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Original Article

AmpC Beta-Lactamases Detection and Genetic Analysis in Gram Negative Urinary Isolates from Six Districts of Bangladesh.

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Abstract

Background: *Gram negative bacteria produce AmpC beta-lactamases (AmpCs) becoming a major therapeutic challenge. So, among multidrug resistant bacteria, AmpCs producers identification by reliable procedure as well as AmpCs encoding genes detection is essential.* ***Objective:*** *This study was carried out to identify AmpC beta-lactamases producing gram negative bacteria isolated from urine through two convenient methods and to detect AmpC beta-lactamases genes by multiplex PCR from September, 2018 to August, 2019.* ***Methodology:*** *In total, 187 isolates were collected from UTI patients of six district areas in Bangladesh. After re-identification, Gram negative isolates were tested for drug resistance against common antimicrobial drugs in microbiology laboratory of BIRDEM General Hospital, Dhaka. Cefoxitin was assessed as primary screening markers for detection of AmpCs production. As a confirmation test, Modified three dimensional test (MTDT) and AmpC disc test were compared. AmpC β-lactamases genes (blaCIT, blaDHA, blaACC, blaEBC, blaMOX, blaFOX) were detected by multiplex PCR.* ***Results:*** *Among the total 187 isolates, 181 (91.8%) were gram negative and 6 (9.2%) were gram positive bacteria. Among 181 gram negative bacteria, 37 (20.4%) isolates were cefoxitin resistant of which AmpC beta-lactamases were detected in 18 (48.6%) by AmpC Disc Test and MTDT separately. Among urinary isolates, blaDHA and blaCIT were predominant genes. AmpCs producers were detected among 16% gram negative bacteria from community acquired UTI. Conclusion: Demonstration of AmpCs producers among uropathogenic isolates and AmpCs genes in significant number from this study indicate prevalence of AmpCs producing bacteria causing UTI and transmission of their resistance genes have increased that signify detection of AmpCs producers by a potential but convenient method will ensure physician to give appropriate antibiotic regimens.*

Keywords: *Urinary tract infection; AmpC beta-lactamases; Modified Three Dimentional Test; AmpC disc test; blaDHA.*

Introduction: Urinary Tract Infection (UTI) are a severe public health problem caused by both Gram-negative and Gram-positive species[1]. In 2007, there were 10.5 million ambulatory visits for UTI, accounting for 0.9% of all ambulatory visit in the United States[2]. *E.coli* causes 75-95% of uncomplicated UTI and *Klebsiella*, *Enterobacter, Staphylococcus, Enterococcus, Streptococcus* are accountable for remaining cases[3]. UTIs are generally self-limiting, with tendency to risk of recurrence. As antibiotics treatment in UTI leads to a more rapid resolution of symptoms and is more likely to clear bacteriuria, uropathogens are becoming resistant to currently available antibiotics[4]. Although antimicrobial resistance is a natural biological phenomenon, antibiotic usage is considered as one of the most important fact in development of antibiotic resistant organisms around the world[5]. One of the most prevalent mechanisms of drug resistance is the production of AmpC β-lactamases enzymes in gram negative bacteria[6] (Gupta et al., 2014).

AmpC β-lactamases are clinically important cephalosporinase that mediate resistance to cephalothin, cefazolin, cefoxitin, most penicillins, and

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β-lactamase inhibitor-β-lactam combinations[7]. Such enzymes provide a broader spectrum of resistance than ESBLs and are not blocked by commercially available inhibitors. AmpCs may be chromosomally mediated (inducible or constitutive) or plasmid mediated[8]. *Enterobacter*, *Citrobacter, Shigella, Morganella, Serratia* and *Pseudomonas aeruginosa* may possess AmpC beta-lactamases on their chromosome (inducible)[9]. In 1989, *K. pneumoniae* was isolated that could transfer resistance to *E. coli* against penicillins, methoxy-cephalosporins, oxyimino-cephalosporins, and monobactams in South Korea[10]. There is structural similarity between plasmid derived AmpCs that usually originated from chromosome derived AmpCs can transfer to same species or to *Klebsiella* spp., *Salmonella* spp., *Proteus mirabilis* via horizontal transfer[11,12]. Usually AmpCs production in *Klebsiella* spp., *Salmonella*, *Proteus, Citrobacter koseri* is confirmatory for plasmid-carried AmpCs genes as these organisms lack a chromosomal AmpCs[13]. Promoter or attenuator mutation, a resistance mechanisms in *E. coli* have been identified which results in upregulation of naturally occurring chromosomal AmpC beta-lactamases production; *E. coli* can also contain plasmid carried AmpC beta-lactamases[14]. Commonly reported plasmid mediated enzymes are CMY, MOX, FOX, EBC, ACC, DHA and CIT[15]. Identification of AmpCs producers is essential for appropriate management of infection that consequently control infection as incidence of AmpCs genes transmission among different Gram negative bacteria is increasing threateningly.

