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Phenotypic and Molecular Characterization of Uropathogenic Extended-Spectrum Beta-Lactamases Producing Isolates from Community in Different Regions of Bangladesh

Saieda Ferdous[1], KM Shahidul Islam[2], Arifur Rahman[3], Rafia Afreen Jalil[4], Adeeba Khanduker[5], Robayet Sharmin[6]

[1]Assistant Professor, Department of Microbiology, Central Medical College, Cumilla, Bangladesh; [2]Ex-Professor, Department of Microbiology, BIRDEM General Hospital, Dhaka, Bangladesh; [3]Associate Professor, Department of Microbiology, Brahmanbaria Medical College, Brahmanbaria, Bangladesh; [4]Associate Professor, Department of Microbiology, Green Life Medical College, Dhaka, Bangladesh; [5]Lecturer, Department of Microbiology, Anwar Khan Modern Medical College, Dhaka, Bangladesh; [6]Laboratory Consultant, National Institute of Preventive and Social Medicine, Dhaka, Bangladesh

Abstract

Background: Prevalence of Extended-spectrum ß-lactamases (ESBL) producing uropathogenic strains have been found to be increased rapidly across the world including Bangladesh. **Objective:** This study was aimed to presents phenotypic and molecular characterization of ESBL producing Enterobacteriaceae and their antibiogram profile isolated from UTI patients of six different districts of Bangladesh. **Methodology:** This cross-sectional study was conducted in Microbiology Laboratory of BIRDEM general hospital, Dhaka, Bangladesh with 187 culture positive cases collected from six laboratories of Feni, Faridpur, Kishoreganj, Sirajganj, Shatkhira and Brahmanbaria during the period from September, 2018 to August, 2019 for one year. Different members of Enterobacteriaceae were isolated and susceptibilities of these isolates to 17 different antimicrobial agents were determined. Ceftazidime (CAZ), cefotaxime (CTX), ceftriaxone (CRO) and amoxiclave (AMC) were used for confirmation of ESBL producing isolates in Double Disc Synergy Test (DDST). Frequency of *bla*$_{\text{CTX-M}}$, *bla*$_{\text{TEM}}$ and *bla*$_{\text{SHV}}$ among these isolates were further detected in this study. **Results:** Out of 187 culture positive cases, 171 samples were Enterobacteriaceae including *Escherichia coli, Klebsiella species, Enterobacter species* and *Proteus mirabilis*. Among them, 85 isolates were screened to be ESBLs producing Enterobacteriaceae and 52 isolates were confirmed as ESBL isolates in DDST. MIC of ceftazidime and MIC reduction of ceftazidime-clavulanic acid for confirmed ESBL producers was found ranged from 0.125ug/ml to 64ug/ml and 0.0156ug/ml to 32ug/ml respectively. Among 52 ESBL isolates, the *bla*$_{\text{CTX-M}}$ (86.3%) gene was predominant followed by *bla*$_{\text{SHV}}$ (22.7%) and *bla*$_{\text{TEM}}$ (18.2%) in ESBLs producing *E. coli.* All three bla genes were harboured in 2.3% and *bla*$_{\textit{CTX-M+SHV}}$ were in 18.2% *E. coli.* **Conclusion:** Alarmingly increasing spread of single gene type *bla*$_{\text{CTX-M}}$ and *bla*$_{\text{SHV}}$ harboring multidrug-resistant ESBL producing Enterobacteriaceae in six district regions of Bangladesh emphasize ESBL detection routinely in all microbiology laboratories by DDST as rapid and cost-effective method and development of rational use of antibiotic strategies in UTI to control spread of ESBL production in community of Bangladesh.

