *K.pneumoniae* and *E. coli*.<sup>1</sup> Carbapenemase producing isolates are usually multidrug resistant as they frequently carry additional resistance genes to other classes of antibiotics including aminoglycosides, fluoroquinolones, macrolides, rifampicin, trimethoprim-sulfamethoxazole and constitute a threat to continuous use of this class of antibiotics in medical practice.<sup>1,4,5</sup> The aim of this study was to highlight the antibiotic resistance pattern of NDM and OXA-48 carbapenemase producing carbapenem resistant *Enterobacteriaceae*.

### Materials and Methods

This cross-sectional study was conducted at Department of Microbiology and Immunology, BSMMU over a period of one year from September 2018 to August 2019. A total 145 isolates of *Enterobacteriaceae* from different

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clinical samples such as urine, blood, tracheal aspirate, wound swab, pus, sputum, throat swab, bile, drain fluid which were resistant to any of the three carbapenems according to CLSI 2019<sup>6</sup> disk diffusion break point criteria (zone diameter  $\leq$  19 mm for meropenem and imipenem; zone diameter  $\leq$  18 mm for ertapenem) were collected for this study. Antimicrobial susceptibility test was done by modified disc diffusion method against ciprofloxacin, co-trimoxazole, ceftriaxone, cefotaxime, ceftazidime, cefuroxime, gentamicin, amikacin, nitrofurantoin, amoxicillin-clavulanate, aztreonam, meropenem, imipenem, ertapenem, mecillinam, nalidixic acid, netilmicin, tigecycline, piperacillin-tazobactam, polymyxin B and the result was interpreted according to CLSI 2019<sup>6</sup> except polymyxin B (according to CLSI 2007<sup>7</sup>); tigecycline and MIC of colistin by broth microdilution method according to EUCAST 2016<sup>8</sup> guidelines. *Escherichia coli* ATCC 25922 was used as a quality control strain for antimicrobial susceptibility testing.

Carbapenem resistant *Enterobacteriaceae* isolates were screened by conventional PCR for the presence of NDM and OXA-48 carbapenemase genes by targeting 813bp<sup>9</sup> and 743bp<sup>10</sup> respectively, using primers<sup>9,10</sup> specific for genes respectively.

## Results

A total of 145 CRE isolates according to CLSI<sup>6</sup> and CDC<sup>11</sup> screening criteria for carbapenem resistant *Enterobacteriaceae* were identified in which 109 isolates were *K. pneumoniae*, 23 *E. coli*, 6 *E. aerogenes*, 5 *E. cloacae* and 2 *K. oxytoca*. Out of 145 CRE isolates, 16.6% (24) isolates were multidrug resistant (MDR), 82.1% (119) extensively drug resistant (XDR) and 1.3% (2) were pan drug resistant (PDR) isolates (Table I).

**Table-I: Drug resistance types of CRE isolates (n=145)**

CRE isolates	Drug resistance type n (%)		
	MDR	XDR	PDR
<i>K.pneumoniae</i> , n=109	7 (6.4)	100 (91.8)	2 (1.8)
<i>K. oxytoca</i> , n=2	1 (50.0)	1 (50.0)	0
<i>E.coli</i> , n=23	13 (56.5)	10 (43.5)	0
<i>E. aerogenes</i> , n=6	0	6 (100.0)	0
<i>E.cloacae</i> , n=5	3 (60.0)	2 (40.0)	0
<b>Total (n=145)</b>	<b>24 (16.6)</b>	<b>119 (82.1)</b>	<b>2 (1.3)</b>

**Table-II: Distribution of MDR and XDR isolates among the carbapenemase genes**

Resistance Type	NDM n (%)	OXA-48 n (%)	NDM, OXA-48 n (%)
<b>MDR, n=24</b>	18 (75)	8 (33.3)	2 (5.9)
<b>XDR, n= 119</b>	85 (71.2)	63 (57)	31 (91.2)

**Table-III: Antimicrobial resistance pattern of NDM and OXA-48 encoding CRE isolates**

Antimicrobial agents	No. of resistant isolates by carbapenemase encoding genes	
	NDM (n=104) n (%)	OXA-48 (n=73) n (%)
Meropenem	104 (100)	73 (100)
Imipenem	104 (100)	73 (100)
Ertapenem	104 (100)	73 (100)
Ceftriaxone	104 (100)	73 (100)
Cefotaxime	104 (100)	73 (100)
Cefuroxime	104 (100)	73 (100)
Ceftazidime	104 (100)	73 (100)
Amoxicillin + clavulanic acid	104 (100)	73 (100)
Piperacillin + tazobactam	104 (100)	73 (100)
Ciprofloxacin	103 (99.0)	73 (100)
Gentamicin	98 (94.2)	70 (95.9)
Amikacin	94 (90.4)	73 (100)
Cotrimoxazole	100 (96.2)	66 (90.4)
Netilmicin	94 (90.4)	73 (100)
Nalidixic acid	104 (100)	73 (100)
Aztreonam	102 (98.1)	72 (98.6)
Nitrofurantoin (urinary isolates)	14 (56.0), n=25	11(91.7), n=12
Mecillinam (urinary E.coli)	11 (91.7), n=12	1 (100), n=1
Colistin (MIC)	27 (26.0)	27 (37.0)
Polymyxin B	11 (10.6)	7 (9.6)
Tigecycline	3 (2.9)	4 (5.5)

Among the MDR isolates 75% (18) and 33.3% (8) of isolates were positive for NDM and OXA-48 respectively. Fifty three percent and 71.2% of XDR isolates were positive for OXA-48 and NDM respectively (Table II).