AmpCs producers may appear susceptible to extended spectrum cephalosporins when initially tested in laboratory that may lead to inappropriate antimicrobial regimens application and therapeutic failure[16] . Available techniques to identify AmpCs producers are not yet optimized for the clinical microbiological laboratory. Different phenotypic AmpC confirmation test have been reported: Disc Potentiation Test, Cefoxitin Agar Based Test, Cefoxitin-Cloxacillin Double Disc Synergy Test, Modified Three Dimentional Test (MTDT)[10,17,18]. MTDT extract test reliably detect AmpCs among enterobacteriaceae, can be adapted in routine laboratory, but it is technically demanding[10].

On contrary, AmpC disk test exhibit good sensitivity-specificity for detection of plasmid-mediated AmpC β- lactamases in *Klebsiella* spp., *Proteus mirabilis* and *Salmonella* spp. and the test is also found easier, reliable and rapid method[17].

In Bangladesh, AmpCs producers were highest isolated in urine among other samples by disc approximation test[19]. But there are no studies on the prevalence of AmpC beta-lactamases producing isolates in UTI collected from different districts of Bangladesh. The present study was conducted to evaluate the prevalence of AmpCs producers and their encoding genes by multiplex PCR in gram negative isolates from UTI cases from six districts in Bangladesh. Special emphasis was given in confirmation of AmpCs production by comparison of two easy and sensitive phenotypic methods for implementation in the routine diagnostic laboratory.

Materials and methods:

This cross-sectional study was approved by ethics committee of BIRDEM ACADEMY.

Clinical Isolates: In this study, Culture positive and microscopy positive (pus cell >5/HPF) samples of UTI patients attended in Vital Research Laboratory (Feni), Diabetic Association Medical College (Faridpur), Jahurul Islam Medical College (Kishoreganj), Khaja Yunus Ali Medical College (Sirajganj), Satkhira Diagnostic Center (Shatkhira) and Brahmanbaria Medical College (Brahmanbaria) were processed. Clinical isolates were inoculated on Mueller Hinton Slant in screw cap tubes and incubated at 37°C then stored at -10°c of refrigerator. Total 187 inoculated isolates on Mueller Hinton slant of screw cap tubes were transported to BIRDEM General Hospital in ice pack box by courier within 2 weeks of preservation with adequate information on data sheet from 6 centers for further analysis within September, 2018 to August, 2019.

Reidentification and antimicrobial susceptibility test at BIRDEM microbiology laboratory:

From inoculated Mueller Hinton slants, culture were done in Blood agar and MacConkey agar media incubated at 37°C for 24 hours. The uropathogenic isolates were further identified by colony morphology, hemolytic criteria, staining character, pigment production and biochemical test as per standard techniques[20].

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Antimicrobial susceptibility test:
Antimicrobial susceptibility tests were performed by Kriby-Bauer modified disc diffusion technique on Mueller Hinton agar media for *E. coli, Klebsiella*, *Enterobacter* using cefuroxime, ceftazidime, ceftriaxone, cefotaxime, cefixime, cefepime, amoxiclave, amikacin, gentamicin, piperacillin-tazobactam, imipenem, colistin, ciprofloxacin, cotrimoxazole, nitrofurantoin, fosfomycin. For *Pseudomonas* spp. piperacillin- tazobactam, ceftazidime, aztreonam, amikacin, gentamicin, netilmicin, imipenem, ciprofloxacin, cotrimoxazole, fosfomycin were tested. ATCC strains *E. coli* 25922 and *Pseudomonas aeruginosa* 23853 were used to assess the potency of the discs. Interpretation was done according to 2017 CLSI giudline[21].

Phenotypic tests for AmpC beta-lactamases:
Those isolates showed less than 18mm of zone diameters for cefoxitin discs by disc-diffusion method were selected for confirmatory test.

Modified three dimensional test
Crude enzyme preparation: From a fresh overnight growth of test organism on MacConkey agar plate, 0.5 McFarland standard bacterial suspension was prepared. Twelve ml Tryptic soya broth was inoculated with 50 µl of 0.5 McFarland adjusted bacterial suspension and was incubated for 4 hours at 37°C. Then concentrate cells were prepared by centrifugation and freezing-thawing cycles for 5 times[9].

Procedure: Lawn culture of *E. coli* ATCC 25922 was prepared on Mueller-Hinton agar plate and a cefoxitin (30µg) disc was placed on the surface of the media. Linear slit beginning 5 mm from the edge of the disc was cut radially with a sterile scalpel blade. At the other end of the slit a small circular well was made. By using a pipette, 25- 30µl of enzyme preparation was loaded into well. Inoculated media was incubated overnight at 37°C. Three different kind of results were recorded9. Isolate that showed clear distortion of zone of inhibition of cefoxitin (Figure I) was taken as AmpCs producer. Isolate with no distortion was taken as AmpCs non-producer and isolate with mild distortion was taken as intermediate strain.

AmpC disc test
AmpC disc preparation: Saline and 100× Tris-EDTA mixture (20 µl; 1:1) was applied on sterile filter paper disc and allowed to dry and stored them at 2◦ to 8°C. Procedure: Lawn of *E. coli* ATCC 25922 strain was inoculated on surface of a Mueller-Hinton agar plate. Immediately prior to use, AmpC discs were rehydrated with 20 µl of normal saline and several colonies of each test organism were applied on surface of a disc. A 30-µg cefoxitin disc was placed on inoculated Mueller-Hinton agar. The inoculated AmpC discs were placed almost touching the cefoxitin disc with the inoculated face in contact with the agar surface. The plate was inverted and incubated overnight at 37°C. After incubation, plate was examined[17]. Isolates that showed indentation or flattening of the zone of inhibition (Figure I) were taken AmpCs producers. Isolates with absence of distortion were taken as AmpCs nonproducers.