Keywords: Urinary tract infection; Extended-spectrum β-lactamase; Clinical and Laboratory Standards Institute; double disc synergy test; *bla*$_{\text{TEM}}$, *bla*$_{\text{CTX-M}}$, *bla*$_{\text{SHV}}$

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Introduction

Urinary tract infection (UTI) is generally defined as the occurrence of pathogenic microbes in the urinary tract associated symptoms[1]. As much as 35.0% of all the infectious disease, constitutes UTI infection along which reveals that UTI is one of the most common infectious disease[2]. One in three women will have at least one UTI diagnosed requiring antibacterial treatment by the age of 24 years and 40.0% to 50.0% of women will certainly experience at least one event

Correspondence: Dr. Saieda Ferdous, Assistant Professor, Department of Microbiology, Central Medical College, Cumilla, Bangladesh; Cell no.: +8801742060050.
Email: saiedaferdousfmc16@gmail.com;
ORCID: https://orcid.org/0009-0005-8448-9169

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of UTI during their lifetime[3]. Prevalence of the pathogens attributed to uncomplicated UTIs are *E. coli* followed by *Klebsiella pneumoniae*, *Staphylococcus saprophyticus, Enterococcus faecalis, Streptococcus, Proteus mirabilis, Pseudomonas aeruginosa, S. aureus* and *Candida species*[4]. Overuse and misuse of antibiotics for treatment of UTI cause antibiotic selective pressure which result in rapid increase and spread of multidrug resistant bacteria. There have been significant changes in the resistance patterns of uropathogens over past few decades that makes empirical treatment of community acquired UTI difficult now[5]. One of the most prevalent mechanisms of resistance among Gram negative bacteria is production of ß-lactamase enzyme.

Gram-negative bacteria of Enterobacteriaceae family carry ESBL genes in their plasmids or chromosomes, produce β-lactam hydrolyzing enzymes and are rightly considered to be among the most challenging pathogens by the World Health Organization[6]. ESBL have broad activity against penicillin, cephalosporins and monobactam. They inactivate ß-lactam antibiotic by breaking amide bond of ß-lactam ring[7]. Moreover, ESBL producing organisms are often resistant to several other non β-lactam antibiotics, plasmids with the gene encoding ESBL often carry other resistance determinants[8]. Some risk factors have been identified that render patients prone to community-associated ESBL infections, including old age, being female, diabetes mellitus, previous antibiotic usage, recurrent urinary tract infections and prior instrumentation to urinary tract[9]. During the late 1990s and 2000s, Enterobacteriaceae that produce ESBLs have been identified predominantly as cause of community-acquired urinary tract infections[10]. *Escherichia coli* and *Klebsiella pneumoniae* are the most prevalent ESBL producers than other gram-negative bacteria in urinary tract infections specially among outpatients[11].

Specific detection of ESBL isolates such as double disc synergy test (DDST), three-dimensional test, E-test, combined disc test, MIC reduction test either by agar dilution or by broth dilution have been described[12,13,14,15]. In previous studies, different phenotypic methods have been compared to the various clinical isolates[16]. ESBL detection rate for above mentioned each particular method vary with the types of ESBL produced and geographical area where these clinical isolates are prevalent[14]. For maximal detection of ESBL producer, detection methods are needed to be standardized.

Although TEM and SHV have been most commonly reported from many countries, since 2000, CTX-M enzymes have emerged worldwide which have replaced TEM, SHV and OXA variants and are now the most predominant type of ESBL found particularly in community acquired UTI[17.18]. Other rarely found ESBL that are transmitted through plasmids are Pseudomonas extended resistant (PER), Vietnam ESBL (VEB), Guiana extended-spectrum (GES), and integron-borne cephalosporinase (IBC)[19]. Bacteria carrying plasmid encoded ESBLs spread their resistance rapidly. These plasmids also can carry other antibiotic resistance genes such as aminoglycosides, fluoroquinolones, tetracyclines, chloramphenicol and sulfamethoxazole-trimethoprim[20]. PCR is highly reliable method to detect ESBLs more accurately than any other phenotypic method. The $bla_{\text{CTX-M}}$ followed by bla_{TEM} were detected the most prevalent ESBL encoding genes in community acquired UTI in previous studies in Bangladesh[21-22]. An infection with ESBL bacteria is related to a worse clinical course that entails deferred clinical and microbiological response, longer hospitalizations, higher costs and higher death toll[23].

