Genetic Detection of Carbapenemase Producing Imipenem Resistant *E. coli* of Urine Sample in Dhaka Medical College.

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Cite this as:
BMCJ 2023; 8(1): 12-16

Received: 19 October 2022
Accepted: 18 Decemberl 2022

Abstract

Background: Antimicrobial resistance is a serious public health problem. The emergence of metallo-β-lactamase-producing *Enterobacteriaceae* is a worldwide health concern. **Objectives:** This study aims to determine genetic detection of carbapenemase producing imipenem resistant *E.coli* in Dhaka Medical College & Hospital. **Methods:** A cross-sectional observational study was done over a period of one year in Dhaka Medical College and Hospital. The clinically suspected urinary tract infected (UTI) patients attending at the outpatient department of Dhaka Medical College Hospital and the Microbiology Department of Dhaka Medical College constituted the study population. A total 280 urine samples were collected by purposive sampling with aseptic precaution from the attended patients. Antimicrobial resistant patterns of uropathogenic *E.coli* with genetic detection of Carbapenemase Producing Imipenem Resistant *E. coli* was determined by PCR in Microbiology Department Dhaka Medical College. Antibiotic susceptibility pattern was determined by double disk method for all the isolated *E.coli* strains. **Results:** A total of 280 urine samples, 83 (29.6%) samples were identified as culture positive. Among the microorganisms identified in this study, 77 (92.8%) were gram negative and the rest 6 (7.2%) were gram positive. The most resistance was found against Cotrimoxazole (90%) and lowest resistant was found against Tigecyline (6.67%). Genotyping detection of carbapenemase encoding genes among imipenem resistant uropathogenic *Esch.coli,* out of nine imipenem resistant *Esch.coli,* seven has positive encoding genes with 55.56% bla NDM-1 and 44.44% bla NDM-2 i.e., 22.22% bla VIM and 22.22% blaOXA-48. There was no bla IMP gene. **Conclusion:** Antimicrobial resistance has become a global issue now a days. So, we should use appropriate antibiotic according to the sensitivity pattern for bacteria to prevent emergence of resistance.

Key words: Uropathogenic *E.coli,* antimicrobial susceptibility pattern, antimicrobial resistance pattern, carbapenemase producing imipenem resistant uropathogenic *E. coli.*

Introduction

Urinary tract infections (UTIs) exclusively cause of emergence of antimicrobial resistance.[1] Antimicrobial resistance (AMR) is a worldwide

nuisance to health community and globally 700,000 deaths are annually reported.[2] *Esch.coli* is increasingly associated with multi-drug resistance, including the resistance to the last-resort carbapenems.[3] This antimicrobial resistance occurs because UTI treatment usually starts without culture and antimicrobial susceptibility testing in developing countries. Secondly, poverty and illiteracy are two factors for increasing trends of inadequate dosing of antibiotics and incomplete course of treatment that cause increase rate of antibiotic resistance.[4] The prevalence of ESBLs producing Esch.coli is reported in 62.9-100% from Asia.[5,6] The novel antibiotic resistant genes have emerged that encode resistance to carbapenems *(blaNDM, blaIMP, blaVIM, and blaKPC)* thus limiting the treatment options. These resistance genes containing bacteria are super bugs and are termed as carbapenem-resistant Enterobacteriaceae (CRE) challenging the empiric treatment worldwide.[7] This study intended to determine genetic detection of carbapenemase producing imipenem resistant *E.coli* in Dhaka Medical College and Hospital in Bangladesh.

Methods

This cross-sectional observational study was carried out in Dhaka Medical College Hospital (DMCH) and Dhaka Medical College (DMC) over a period of one year. The clinically suspected urinary tract infected (UTI) patients attending at the outpatient department of DMCH and the Microbiology Department of DMC constituted the study population. A total 280 urine samples were collected by purposive sampling with aseptic precaution from the attended patients. The samples from the patients were collected by aseptic ways. The specimen was inoculated in blood agar, nutrient agar and Mac Conkey agar media and incubated aerobically at 37^0C for 24 hours. Antibiotic suscepti bility pattern was determined by double disk method for all the isolated *E.coli* strains. Imipenem resistant genes *(blaNDM, blaIMP, blaVIM, and blaKPC)* were detected by polymerase chain reaction (PCR) in Microbiology Department of DMC.

Results

Table I: Distribution of bacteria isolated from urine by culture (n=83).

Bacteria	Frequency (percentage) N (%)
Escherichia coli	60 (72.3)
Klebsiella spp.	6 (7.2)
Pseudomonas spp.	5 (6.0)
Proteus spp.	3 (3.6)
Enterobacter spp.	2 (2.4)
Acinetobacter spp.	1 (1.2)
CONS	3 (3.6)
Staphylococcus aureus	2 (2.4)
Enterococcus spp.	1 (1.2)

A total of 83 culture positive urine, 60 (72.2%) were *E.coli,* followed by 6(7.2%) *Klebsiella spp,* 5(6.0%) *Pseudomonas spp,* 3(3.6%) were *Proteus spp* and CONS (Table I).

Among the isolated uropathogenic *Esch.coli,* 90.0% were resistant to cotrimoxazole followed by 85.0% to ciprofloxacin, 83.3% to ceftriaxone, 80.0% toaztreonamand ceftazidime, 70.0% tocefoxitin, cefotaxime, amoxyclav and gentamicin, 65.0% topiperacillin/tazobactum, 40.0% toamikacin and nitrofurantoin, 23.3% to fosfomycin, 15% toimipenem, 13.3% to colistin and 6.7% resistant totigecycline (Table II).