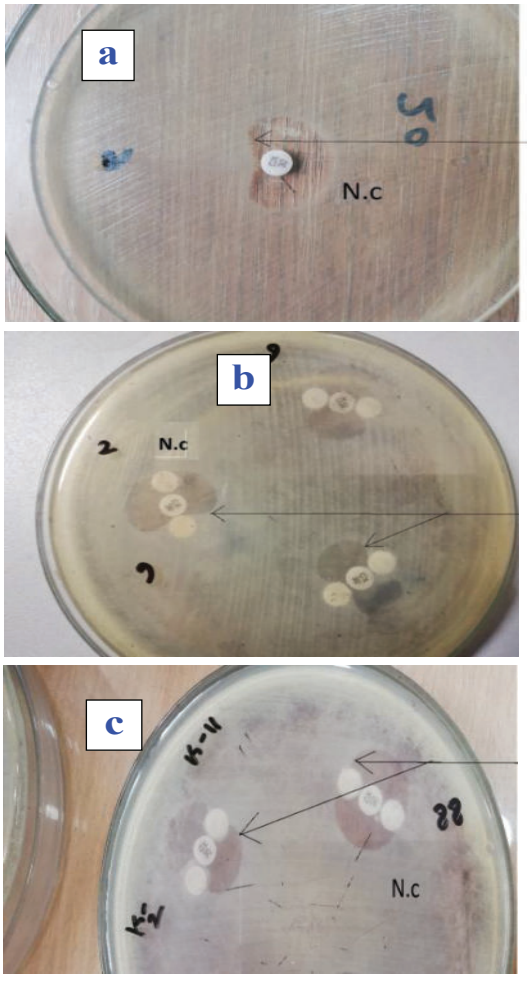

Figure I: Pattern of zone of cefoxitin
a) Clear distortion in MTDT.
b) Indentation and c) Flattening in AmpC disc test.

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Molecular test for AmpC beta-lactamases genes:

Cefoxitin resistant isolates were tested by multiplex PCR which detect six AmpC beta-lactamases genes families (*bla*DHA, *bla*CIT, *bla*ACC, *bla*EBC, *bla*MOX, *bla*FOX). Total 25µl of reaction mixture was prepared by mixing of 12.5µl of mastermix (mixture of dNTP, taq polymerase Mgcl2 and PCR buffer), 2µl of each of selected forward and reverse primers (Promega corporation, USA) (Table 1), 2µl of DNA template and 6.5µl of sterile distilled water in a PCR tube. In thermal cycler amplification program consisted of an initial denaturation step at 94°C for 3 min, followed by 25 cycles of DNA denaturation at 94°C for 30s, primer annealing at 64°C for 30s, and primer extension at 72°C for 1 min. After the last cycle, a final extension step at 72°C for 7 min was added. Amplified PCR products and DNA ladder were loaded into separate wells of 1.5% agarose gel stained with ethidium bromide. PCR products were analyzed using 1.5% agarose gel electrophoresis and visualized by UV transilluminator.

Table 1. Primers Used in Multiplex PCR

Family	Genes	Sequence (5' to 3')	bp
DHA	DHAMF DHAMR	AAC TTT CAC AGG TGT GCT GGG T CCG TAC GCA TAC TGG CTT TGC	405
CIT	CITMF CITMR	TGG CCA GAA CTG ACA GGC AAA TTT CTC CTG AAC GTG GCT GGC	462
ACC	ACCMF ACCMR	AACAGCCTCAGCAGCCGGTTA TTCGCCGCAATCATCCCTAGC	346
EBC	EBCMF EBCMR	TCG GTA AAG CCG ATG TTG CGG CTT CCA CTG CGG CTG CCA GTT	302
MOX	MOXMF MOXMR	GCT GCT CAA GGA GCA CAG GAT CAC ATT GAC ATA GGT GTG GTG C	520
FOX	FOXMF FOXMR	AAC ATG GGG TAT CAG GGA GAT G CAA AGC GCG TAA CCG GAT TGG	190

Results:

After culture and biochemical test from inoculated Mueller Hinton Slant in screw cap tubes collected from districts laboratories, 181 isolates (96.8%) were identified gram negative bacteria and rest (3.2%) isolates were identified gram positive bacteria. *E. coli* was prevailing followed by *Klebsiella* spp. and *Enterobacter* spp. among gram negative isolates. Of 181 gram negative bacteria, 37 (20.4%) were screened for AmpC beta-lactamases production (Table 2).

