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# Phenotypic and Genotypic Detection of Carbapenems Producing Imipenem Resistant Uropathogenic *E. coli* with Their Antimicrobial Resistance Pattern in Dhaka Medical College

**Md. Sirazum Munir[1]*, Md. Ahsanul Haque[2]**
[1]Lecturer, Department of Virology, Rajshahi Medical College, Rajshahi, Bangladesh
[2]Medical Officer, Department of Microbiology, Rajshahi Medical College, Rajshahi, Bangladesh

**Abstract:** ***Background:*** Antimicrobial resistance (AMR) is a worldwide disaster to health community. Uropathogenic *E.coli* is increasingly related with multidrug resistance (MDR), including the resistance to the last resort carbapenems. This study aims to determine the antibiotic resistant pattern and detection of carbapenemase producing imipenem resistant *E.coli* in Dhaka Medical College and Hospital. ***Methods:*** An observational study was done over a period of one year in Dhaka Medical College that involve 280 patients. The gram positive and gram-negative bacteria were identified, their antimicrobial resistant patterns were determined and detection of antimicrobial resistant pattern from urine samples. ***Results:*** A total of 280 microorganisms were identified among 83 culture positive cases. The microorganisms identified were 92.77% (n=77) gram negative and 7.23% (n=6) gram positive. Antibiotic susceptibility pattern was determined by double disk method for all the isolated *E.coli* strains. The most resistance was found against cotrimoxazole (90%) and lowest resistant was found against tigecyline (6.67%). Phenotypic detection of imipenem resistant Esch.coli, 55.56% carbapenemase producers were detected by DDS test, 66.67% were detected by CD assay and 22.22% were detected by MHT. Genotyping detection 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, 44.44% *bla* NDM-2 like, 22.22% *bla* VIM, 22.22% *bla* OXA-48 and 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.

**Keywords:** Uropathogenic *E.coli*, antimicrobial susceptibility pattern, antimicrobial resistance pattern, Phenotypic & genotypic detection of carbapenemase producing imipenem resistant uropathogenic *E. Coli*.

**Original Researcher Article**

***Correspondence:***
***Dr. Md. Sirazum Munir***
*Lecturer Department of Virology, Rajshahi Medical College Rajshahi, Bangladesh*
*Email: sirazummunir2018@gmail.com*

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**Article at a glance:**

***Study Purpose:*** *To contribute to existing knowledge or propose new ideas.*

***Key findings:*** *Phenotypic detection of imipenem resistant Esch.coli, 55.56% carbapenemase producers were detected by DDS test, 66.67% were detected by CD assay and 22.22% were detected by MHT. Highest genotyping detection of carbapenemase encoding genes among imipenem resistant uropathogenic Esch.coli, were 55.56% bla NDM-1.*

***Newer findings:*** *Increased resistant rate of bla NDM-1 gene from previous study (40%) to 55% in this study.*

***Abbreviations:*** *DDS= Double disc synergy test, CD assay= Combined disc assay, MHT= Modified Hodge test.*



## INTRODUCTION

UTIs are the common bacterial infections worldwide and affects around a lot of peoples annually and contribute a huge financial burden on community and health system.[1,2] Urinary tract infections (UTIs) exclusively cause of emergence of antimicrobial resistance.[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] Colistin isthe drug of last resort for carbapenem resistant *enterobacteriaceae*. Colistin resistance causes a pan drug resistant state, with virtually no therapeutic options.[5]Recently the drug, fosfomycin has attracted renewed interest for the treatment of serious systemic infection caused multi drug resistance *enterobacteriaceae*.[6]

## METHODS

An observational study was done in the Microbiology department of Dhaka Medical College from January 2022 to December 2022. The samples from the patients were collected by aseptic ways. The specimen was inoculated in blood agar, nutrient agar and MacConkey agar media and incubated aerobically at 37$^0$C for 24 hours.

## RESULTS

**Table1: Results of urine culture in relation with pus cells in microscopic examination (N=280)**

| Pus cells/HPF | Numbers of samples | Culture | |
|---|---|---|---|
| | | Positives (%) | Negatives (%) |
| 5-10 | 135 | 18(13.33) | 117(86.67) |
| 11-20 | 85 | 21(24.71) | 64(75.29) |
| >20 | 60 | 44(73.33) | 16(26.67) |
| Total | 280 | 83(29.64) | 197(70.36) |

Out of 280 urine samples had pus cells ≥5/HPF, 83(29.64%) yielded significant growth of different organisms. Out of 135 samples with pus cells 5-10/HPF, 18(13.33%) were culture positive, 85 samples with pus cells 11-20/HPF, 21(24.71%) were culture positive. Out of 60 samples with pus cells >20/HPF, 44(73.33%) were culture positive.