Treatment of UTI cases is often started empirically based on the antimicrobial resistance patterns of urinary pathogens from existing surveillance report. A limited number of studies on the prevalence and susceptibility patterns of ESBL producing isolates in UTI cases of different districts in Bangladesh. No study has been found to detect their susceptibility pattern to fosfomycin in Bangladesh. In view of this background, this study has been carried out to determine the prevalence of ESBL producing Enterobacteriaceae in UTI patients in different districts of Bangladesh and their resistance patterns to antibiotics generally used for the treatment of UTI and to determine prevalence of $bla_{\text{CTX-M}}$, bla_{TEM}, bla_{SHV} genes in Bangladesh. This study was further carried out to evaluate better phenotypic method between DDST and MIC reduction test for detection of ESBL in isolated strains from patients of UTI.

Methodology

Study Settings and Population: This cross-sectional study was conducted in BIRDEM General Hospital, Dhaka, Bangladesh from September, 2018 to August, 2019 for one year. This study enrolled 971 urine samples of clinically suspected UTI patients referred for urine culture to Vital Research Laboratory (Feni), Diabetic Association Medical College (Faridpur),

Jahurul Islam Medical College (Kishoreganj), Khaja Yunus Ali Medical College (Sirajganj), Satkhira Diagnostic Center (Satkhira) and Brahmanbaria Medical College, Brahmanbaria, Bangladesh.

Sample Processing in Districts Level: From clinically suspected UTI patients attended in the selected laboratories of six districts, bacteria having both microscopy positive (>5 pus cells/ HPF) and culture positive (Colony count: $\geq 10^5$ cfu/ml) were isolated for this study. Cultured isolates from Feni, Faridpur, Satkhira, Kishoreganj, Sirajganj and Brahmanbaria contributed 43, 32, 18, 46, 21, 27 respectively. Isolates were inoculated on sterile Mueller Hinton slant in individual screw cap tubes and incubated overnight at 37°C. Then, screw cap tubes were stored in -10°C temperature of refrigerator at respective district laboratories.

Transportation: The screw cap tubes were transported to microbiology laboratory of BIRDEM General Hospital in ice pack box by courier within 2 to 4 weeks' time with adequate information about cultured isolates on data sheet.

Reidentification in BIRDEM General Hospital: Inoculation was done in Blood agar and MacConkey agar plate consecutively from cultured colonies on Mueller Hinton slants in laboratories of BIRDEM General Hospital. After overnight incubation isolates were reidentified by colony morphology, staining character and biochemical test.

Antimicrobial Susceptibility Test: Antimicrobial susceptibility was performed by Kriby-Bauer modified disc-diffusion test. Antibiotic disc (Himedia Ltd, India) used for Enterobacteriaceae were ampicillin (10ug), cefuroxime (30ug), ceftazidime (30ug), cefotaxime (30ug), ceftriaxone (30ug), cefixime (5ug), cefepime (30ug), amoxiclave (20/10ug), gentamicin (10ug), amikacin (30ug), piperacillin/tazobactam (100/10ug), imipenem (10ug), ciprofloxacin (5ug), cotrimoxazole (1.25/23.75ug), colistin (10ug), nitrofurantoin (300ug) and fosfomycin (200ug). Quality control was achieved by using ATCC strain of *E. coli* 25922 and zone sizes were interpreted according to CLSI[12].

Screening Test for ESBLs detection: All the Enterobacteriaceae were screened for ESBL producers by standard disc diffusion test (SDDT) according to CLSI[12]. Test was performed using cefotaxime (CTX) (30ug), ceftriaxone (CRO) (30ug) and ceftazidime (CAZ) (30ug) on inoculated Mueller Hinton agar plate. Those isolates fulfilled the criteria for ceftriaxone ≤ 25mm, ceftazidime ≤22mm and cefotaxime ≤27mm were considered as suspected ESBL producers.