Table 2: Frequency of cefoxitin resistance

Gram negative isolates	Total No.	Frequency (percent)
E. coli	150	26 (17.3%)
Klebsiella spp.	10	3 (30.0%)
Enterobacter spp.	10	3 (30.0%)
Proteus mirabilis	1	0 (0.0%)
Pseudomonas spp.	10	5 (50.0%)
Total	**181**	**37 (20.4%)**

Antibiotic sensitivity pattern of these 37 cefoxitin-insusceptible isolates revealed higher resistance rate to amoxiclave, all cephalosporins, cotrimoxazole, piperacillin-Tazobactam, gentamicin and lower resistance rate to amikacin, imipenem, nitrofurantoin, fosfomycin, colistin (Figure II). *Klebsiella* spp. exhibited highest resistance to maximum drugs among all other spp.. *Pseudomonas* spp. exhibited notable resistance to cotrimoxazol 80%, piperacillin-tazobactam 60% (Figure III). As *Pseudomonas* intrinsically resistant to amoxiclave, most of cephalosporin and nitrofurantoin, these discs were not used. These 37 isolates were confirmed for AmpC beta-lactamases detection by confirmatory phenotypic and molecular test.

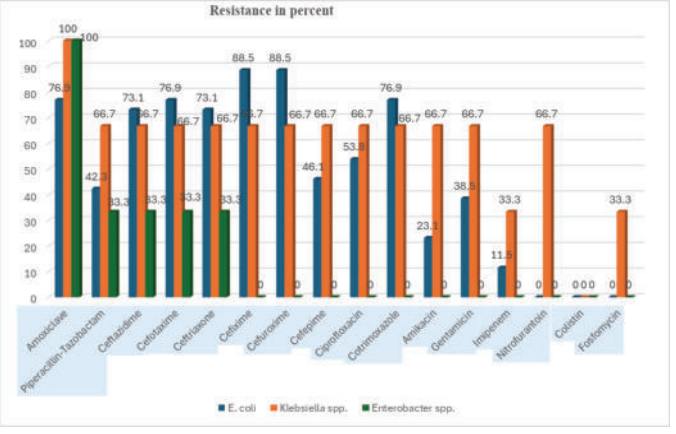

Figure II: Resistance pattern of cefoxitin resistant isolates.

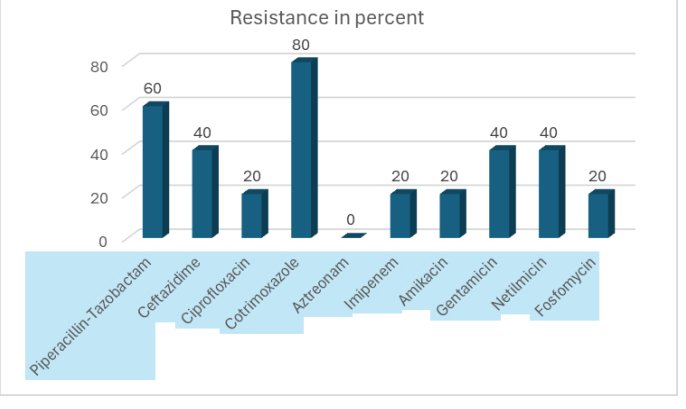

Figure III: Resistance pattern of cefoxitin resistant *Pseudomonas* spp.

The AmpC disc test and MTDT both detected 18(48.6%) isolates as AmpCs producers respectively (Table 3). Both two tests detected ten *E. coli*, three *Enterobacter*, one *Klebsiella* and one *Pseudomonas* spp.. AmpC disc test alone detected three *E. coli* that were not detected by MTDT. On contrary, MTDT detected alone another three organisms including *E. coli, Klebsiella* spp., *Pseudomonas* spp. that were not detected by AmpC disc test (Figure IV).

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Table 3: Detection rate of AmpCs producers by phenotypic tests

Gram negative organisms	Total No.	AmpC disc test	MTDT
E. coli	26	13 (50%)	11 (42.3%)
Klebsiella spp	3	1 (33.3%)	2 (66.7%)
Enterobacter spp.	3	3 (100%)	3 (100%)
Pseudomonas spp.	5	1 (20%)	2 (40%)
Total	37	18 (48.6%)	18 (48.6%)

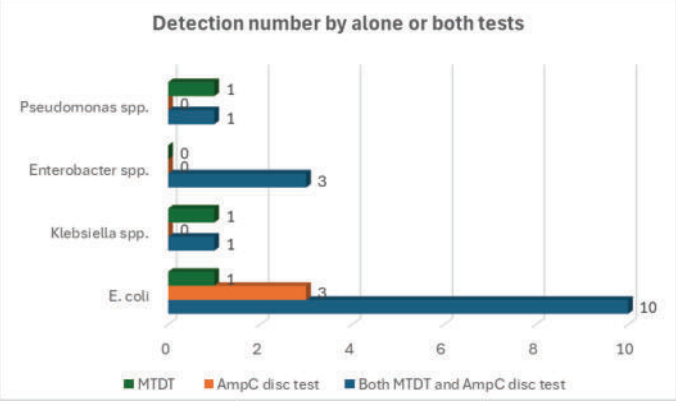
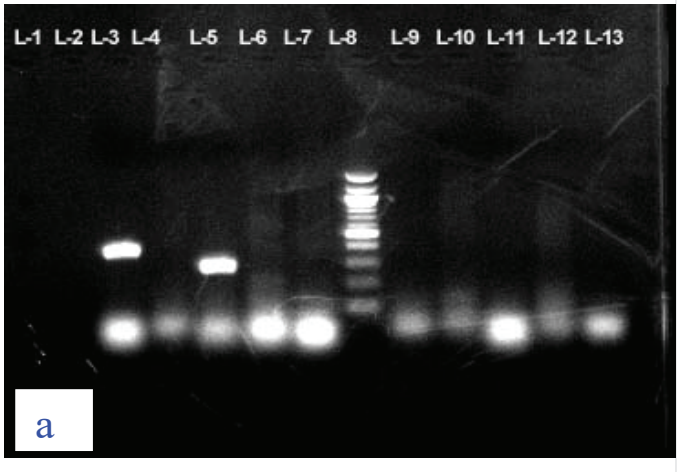