**Table 2: Distribution of bacteria isolated from urine by culture (N=83)**

| Bacteria | Number (%) |
|---|---|
| *Escherichia coli* | 60(72.29) |
| *Klebsiella spp.* | 6(7.23) |
| *Pseudomonas spp.* | 5(6.02) |
| *Proteus spp* | 3(3.61) |
| *Enterobacterspp* | 2(2.41) |
| *Acinetobacterspp* | 1(1.20) |
| *CONS* | 3(3.61) |
| *Staphylococcus aureus* | 2(2.41) |
| *Enterococcus spp* | 1(1.20) |
| Total | 83(100.00) |

Table 2 shows the pattern of organisms isolated from urine. Among 83 culture positive urine, 60(72.29%) were *Esch.coli*, followed by 6(7.23%) *Klebsiella spp*, 5(6.02%) *Pseudomonas spp.*, 3(3.61%) were *Proteus spp* and *CONS.*

**Table 3: Antibiotic resistance patterns of isolated uropathogenic *Esch.coli* (N=60)**

| Antimicrobial drugs | Resistant (%) |
|---|---|
| Amikacin | 24(40.00) |
| Amoxiclav | 42(70.00) |
| Aztreonam | 48(80.00) |
| Cefotaxime | 42(70.00) |
| Cefoxitin | 42(70.00) |
| Ceftazidime | 48(80.00) |
| Cotrimoxazole | 54(90.00) |
| Ceftriaxone | 50(83.33) |

| | |
|---|---|
| Ciprofloxacin | 51(85.00) |
| Gentamicin | 42(70.00) |
| Piperacillin/Tazobactam | 39(65.00) |
| Nitrofurantoin | 24(40.00) |
| Colistin | 08(13.33) |
| Imipenem | 09(15.00) |
| Fosfomycin | 14(23.33) |
| Tigecycline | 04(06.67) |

Antibacterial resistance pattern of the isolated uropathogenic *Esch.coli* are shown in Table 3. Among the isolated uropathogenic *Esch.coli*, 90% were resistant to cotrimoxazole followed by 85% ciprofloxacin, 83.33% ceftriaxone, 80% aztreonam and ceftazidime, 70% cefoxitin, cefotaxime, amoxyclav and gentamicin, 65% piperacillin/tazobactum, 40% amikacin and nitrofurantoin, 23.33% fosfomycin, 15% imipenem, 13.33% colistin and 6.67% was resistant to tigecycline.

**Table 4: Detection of carbapenemase producers among imipenem resistant uropathogenic *Esch.coli* by phenotypic method (N=9)**

| Method | Positive (%) | Negativen (%) |
|---|---|---|
| DDS test | 5 [1+3*+1**] (55.56) | 4(44.44) |
| CD assay | 6 [2+3*+1**] (66.67) | 3(33.33) |
| MHT | 2 [1+1**] (22.22) | 7(77.78) |

Note: '*' denotes positive for both DDS test and CD assay
'**' denotes positive for DDS test, CD assay and MHT

DDS= Double disc synergy test, CD assay= Combined disc assay, MHT= Modified Hodge test. Here, demonstrates carbapenems producers among imipenem resistant uropathogenic *Esch.coli* by phenotypic method. Among nine imipenem resistant *Esch.coli*, 5(55.56%) carbapenemaseproducers were detected by DDS test, 6(66.67%) were detected by CD assay and 2(22.22%) were detected by MHT.

**Table 5: Distribution of carbapenemase encoding genes (*bla*NDM-1, *bla* NDM-2like, *bla* IMP, *bla* VIM, *bla* OXA-48 genes) among imipenem resistant uropathogenic *Esch.coli*(N=9)**

| *bla*NDM-1 | *bla* NDM-2like | *bla*IMP | *bla*VIM | *bla*OXA-48 | Total |
|---|---|---|---|---|---|
| + | - | - | - | - | 3 |
| - | + | - | - | - | 2 |
| + | + | - | + | + | 2 |
| - | - | - | - | - | 2 |

N=Total number of imipenem resistant *Esch.coli* in urine.
n=number of imipenem resistant *Esch.coli* in urine having respective carbapenemase genes,
'+'=present, '_'=absent.