Double Disc Synergy Test (DDST) for ESBLs Detection: The test was performed among screened ESBL producers[15]. Mueller Hinton agar plate was inoculated with standardized inoculum of the organism. Disc containing amoxiclave (AMC) (20/10ug) was placed in the center of the inoculated plate. Third generation cephalosporin discs of ceftazidme (CAZ), cefriaxone (CRO) and cefotaxime (CTX) were placed 20 mm apart from the center disc then the plate was incubated overnight at 37°C. Extension of the inhibition zone of three 3rd generation cephalosporin disc towards amoxiclave was interpreted positive for ESBL production.

MIC Reduction Test: MIC of ceftazidime (CAZ) and MIC of ceftazidime (CAZ) with 4 ug/ml clavulanic acid (CA) were determined by agar dilution test using range of concentration 0.015-128ug/ml and 0.015/4-64/4ug/ml respectively. MIC reduction test was performed among DDST positive isolates. Ceftazidime and clavulanic acid powder were collected from Square pharmaceuticals Ltd, Dhaka. *Klebsiella pneumoniae* ATCC 700603 was used as ESBL positive control and *Escherichia coli* ATCC 25922 was used as ESBL negative control. In MIC of ceftazidime, cut-off point >2ug/ml for ESBL producers were taken according to CLSI[12]. MIC reduction of two times and three times doubling dilutions were assessed for cut-off to be taken[12,14].

Polymerase Chain Reaction: ESBLs producing isolates were assessed by PCR to ensure the presence of $bla_{\text{CTX-M}}$, bla_{TEM} and bla_{SHV} genes. 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. Thermal cycling conditions followed[24] in this study were listed in Table 2.

Table 1: List of primers for ESBL encoding genes

Genes	Sequence (5'-3')	bp
CTX-M-F	ACGCTGTTGTTAGGAAGTG	857
CTX-M-R	TTGAGGCTGGGTGAAGT	
TEM-F	TCGGGGAAATGTGCGCG	972
TEM-R	TGCTTAATCAGTGAGGCACC	
SHV-F	GGGTTATTCTTATTTGTCGC	615
SHV-R	TTAGCGTTGCCAGTGCTC	

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 2: Thermal cycles used in the study

Genes	Programs	Number of cycles
bla_{TEM}	5 min 94°C initial denaturation	35
	45 s 94°C denaturation	
	30s 54°C annealing	
	1 min 72°C extension	
	5 min 72°C final extentsion	
bla_{SHV}	5 min 94°C initial denaturation	30
	45s 94°C denaturation	
	1 min 56°C annealing	
	1 min 72°C extension	
	5 min 72°C final extension	
bla_{CTX-M}	3 min 94°C initial denaturation	36
	1 min 94°C denaturation	
	30s 58°C annealing	
	1 min 72°C extension	
	10 min 72°C final extension	

Statistical Analysis: Collected data was compiled, checked and edited. Data processing and analysis was done with the help of computer using statistical software IBM SPSS (Statistical Package for Social Science) version 15.0 for windows. The test statistic used to analyze the data was descriptive statistics and Chi-square test. Level of significance was set at 0.05 and $P < 0.05$ was considered significant.

Ethical Clearance: All procedure of the present study were carried out in accordance with the principles for human investigations (i.e., Helsinki Declaration) and also with the ethical guidelines of the Institutional research ethics. Formal ethics approval was granted by IRB of BIRDEM ACADEMY. Participants in the study were informed about the procedure and purpose of the study and confidentiality of information provided. All participants consented willingly to be a part of the study during data collection periods. All data were collected anonymously and analyzed using the coding system.

Results

The rate of positive urine culture was 19.3% (187/971). Among the positive cases, 70.1% were female and 29.9% were male. Of 187 cultured isolates, gram negative bacteria were 181 (96.8%) and gram-positive bacteria were 6 (3.2%). *E. coli* (80.2%) was found to be the most common pathogen followed by *Klebsiella spp.* (5.4%), *Enterobacter species* (5.34%) and coagulase negative *Staphylococcus* (CoNS) (2.7%) (Table 3).