Figure IV: Comparison of two phenotypic tests to detect AmpCs producers.

In multiplex PCR, *bla*DHA was found predominantly followed by *bla*CIT among *E. coli* (30.8% and 23.1%) and *Klebsiella* spp. (66.7% and 33.3%). Each of *bla*CIT, *bla*ACC or *bla*EBC was found individually to contain three *Enterobacter* spp. One *Pseudomonas* spp. belonged to *bla*EBC. Total twenty-four (24/37) cefoxitin resistant isolates were confirmed as plasmid carried AmpC beta-lactamases producers (Table 4). No *bla*MOX and *bla*FOX were detected among gram negative organisms. In addition, two *E. coli* were found to contain *bla*DHA combination with either *bla*ACC or *bla*CIT.



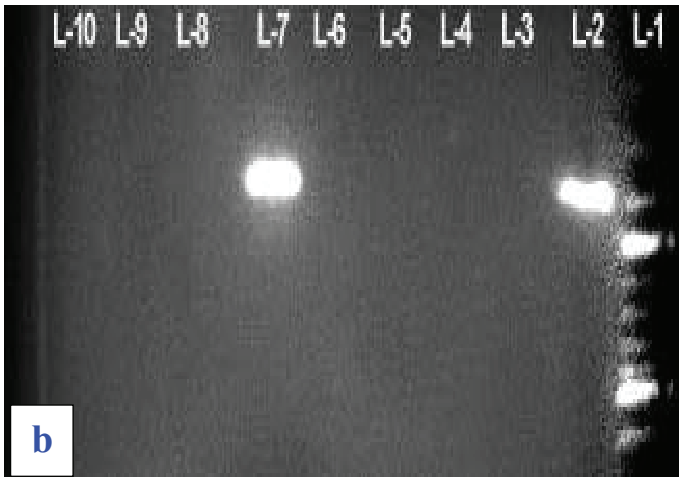


Figure V: Gel electrophoresis picture showing the results for PCR amplification. (a) Lane 3 = positive for DHA (405bp); Lane 5 = positive for ACC (356bp); Lane 4 = negative control; Lane 1,2,6,7,9,10,11,12,13 = negative; Lane 8 = 1500bp DNA ladder. (b) Lane 2 positive for CIT (462bp); Lane 7 positive for EBC (302bp); Lane 3, 5,6,8,9,10 Negative; Lane 4 negative control; Lane 1 1500bp DNA ladder.

Table 4: Pattern of AmpCs encoding genes.

Genes	*E.coli* (26)	*Klebsiella* spp. (3)	*Enterobacter* spp. (3)	*Pseudomonas* spp. (5)
*bla*DHA	8 (30.8%)	2 (66.7%)	0 (0.0%)	0 (0.0%)
*bla*CIT	6 (23.1%)	1 (33.3%)	1 (33.3%)	0 (0.0%)
*bla*EBC	2 (7.7%)	0 (0.0%)	1 (33.3%)	1 (20%)
*bla*ACC	1 (3.8%)	0 (0.0%)	1 (33.3%)	0 (0.0%)
*bla*MOX	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
*bla*FOX	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Total	17 (65.4%)	3 (100%)	3 (100%)	1 (20%)

MTDT and AmpC disc test both detected four *E. coli* and one *Pseudomonas* spp., that were not detected by PCR (Table 5:a). Five *E. coli* and one *Klebsiella* spp. were detected only by multiplex PCR that are not detected by phenotypic method (Table 5:b).

Table 5: Comparison of MTDT and Amp C disk test with PCR.

		PCR											
		E. coli			*Klebsiella spp.*			*Enterobacter* spp.			*Pseudomonas spp.*		
		Positive	Negative	Total	Positive	Negative	Total	Positive	Negative	Total	Positive	Negative	Total
MTDT	Positive	7	[a]4	11	2	0	2	3	0	3	1	[a]1	2
	Negative	[b]8	7	15	[b]1	0	1	0	0	0	0	3	3
AmpC Disc test	Positive	9	[a]4	13	1	0	1	3	0	3	0	[a]1	1
	Negative	1, [b]5	7	13	1,[b]1	0	2	0	0	0	1	3	4

a) Detected by both MTDT and AmpC disc test, but not detected by PCR. b) Detected by PCR, but not detected by MTDT or AmpC disc test.

