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.

Keywords: 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 infections¹². 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 antibiotics¹³. 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 world³. 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 worldwide⁴. Over the last decade, NDM producers with susceptibility to a few antibiotics including colistin have undergone rapid spread in the South-Asian continent⁵. 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 Asia⁶. 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.

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) 2019⁷ guidelines; for polymyxin B and colistin according to CLSI 2007⁸ and tigecycline according to EUCAST 2016⁹ 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 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 | TTTGTCGATGATTACGCGG | 743 | 12 |
|        | OXA-48 reverse | GAGCA CTT CTG TAGG GCCC | | |
| KPC    | KPC forward | ATG TCA CTG ATG CCG GTCT | 887 | 13 |
|        | KPC reverse | TTTTCA AGG CCGT AAC CCG | | |
| IMP    | IMP forward | GAATAG(A/G)(A/G)TGGCTTAA(C/T)TCT | 188 | 14 |
|        | IMP reverse | CCAAA C(CT/CA)TAC GAC 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 | 3                      | 13.0           |

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)     | 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      | 4 (36.4), n=11                                          | 0 (0.0), n=1 |
| (urinary isolates)  |                                                        |              |
| 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^{1,2}. 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*\(^6\). Khajuria et al (2014) reported 100% of *NDM* and 55% of *NDM* and *OXA*-48 co-producers among multidrug resistant urin ary *E. coli* isolates\(^3\). The prevalence of *NDM*-producing *E. coli* in India, China, Pakistan and Nepal were 50.3%, 21.4%, 7.4% and 6.8% respectively\(^4\). 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\(^5\).

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\(^6\). All of the isolates included in this study were 100% resistant to most of the tested antibiotics (amoxicillin, aztreonam, carbapenems, cephalosporins, ciprofloxcin, nalidixic acid, piperacillin+ tazobactum). Currently polymyxins, aminoglycosides and tigecycline are regarded as the main treatment options for the treatment of invasive CRE infections\(^7\). 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\(^8\). 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\(^9\). 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\(^3\).

**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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