Table 3: Distribution of isolated bacteria from culture (n=187)

Pattern of Isolated Organisms	Frequency	Percent
Gram negative organisms	181	96.8
E. coli	150	80.2
Klebsiella spp.	10	5.4
Enterobacter spp.	10	5.4
Pseudomonas spp.	10	5.4
Proteus mirabilis	1	0.5
Gram positive organisms	6	3.2
S. aureus	1	0.5
CoNS	5	2.7

Among isolated Enterobacteriaceae, Proteus mirabilis was not ESBL producers. Of 150 *E. coli*, 10 *Klebsiella spp.* and 10 *Enterobacter spp.*, 44 (29.3%), 5 (50%), 3 (30%) were respectively ESBLs producers (Table 4).

Figure I demonstrates the frequency of ESBL producing Enterobacteriaceae more in Satkhira (44.44%), Faridpur (40.83%), Sirajganj (33.3%) and comparatively low in Kishoerganj (17.39%) and Brahmanbaria (11.11%).

Table 4: Rate of ESBL Producing Isolates among Enterobacteriaceae

Enterobacteriaceae	Total	ESBL producing isolates		Non-ESBL producing isolates	
		Frequency	Percent	Frequency	Percent
E. coli	150	44	29.3	106	70.7
Klebsiella spp.	10	5	50. 0	5	50.0
Enterobacter spp.	10	3	30.0	7	70.0
Proteus mirabilis	1	0	0.0	1	100
Total	**171**	**52**	**30.4**	**119**	**69.6**

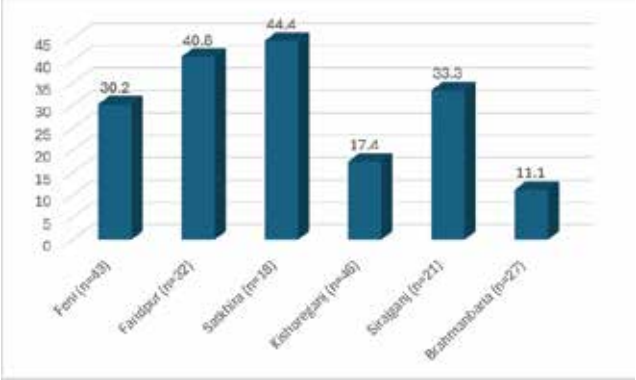

Figure I: Frequency of ESBL producing Enterobacteriaceae in different district

ESBLs producing isolates were all found to be resistant to ampicillin, cefuroxime, ceftazidime, cefotaxime, ceftraixone and cefixime. These isolates exhibited comparatively more resistant to cefepime (48.1%%), gentamicin (19.2%), ciprofloxacin (57.7%) and cotrimoxazole (59.6%) than non-ESBLs producing isolates ($p < 0.05$). Lowest rate of resistance against piperacillin/tazobactam (5.8%), amikacin (7.7%), nitrofurantoin (1.9%) and fosfomycin (1.9%) was found in ESBLs producing isolates. No ESBLs producing isolates was found to be resistant to imipenem and colistin. Difference in resistance patterns between ESBLs producers and non-ESBL producers to these six drugs was not found statistically significant (Table 5).

Distribution of MIC of ceftazidime (CAZ) and ceftazidime with clavulanic acid (CAZ-CA) for ESBLs producing Enterobacteriaceae was recorded. Of DDST positive ESBL producing Enterobacetriaceae, MIC of CAZ for 43 isolates fulfilled the CLSI recommended breakpoint level (≥ 2ug/ml). But MIC of < 2ug/ml was found in seven E. coli, one klebsiella spp. and one Enterobacter spp. In MIC reduction test using CAZ-CA, 45 (86.5%) reduced MIC at ≥ 3 doubling dilution. Among rest seven isolates, four E. coli, one klebsiella spp. and one Enterobacter spp. reduced MIC at 2 doubling dilution. However, use of ≥ 2 doubling dilution MIC reduction criteria increased the sensitivity of ESBL producing isolates detection. One Enterobacter spp. reduced MIC at one doubling dilution that did not fulfill ≥ 3 or ≥ 2 doubling dilution MIC reduction criteria (Table 6).