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Discussion:

Detection of AmpCs producers from gram negative organisms is necessary for ensuring effective antibiotic therapy but renders a challenge to microbiological laboratories[22]. Several screening and confirmatory phenotypic methods have been evaluated for detection of AmpC beta-lactamases production[17,18,22]. In this study, of 181 gram negative bacteria isolated from urine, resistance rate of screened 37 AmpCs producers were shown in Figure II and III. of them, *E. coli* was notably found to show higher resistance 76.9% for cotrimoxazole than ciprofloxacin that was 53.8%. Pattern of their resistance rate to all drugs used in this study corresponds with the study of Pakistan[23]. *Klebsiella* spp. showed high resistance to all drugs except colistin and imipenem that corresponds with another study[24]. One *klebsiella* spp. showed resistance to fosfomycin used to detect its efficacy against urinary isolates in the study. On contrary, Screened AmpCs producing *Enterobacter* spp. revealed good sensitivity to all drugs except amoxiclave. Similar finding demonstrated in a study[25] may be due to *Enterobacter* spp. producing AmpCs in an inducible manner usually appear susceptible to third-generation cephalosporins. *Psuedomonas* spp. showed high resistance to cotrimoxazole, moderate resistance to gentamycin and less resistance to ciprofloxacin and imipenem that agreed with the study of Iraq[26]. This isolates (20%) showed resistance to fosfomycin that signify necessity of further surveillance in our country among urinary isolates for fosfomycin.

Organisms producing AmpC beta-lactamases often go undetected as multiplex PCR cannot yet routinely used in all laboratories as it requires extensive infrastructure and specialised skills apart from its high cost[15]. The present study compared the performances of MTDT and AmpC disc test for accurate identification of AmpCs production and revealed that MTDT and AmpC disc test both detected competently 18 (48.6%) AmpCs producers from cefoxitin resistant gram negative bacteria (Table 3). AmpCs producers were detected 93.8% by MTDT and 100% by AmpC disc test9 which rate is much higher than present study. Study in 2018 showed positive result for 24% isolates by MTDT but showed positivity for 40% isolates by AmpC disc test[27]. Their findings demonstrated AmpC disc test better than MTDT but our study found both test equally competent.

Although AmpCs genes were first reported around late 1960s, majority of health personnel remain unware of their clinical importance[28]. Plasmid carried AmpC beta-lactamases genes derived from the chromosome lead to the continuous emergence of new genotypes through point mutation[29]. Additionally, if combined with decreased outer membrane permeability, bacteria can become resistant to carbapenems also[14]. It is very crucial to know the exact prevalence of AmpC beta-lactamases producing isolates in Bangladesh to control the spread of these organisms. From this study, *bla*DHA and *bla*CIT were found (Table 4) to be prevalent in *E. coli* and *Klebsiella* spp. that was consistant with other studies[30,31]. But *bla*FOX was most prevalent (45%) followed by CMY (20%) and DHA (15%) among urinary *E. coli* and *Klebsiella* spp. in a study of Bangladesh[32] which was not consistent with the present study. In our study *Enterobacter* spp., *bla*CIT, *bla*ACC, *bla*EBC were detected whereas, Enterobacter cloaca containing in *bla*EBC was detected in the study of china[31]. *Pseudomonas* spp. with *bla*EBC isolated in this study which was not consistent with the study in only where *bla*MOX, *bla*FOX, *bla*CIT and *bla*DHA were isolated from the species[33]. On contrary, report from another studies revealed *bla*EBC along with other AmpCs encoding genes in *Pseudomonas* spp.[34,35].

AmpC disc test solely detected three *E. coli* and MTDT solely detected three isolates (Figure IV) that were also confirmed by multiplex PCR but MTDT test is laborious, technically demanding requiring careful cutting of slit and well, time consuming (minimum 48 hours) and needs experience. On contrary AmpC disc test with using direct bacterial colonies distinctly overcome these problems. Therefore, laboratories can use the AmpC disc test routinely in detection of AmpC β-lactamases. Six isolates additionally detected by PCR, not by MTDT and AmpC disc test (Table 5:b) represents multiplex PCR to be highly sensitive. Nonetheless, phenotypic tests are able to detect chromosomal or plasmid mediated AmpC producers[36]. In this study, MTDT and AmpC disc test both detected five isolates, but were not detected by PCR (Table 5:a) may be due to chromosomal origin that causes hyperexpression of AmpCs among *E. coli* and *Pseudomonas* spp. These five isolates confirmed by phenotypic tests and other 24 isolates detected by multiplex PCR, total 29 isolates were detected as AmpCs producers from this present study. The prevalence of AmpC beta lactamases was found 16% (29/181) correlates well

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closer to present finding[36]. Shagufta et al from kashmir reported AmpCs production was seen only in 3.4% isolates among various samples[37]. But higher prevelence were observed in other studies from Mumbai (40%)[35] and Bangalore (42.85%)[38]

Conclusion:

The prevalence of AmpCs producing bacteria in the community acquired UTI is increasing in different districts of Bangladesh. Rising trend of AmpCs producers in community acquired UTI is quite alarming and it limits the choice of empirical antibiotic treatment for clinician in district level. Nitrofurantoin, fosfomycin amikacin, imipenem, colistin showed good efficacy against AmpCs producers in the study and as a oral form fosfomycin can be initiated in community acquired UTI. Prevalence of *bla*DHA and *bla*CIT was found to be highest among six AmpCs genes in *E. coli* and *Klebsiella* spp. causing UTI in different districts of Bangladesh. AmpCs producers yeild challenges in their detection and also in clinical management for their resistance mechanism but the present study emphasize the detection of AmpCs producers in district level. Many clinical laboratories due to manpower shortage and overworked, can implement AmpC disc test in regular detection process as it is easy to perform, convenient and sensitive method than MTDT.