Table 5 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, 44.44% *bla* NDM-2 like, 22.22% *bla* VIM, 22.22% *bla* OXA-48 and no *bla* IMP gene.

## DISCUSSION

A total of 280 specimens (urine) were collected from clinically suspected infected patients from Dhaka Medical College. Table I shows, 280 samples had significant pus cells (≥5/HPF). Out of 280 samples, 83(29.64%) were culture positive and 197(70.36%) were culture negative. This study was nearly similar with Sridhar*et al.*,2017 in

India;[7]Ebongue*et al.*,2019 in Cameroon;[8]Kuruvilla*et al.*,2014 in India[9]found 28.3%, 32.0% and 35.3% were cultures positives and 71.7%, 68.0% and 64.7% were found culture negative respectively.In the present study, Table II shows, majority 92.77% of UTI were due to gram negative bacilli (GNB) and remaining 7.23% due to gram positive cocci (GPC). Another study by Panigrahy*et al.*,2022 reported GNB and GPC among uropathogens were 94.4% and 5.6%, respectively; which is almost similar to with the present findings.[10] In this study, the most common uropathogens isolated were *Esch.coli* (72.29%) followed by *Klebsiella spp*. (7.23%).

A recent study of Department of Microbiology, All India Institute of Medical Sciences, New Delhi, India by Panigrahy*et al.*,2022 reported that prevalence of *Esch.coli* and *Klebsiellapneumoniae* among total isolated uropathogens were 65.57% and 16.19% respectively, which are in accordance with present findings.[10]The other gram-negative bacteria were *Pseudomonas spp.* 6.02%, *Proteus spp.* 3.61%, *Enterobacter spp.* 2.41%, *Acinetobacter spp.* 1.20%. This similar finding wasAkter*et al.*,2016 in Pakistan (*Pseudomonas spp.* 7.61%, *Proteus spp.* 4.01%, *Enterobacter spp.* 2.31%, *Acinetobacter spp.* 1.03%).[11] Table 3 shows, higher rate of resistance exhibited by *Esch.coli* towards Cotrimoxazole and ciprofloxacin was 90% and 85%, respectively. These findings are in agreement with the study by Bhowmik*et al.*,2021 who reported 86.6% resistance of *Esch.coli* to Cotrimoxazole and 79.92% to ciprofloxacin.[12]In the present study, resistance pattern of *Esch.coli* to colistin and fosfomycin were 13.33% and 23.33%, respectively. Padhi *et al.*,2020 from India reported 9.8% resistant to colistin and 15.9% resistant to Fosfomycin for *Esch.coli*.[13] Chowdhury *et al.*,2019 from Bangladesh reported that resistance of *Esch.coli* to colistin and Fosfomycin were 12.19% and 17.47% respectively.[14]These findings are in agreement with the present findings.

In the present study, Table IV shows, among the nine-imipenem resistant *Esch.coli*, 5(55.56%) were positive by DDS, 6(66.67%) were positive by CD assay and 2(22.22%) were positive by MHT. In the present study, the sensitivity of CD assay was more in comparison to DDS test while MHT gave the least satisfactory result. In a previous study in DMC by Begum and Shamsuzzaman, 2016 reported that, 65%, 75% and 35% carbapenemase producers were detected by DDS, CD assay and MHT, respectively.[15]

(Table V) From this study observed that 5(55.56%) *bla*NDM-1 and 1(11.11%) *bla*VIM 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) in DMCH who observed 47.25% *bla*NDM-1 and 9.72% *bla*VIM.[16] In this study, 4(44.44%) *bla*NDM-2like and 1(11.11%) *bla*OXA-48 positive isolates were detected. *Bla* IMP was not detected in imipenem resistant uropathogenic *Esch.coli*. A study conducted by Altayb *et al.* (2020) reported that *bla*OXA-48 gene was detected in 15.5% of the isolates and *bla* IMP gene was not detected.[17]Memon (2021) also reported that 41.34% *bla*NDM-2 like positive isolates were detected. The reason behind not detection of *bla* IMP gene may be due to presence of other carbapenemase encoding genes rather than *bla* IMP in these imipenem resistant strains.[18]

## CONCLUSION

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.

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**Declarations**

I, hereby, declare that the submitted Research Paper is my original work and no part of it has been published anywhere else in the past.

**Ethical approval:** Ethical clearance for the study was taken from the Instutional Review Board and concerned authority, Dhaka Medical College & Hospital.

**Conflict of interest:** None declared.

**Consent:** Informed written consent was taken from each patient or patient's attendant.

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