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

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Abstract

Background: Antimicrobial resistance (AMR) is a worldwide nuisance to health community. Uropathogenic *E.coli* is increasingly associated with multidrug resistance (MDR), including the resistance to the last resort carbapenems. **Objective:** This study aims to determine the antimicrobial resistant pattern and detection of carbapenemase producing imipenem resistant *E.coli* in Rajshahi Medical College and Hospital. **Methods:** A cross-sectional descritive study was performed over a period of one year in Dhaka Medical College that involve 280 patients. The gram positive and gram negative bacteria were isolated, their antimicrobial resistant patterns were determined and detection of antimicrobial resistant pattern from urine samples. **Results:** A total of 280 urine samples, 83 were culture positive cases. The microorganisms isolated were 77 (92.77%) gram negative and 6 (7.23%) gram positive. Antimicrobial susceptibility pattern was determined by double disk method for all the isolated *E.coli* strains. The highest resistance was found against cotrimoxazole (90%) and lowest resistant was found against tigecyline (6.67%). In this study, 71.67% (MDR), 23.33% Extensively drug resistant (XDR) and 5% Pan drug resistance (PDR) Esch.coli were isolated. 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 Modified Hodge test (MHT). **Conclusion:** Antimicrobial resistance has become a global warning now a days. So, appropriate antibiotics should be used according to the sensitivity pattern for bacteria to prevent emergence of resistance.

Key words: uropathogenic E.coli, antimicrobial susceptibility pattern, antimicrobial resistance pattern

Introduction

UTIs are the common bacterial infections worldwide and affects around 150 million peoples annually and contribute a significant financial burden on community and health system.[1,2] Urinary tract infections (UTIs) exclusively contribute to emergence of antimicrobial resistance.[3] This is because of 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 lead to an increase in rate of antibiotic resistance.[4]

Colistin is a drug of last resort for carbapenem resistant enterobacteriaceae. Colistin resistance implies a pan drug resistant state, with virtually no therapeutic options.[5] Recently the use of fosfomycin has attracted renewed interest for the treatment of serious systemic infection caused multi drug resistance enterobacteriaceae.[6] WHO has classified fosfomycin in the category of a critically important antimicrobial for investigation in light of its efficacy. MDR gram negative organism has been identified as an antimicrobial which holds great promise worldwide for managing MDR gram negative infection, due to affordable cost and efficacy as carbapenem sparing regimen.

Methods

A cross-sectional descritive study was conducted in the Microbiology department of Dhaka Medical College. The samples from the patients were collected in aseptic precautions. The specimen (urine) was inoculated in blood agar, nutrient agar and MacConkey agar media and incubated aerobically at 370C for 24 hours. If culture plates showed the growth of bacteria, then subculture was done on blood agar and MacConkey agar for pure colony.

Results

Table I: Results of urine culture in relation with pus cells in microscopic examination (N=280).

Pus cells/HPF	Numbers of samples	Culture	
		Positives n(%)	Negatives n(%)
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)

A total of 280 urine samples having 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. Significant differences were observed in culture positivity between urine having pus cells 5-10/HPF and 11-20/HPF (P=0.02) and between 11-20/HPF and >20/HPF(P<0.0001) (Table I).

Table II: 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 II showed 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.*

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

Antimicrobial drugs	Resistant n (%)
Amikacin	24(40.00)
Amoxyclav	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/Tazobactum	39(65.00)
Nitrofurantoin	24(40.00)
Colistin	08(13.33)
Imipenem	09(15.00)
Fosfomycin	14(23.33)
Tigecycline	04(06.67)

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 III).

Table IV: Types of antibiotic resistance pattern of isolated uropathogenic *Esch.coli* (N=60).

Type of resistant	Number n (%)
MDR	43(71.67)
XDR	14(23.33)
PDR	03(05.00)

Table IV showed resistance pattern of the isolated Esch.coli. Out of 60 uropathogenic *Esch.coli* 43 (71.67%) were multidrug resistance (MDR) followed by 14 (23.33%) and 3 (5.00%) were XDR and PDR respectively.

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

Method	Positive n (%)	Negative n (%)
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 carbapenemase producers among imipenem resistant uropathogenic *Esch.coli* by phenotypic method. Among nine imipenem resistant Esch.coli, 5(55.56%) carbapenemase producers were detected by DDS test, 6(66.67%) were detected by CD assay and 2(22.22%) were detected by MHT.

Discussion

A total of 280 specimens (urine) were collected from clinically suspected infected patients from Dhaka Medical College for isolation, identification and antimicrobial susceptibility pattern.

Table I showed, 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 Ranjan, *et al.* (2017)[7] in India; Nguefack, *et al.* (2019)[8] in Cameroon; Rajaratnam, *et al.* (2014)[9] in India 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 showed, 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 Mohapatra, *et al.* (2020)[10] reported GNB and GPC among uropathogens were 94.4% and 5.6%, respectively; which is almost similar to with the present study findings. 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 Mohapatra, *et al.* (2020)[10] reported that prevalence of *Esch.coli* and Klebsiella pneumoniae among total isolated uropathogens were 65.57% and 16.19% respectively, which are in accordance with present findings. The other gram-negative bacteria were Pseudomonas spp. 6.02%, Proteus spp. 3.61%, Enterobacter spp. 2.41%, Acinetobacter spp. 1.20%. Qadder, *et al.* (2016)[11] in Pakistan Observed Similar Findings in their Study.

Table III showed, higher rate of resistance exhibited by Esch.coli towards Cotrimoxazole and ciprofloxacin was 90% and 85%, respectively. These findings were in agreement with the study by Hossain, *et al.* (2021)[12] who reported 86.6% resistance of *Esch.coli* to Cotrimoxazole and 79.92% to ciprofloxacin. In the present study, resistance pattern of Esch.coli to colistin and fosfomycin were 13.33% and 23.33%, respectively.

Dash, *et al.* (2020)[13] from India reported 9.8% resistant to colistin and 15.9% resistant to Fosfomycin for *Esch.coli.* Rahman, *et al.* (2019)[14] from Bangladesh reported that resistance of *Esch.coli* to colistin and fosfomycin were 12.19% and 17.47% respectively. These findings were in agreement with the present findings.

Table IV showed, among the 60 isolated *Esch.coli*, 43(71.67%) were multidrug resistant (MDR), 14(23.33%) were extensively drug resistant (XDR) and 03 (5.00%) were pandrug resistant (PDR). A study conducted by Kabir, (2019)[15] in DMCH, reported that 68.1% isolates were MDR, 25.32% were XDR and 6.58% were PDR which are almost similar to the findings in present study. In Tehran, a study conducted by Saderi and Owlia, (2015)[16] who reported that 74.5% of isolates were MDR, 21.73% were XDR and 3.77% PDR which are also similar to the findings of this study.

In the present study, Table V showed, 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)[17] reported that, 65%, 75% and 35% carbapenemase producers were detected by DDS, CD assay and MHT, respectively.

Conclusion

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

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