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Conflict of Interest

The authors declared no conflict of interest.

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Reference:

1. Flores-Mireles A, Walker J, Caparon M, Hultgren HJ. Urinary tract infections: epidemiology, mechanisms of infection and treatment options. Nature Review Microbiology.2015;13:269–284.

2. Foxman B. Urinary tract infection syndromes: occurrence, recurrence, bacteriology, risk factors, and disease burden. Infectious Disease Clinics. 2014;28:1–13.

3. Hooton TM. Uncomplicated Urinary Tract Infection. The New England Journal of Medicine. 2012;366:1028-1037.

4. Foxman B. The epidemiology of urinary tract infection. Nature Review Urology. 2010; 7, 653–660.

5. World Health Organization, 2019. WHO Strategic Priorities on Antimicrobial Resistance.

6. Gupta G, Tak V, Mathur P. Detection of AmpC β lactamases in gram-negative bacteria. Journal of laboratory physicians. 2014; 6(1): p.1.

7. Jacoby GA. AmpC β-lactamases. Clinical microbiology reviews. 2009; 22(1):161-182

8. Coudron PE, Moland ES, Thomson KS. Occurrence and detection of AmpC beta-lactamases among Escherichia coli, Klebsiella pneumoniae, and Proteus mirabilis isolates at a veterans medical center. Journal of Clinical Microbiology. 2000;38(5):1791-1796.

9. Rudresh SM, Nagarathnamma T. Two simple modifications of modified threedimensional extract test for detection of AmpC β-lactamases among the members of family Enterobact eriaceae. Chronicles of young Scientists. 2011; 2(1):42.

10. Bauernfeind A, Chong Y, Schweighart S. Extended broad spectrum β-lactamase in Klebsiella pneumoniae including resistance to cephamycins. Infection. 1989;17:316–321

Received date: 06/07/2025
Accepted reviewed version date: 16/09/2025

11. Dehghani M, Haddadi AZ, Shavandi M. Multiplex PCR Study of Plasmid-Mediated AmpC Beta- Lactamase Genes in Clinical Isolates of Escherichia coli. Journal of Medical Bacteriology .2016;5(5–6): 21–28.

12. Philippon A, Arlet G, Jacoby GA. Plasmid-determined AmpC-type β-lactamases. Antimicrobial agents and chemotherapy. 2002; 46(1):1-11.

13. Dhanashree PI, Anuradha B. Phenotypic methods for detection of Amp C β lactamases in Gram negative clinical isolates of a tertiary care hospital. Indian Journal of Microbiology Research. 2020;7(2):125–12.

14. Pitout JD, Le PG, Moore KL, Church DL, Gregson DB. Detection of AmpC beta-lactamases in Escherichia coli, Klebsiella spp., Salmonella spp. and Proteus mirabilis in a regional clinical microbiology laboratory. Clinical Microbiology Infectious. 2010;16 (2):165-70.

15. Pérez-Pérez F.J, Hanson N.D. Detection of plasmid-mediated AmpC β-lactamase genes in clinical isolates by using multiplex PCR. Journal of clinical microbiology. 2002; 40(6):2153-2162.

16. Peter-Getzlaff S, Polsfuss S, Poledica M, Hombach M, Giger J, Böttger EC, Zbinden R, Bloemberg GV. Detection of AmpC beta-lactamase in Escherichia coli: comparison of three phenotypic confirmation assays and genetic analysis. Journal of Clinical Microbiology. 2011;49(8):2924-32.

17. Black, JA., Moland, E.S. and Thomson, KS. (2005) AmpC disk test for detection of plasmidmediated AmpC β-lactamases in Enterobacteriaceae lacking chromosomal AmpC β-lactamases. Journal of clinical microbiology. 43(7), pp.3110-3113.

18. Nasim K, Elsayed S, Pitout JDD, Conly J, Church DL, Gregson DB. New method forlaboratory detection of Amp C beta lactamases in Escherichia coli and Klebsiella pneumoniae. J Clin Microbiol 2004; 42:4799-802.

19. Karim F, Rashid H, Sattar ANI, Ahmed S, Saleh AA, Sattar H, Miah MRA. Phenotypic detection of AmpC β-lactamases in Gram negative bacteria. Bangladesh journal of medical microbiology. 2008; 2(1):28-32.

20. Cheesbrough M. Microbiological technique used in microbiology, Culturing bacterial pathogens, Biochemical test to identify bacteria In: Cheesbrough, M. (2 nd ed.) District Laboratory Practice in Tropical Countries. 2006; 2(1):pp. 35- 70.