All the NDM and OXA-48 producing isolates were 100% resistant to all the  $\beta$ -lactam drugs tested except aztreonam, 98.1% for NDM and 98.6% for OXA-48. Colistin resistance observed in 26% NDM and 37% OXA-48 producers. Resistance to commonly used antibiotics among bla<sub>NDM</sub> and bla<sub>OXA-48</sub> carbapenemase gene encoding isolates are presented in Table III.

## Discussion

In recent time the worldwide emergence of carbapenem resistant *Enterobacteriaceae* constitutes an important growing public health threat.<sup>4</sup> Sporadic outbreaks or endemic situations with carbapenem non susceptible enterobacterial isolates are now reported from both communities and hospitals across the globe. Carbapenemases are the most important determinant of carbapenem resistance and increasing prevalence of NDM type and OXA-48 carbapenemase producing *K.pneumoniae* and *E. coli* are reported frequently.<sup>1,4</sup> In our study bla<sub>NDM</sub> genes were identified from *K. pneumoniae*, *E.coli*, *E.aerogenes*, *E. cloacae*, *K. oxytoca* isolates and bla<sub>OXA-48</sub> genes were identified from *K. pneumoniae*, *E. coli* and *E. aerogenes*. In our country bla<sub>OXA-48</sub> genes were previously identified in *K. oxytoca*, *K. pneumoniae*, *E. aerogenes*, *E. coli* isolates.<sup>12</sup> An association between production of carbapenemase and MDR and XDR are emergent in *Enterobacteriaceae* due to their high propensity to pick and give out mobile resistance element.<sup>13</sup> Our study reveals 24 of 145 isolates (16.6%) were MDR, 119 (82.1%) were XDR and 2 (1.3%) were PDR. MDR was defined as being resistant to three or more antimicrobial categories, XDR was defined as resistant to at least one agent in all but two or fewer antimicrobial categories (i.e. bacterial isolates remain susceptible to only one or two categories) and PDR was defined as non-susceptibility to all agents in all antimicrobial categories.<sup>14</sup> Twelve (63.1%) of 19 extensively drug resistant carbapenemase producing isolates were also reported in a study of Bangladesh.<sup>12</sup>

All the bla<sub>NDM</sub> and bla<sub>OXA-48</sub> positive isolates of our study showed 100% resistant to all beta-lactams drugs, including all the three carbapenems tested (imipenem, meropenem, ertapenem). CRE isolates carrying bla<sub>NDM</sub> and bla<sub>OXA-48</sub> were also highly resistant to the most other classes of antibiotics tested, including quinolones and aminoglycosides. In a study of India, high percentage of resistance to amoxycyclavulanate, ampicillin, aztreonam ciprofloxacin and the third generation cephalosporins<sup>14</sup> were also detected.

Serious infections due to carbapenemase producing *Enterobacteriaceae* in debilitated and immunocompromised patients are associated with prolonged hospital stays

and increased mortality rates where aggressive and rapidly efficacious therapy are needed. However, therapeutic options are obviously limited and colistin, tigecycline, fosfomycin remains the most effective antibacterials against clinical *Enterobacteriaceae* producing either KPC or MBLs. Drug effectiveness differs depending on the extent of spread of resistant isolates in each setting.<sup>15</sup>

Findings of our study revealed that resistance rates of colistin (37%) and tigecycline (5.5%) were quite higher in bla<sub>OXA-48</sub> positive isolates than bla<sub>NDM</sub> positive isolates in which 26% of isolates were resistant to colistin and 2.9% were resistant to tigecycline. In Bangladesh 5.3% of tigecycline resistant CRE isolates were also reported.<sup>12</sup> High resistance rate of netilmicin, amikacin and colistin in bla<sub>NDM</sub> positive isolates of our study was statistically significant ( $p < 0.05$ ).

Our study place, being a tertiary health care hospital, the selection pressure exerted on microorganisms because of wide spread use of broad spectrum antimicrobial and prior exposure of patient to antibiotics, might be the basis for such high level of antimicrobial resistance.

## Conclusion

Majority of the carbapenemase producing isolates of our study are extensively drug resistant and only susceptible to colistin, polymyxin B, tigecycline which are basically used to treat MDR organisms in our country. But resistance rate of these reserved antimicrobials are also alarming which reflect the eminent therapeutic challenge for these superbugs. Thus routine screening of clinical isolates of CRE is essential to understand the local emerging resistance pattern and to offer targeted therapy or changing the empirical treatment protocol.

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