Table II: Antibiotic resistance patterns of isolated uropathogenic *Esch.coli* (n=60).

Antimicrobial drugs	Resistant N (%)
Amikacin	24(40.0)
Amoxyclav	42(70.0)
Aztreonam	48(80.0)
Cefotaxime	42(70.0)
Cefoxitin	42(70.0)
Ceftazidime	48(80.0)
Cotrimoxazole	54(90.0)
Ceftriaxone	50(83.3)
Ciprofloxacin	51(85.0)
Gentamicin	42(70.0)
Piperacillin/Tazobactum	39(65.0)
Nitrofurantoin	24(40.0)
Colistin	08(13.3)
Imipenem	09(15.0)
Fosfomycin	14(23.3)
Tigecycline	04(06.6)

Table III: Distribution of carbapenemase encoding genes among imipenem resistant uropathogenic *Esch.coli* (n=9).

blaNDM-1	blaNDM-2like	blaIMP	blaVIM	blaOXA-48	TotalN(%)
Present (+)	Absent (-)	Absent (-)	Absent (-)	Absent (-)	3 (33.3)
Absent (-)	Present (+)	Absent (-)	Absent (-)	Absent (-)	2 (22.2)
Present (+)	Present (+)	Absent (-)	Present (+)	Present (+)	2 (22.2)
Absent (-)	Absent (-)	Absent (-)	Absent (-)	Absent (-)	2 (22.2)

Table III shows the distribution of carbapenemase encoding genes among imipenem resistant uropathogenic *Esch.coli.* Out of nine imipenem resistant Esch.coli, seven has positive encoding genes where has 55.56% bla NDM-1 and 44.44% bla NDM-2 I.e., 22.22% bla VIM and 22.22% blaOXA-48. There was no bla IMP geneisolated among imipenem resistant uropathogenic *Esch.coli.*

DISCUSSION

The findings of the present study suggests that majority of UTI were due to gram negative bacilli(GNB) and only 7.2% were due to gram positive cocci (GPC).

In a recent study[8], it was reported that GNB and GPC among uropathogens were 94.4% and 5.6%, respectively, which is almost similar to the present study findings. In the present study, the most common uropathogens isolated were *Esch.coli* (72.3%) followed by *Klebsiella spp.* (7.2%). Mohapatraet *al.(2022)*[9] reported that prevalence of *Esch.coli* and *Klebsiella pneumoniae* among total isolated uropathogens were 65.6% and 16.2% respectively in India, which are in accordance with present findings.

The other gram-negative bacteria noted in this present study were *Pseudomonas spp.* 6.0%, *Proteus spp.* 3.6%, *Enterobacter spp.* 2.4%, *Acinetobacter spp.* 1.2%. This finding is consistent with Akteret *al. (2016)*[10] in Pakistan (*Pseudomonas spp.* 7.6%, *Proteus spp.* 4.0%, *Enterobacter spp*. 2.3%, *Acinetobacter spp.*

1.0%). The present study revealed the high resistance of *Esch.coli* towards cotrimoxazole and ciprofloxacin i.e. 90% and 85%, respectively. These findings are in agreement with the study by Bhowmik *et al, (2021)*[11] who reported 86.6% resistance of *Esch.coli* to Cotrimoxazole and 79.9% to ciprofloxacin. In the present study, resistance pattern of *Esch.coli* to colistin and fosfomycin were 13.33% and 23.33%, respectively. Padhi *et al. (2020)*[12] from India reported 9.8% of *Esch.coli* resistant to colistin and 15.9% resistant to fosfomycin. Chowdhury *et al. (2019)*[13] reported that resistance of *Esch.coli* to colistin and fosfomycin were 12.19% and 17.47% respectively in Bangladesh.[13] The findings of Padhi *et al.(2020)*[12] and Chowdhury *et al. (2019)*[13] suggest that the scenario of *Esch.coli* resistant to colistin and fosfomycin in Bangladesh is the same as India. In the present study, it was observed that 5(55.56%) bla NDM-1 and 1(11.11%) blaVIM positive isolates, each of them was detected by PCR from 9(100%) imipenem resistant *Esch.coli* which were in agreement with the study conducted by Marufa (2016)16 in DMCH, who observed 47.25% blaNDM-1 and 9.72% blaVIM genes in imipenem resistant uropathogenic *Esch.coli.*[14] In this study, 4(44.44%) blaNDM-2like and 1(11.11%) blaOXA-48 positive genes were detected in imipenem resistant uropathogenic *Esch.coli.* No blaIMP gene was detected in the present study. A study conducted by Altayb *et al. (2020)*[15] reported that blaOXA-48 gene was detected in 15.5% of the isolates and blaIMP gene was not detected. Memon (2021)[16] also reported that 41.34% blaNDM-2 like positive isolates were detected. The reason behind not detection of blaIMP gene may be due to presence of other carbapenemase encoding genes rather than blaIMP in these imipenem resistant strains.

Antimicrobial resistance has become a global issue for all of us. That's why, we should use appropriate antibiotics according to the sensitivity pattern for bacteria to prevent emergence of resistance.

ACKNOWLEDGEMENTS

I express my profound gratitude to The Almighty Allah for giving me the opportunity to carry out and complete the study I acknowledge the support of Department of Microbiology, Dhaka Medical College, Dhaka for providing me the opportunity and resources to undertake my study. I express my gratitude to all the persons from whom I collected samples.

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