21. Clinical and Laboratory Standard Institute (CLSI). Performance standards for antimicrobial susceptibility testing. M100-S27.Twenty-Third Informational Supplement. Wayne, Pennsylva nia. CLSI: 2017.

22. Polsfuss S, Bloemberg GV, Giger J, Meyer V, Böttger EC and Hombach M. (2011) Practical approach for reliable detection of AmpC beta-lactamase-producing Enterobacte riaceae. Journal of clinical microbiology. 49(8), pp.2798-2803.

23. Jameel NU, Ejaz H, Zafar A, Amin H. Multidrug resistant AmpC β-lactamase producing Eschericia coli isolated from a paediatric hospital. Pakistan Journal of Medical Science. 2014; 30(1):181-4.

24. Gupta V, Kumarasamy K, Gulati N, Garg R, Krishnan P, Chander J. AmpC β-lactamases in nosocomial isolates of Klebsiella pneumoniae from India. Indian Journal of Medical Research. 2012;136(2):237-41.

25. Meini S, Tascini C, Cei M, Sozio E, Rossolini GM. AmpC β-lactamase-producing Enterobacterales: what a clinician should know. Infection. 2019 Jun;47(3):363-375.

26. Saleh MK, Suood AM, Saeed AY. Prevalence of β-lactamase enzymes and molecular detection of the AmpC gene in Pseudomonas aeruginosa isolated from various clinical infections. Journal of Applied and Natural Science, 16(2), 470 - 477.

27. Naik D, De A, Naik B. Detection of AmpC β-lactamase in Gram Negative Bacteria with their Predisposing Factors and Antimicrobial Susceptibility Pattern. The Indian Practitioner. 2018: 71(2):16-24.

Received date: 06/07/2025
Accepted reviewed version date: 16/09/2025

28. Mata C, Miró E, Alvarado A, Garcillán-Barcia P, Toleman M, Walsh TR, Cruz FDL, Navarro F Plasmid typing and genetic context of AmpC β-lactamases in Enterobacteriaceae lacking inducible chromosomal ampC genes: findings from a Spanish hospital 1999-2007. Journal of Antimicrobial Chemotherapy. 2012; 67(1):1 15-22.

29. Coertze RD, Bezuidenhout CC. The prevalence and diversity of AmpC β-lactamase genes in plasmids from aquatic systems. Water Science and Technology.2018. 2017(2), 603–611.

30. Helmy MM and Wasfi R (2014). Phenotypic and molecular characterization of plasmid mediated AmpC β-lactamases among Escherichia coli, Klebsiella spp, and Proteus mirabilis isolated from urinary tract infections in Egyptian hospitals. BioMed research international. vol 2014 (171548), p.8.

31. Zhou Q, Tang M, Zhang X, Lu J, Tang X, Gao Y. Detection of AmpC beta-lactamases in gram-negative bacteria. Heliyon. 2022;8 (12): 12245.

32. Rahman MH, Begum S, Ahmed M, Islam A. The Status of Extended-spectrum β-lactamase and AmpC β-lactamase production in uropathogenic isolates of Escherichia coli and Klebsiella pneumonia in Bangladesh. Journal of Integrative Microbial Science. 2022;5 (1):181-189.

33. Jamali S, Shahid M, Farrukh S, Singh A, Khan HM. Molecular characterization of genes encoding AmpC beta-lactamases in clinical isolates of Pseudomonas and Acinetobacter species. Journal of Applied Pharmaceutical Science. 2015;5(10):048-051.

34. Bajpai V, Govindaswamy A, Khurana S, Batra P, Aravinda A, Katoch O, F Hasan, Malhotra R, Mathur P. Phenotypic & genotypic profile of antimicrobial resistance in Pseudomonas species in hospitalized patients. Indian Journal of Medical Research. 2019;149: 216-221.

35. Wassef M, Behiry I, Younan M; El NG, Mostafa S, Abada E. Genotypic Identification of AmpC β-Lactamases Production in Gram- Negative Bacilli Isolates. Jundishapur Journal of Microbiology. 2014; 7(1): 8556.

36. Saffar H, Asgari NN, Ghahroudi TA, Baseri Z, Abdollahi A, Prevalence of AmpC beta-lactamase in Clinical Isolates of Escherichia coli, Klebsiella spp. and Proteus mirabilis in a Tertiary Hospital in Tehran, Iran. Jundishapur Journal of Microbiology. 2016;9(12):39121.

37. Shagufta1 R, Bashir AF, Gulnaz1 B, Lubna1 S, Jan1 A, Mohd1 S, Junaid A. Prevalence of AmpC Beta-lactamase in Gram Negative Bacilli by Different Phenotypic Methods in a Tertiary Care Institute in Kashmir. British Journal of Medicine & Medical Research.2017;19(2:1-9.

38. Bhattacharjee A, Anupurba S, Gaur A, Sen MR. Prevalence of Inducible AmpC β-lactamase Producing Pseudomonas aeruginosa in a Tertiary Care Hospital in Northern India. Indian Journal of Medical Microbiology. 2008; 26(1): 89-98 .

Received date: 06/07/2025
Accepted reviewed version date: 16/09/2025