Confirmed ESBLs producing Enterobacteriaceae by DDST were further evalauted by PCR for presence of bla_{CTX-M}, bla_{TEM}, bla_{SHV}. The bla_{CTX-M} was found the most prevalent gene among isolated ESBL producing *E. coli*, *Klebsiella spp.*, *Enterobacter spp.*. Single bla gene was found to have in thirty one ESBL producing isolates total, in contrast two or three bla genes were found in seventeen different isolates (Table 7).

Table 5: Antibiotic Resistance Patterns of Isolated Enterobacteriaceae

Name of Antibiotics	ESBL producing isolates				Non-ESBL producing isolates					
	E. coli (n=44)	*Klebsiella spp.* (n=5)	*Enterobacter spp. (n=3)*	Total (n=52)	*E. coli* (n=106)	*Klebsiella spp.* (n=5)	*Enterobacter spp.* (n=7)	*Proteus mirabilis* (n=1)	Total (n=119)	P value
Ampicillin	100%	100%	100%	100%	58.5%	80%	57.1%	100%	59.7%	0.00
Cefuroxime	100%	100%	100%	100%	58.5%	80%	57.1%	100%	59.7%	0.00
Ceftazidime	100%	100%	100%	100%	26.4%	40%	28.6%	0.0%	26.9%	0.00
Ceftriaxone	100%	100%	100%	100%	26.4%	60%	28.6%	0.0%	27.7%	0.00
Cefotaxime	100%	100%	100%	100%	29.2%	60%	28.6%	0.0%	30.3%	0.00
Cefixime	100%	100%	100%	100%	33.9%	60%	42.9%	0.0%	35.3%	0.00
Cefepime	45.5%	80 %	33.3%	48.1%	22.6%	60%	0.0%	0.0%	22.7%	0.00
Amoxiclave	100%	100%	100%	100%	33.9%	60%	28.6%	0.0%	34.5%	0.00
Piperacillin/ Tazobactum	2.3%	20%	33.3%	5.8%	10.4%	20%	14.3%	0.0%	10.9%	0.29
Gentamicin	13.7%	40%	66.7 %	19.2%	8.5%	40%	14.3%	0.0%	10.1%	0.00
Amikacin	6.8%	20%	0.0%	7.7%	7.6%	40%	28.6%	0.0%	10.1%	0.62
Ciprofloxacin	59.1%	60%	33.3%	57.7%	40.6%	20%	0.0%	100%	37.8%	0.00
Cotrimoxazole	61.4%	40 %	66.7%	59.6%	39.6%	20%	0.0%	100%	36.9%	0.00
Nitrofurantoin	0.0%	20%	0.0%	1.9%	4.7%	0.0%	0.0%	0.0%	4.2%	0.46
Imipenem	0.0%	0.0%	0.0%	0.0%	1.9%	20%	0.0%	0.0%	2.5%	0.25
Colistin	0.0%	0.0%	0.0%	0.0%	0.9%	0.0%	0.0%	0.0%	0.8%	0.51
Fosfomycin	0.0%	20%	0.0%	1.9%	0.0%	0.0%	0.0%	0.0%	0.0%	0.13

Table 6: MIC of ceftazidime and ceftazidime-clavulanic acid of ESBL producing isolates.

ESBL producing isolates	ESBL producing isolates Frequency Percent													N (%) of isolates in MIC reduction of doubling dilution	
	Drugs	.0156	.0312	.0625	.125	.25	.5	1	2	4	8	16	32	64	≥ 2	≥ 3
E. coli (44)	CAZ	0	0	0	1	3	2	1	1	6	7	16	7	0		
	CAZ-CA	2	3	12	10	10	6	2	1	0	0	0	0	0	44 (100%)	40 (90.9%)
Klebsiella spp. (n=5)	CAZ	0	0	0	0	1	0	0	0	1	2	1	0	0		
	CAZ-CA	0	0	1	1	1	2	0	0	0	0	0	0	0	5 (100%)	4 (80%)
Enterobacter spp. (n=3)	CAZ	0	0	0	0	1	0	0	0	0	0	1	0	1		
	CAZ-CA	1	0	0	0	0	0	0	0	1	0	0	1	0	2 (66.7%)	1 (33.3%)

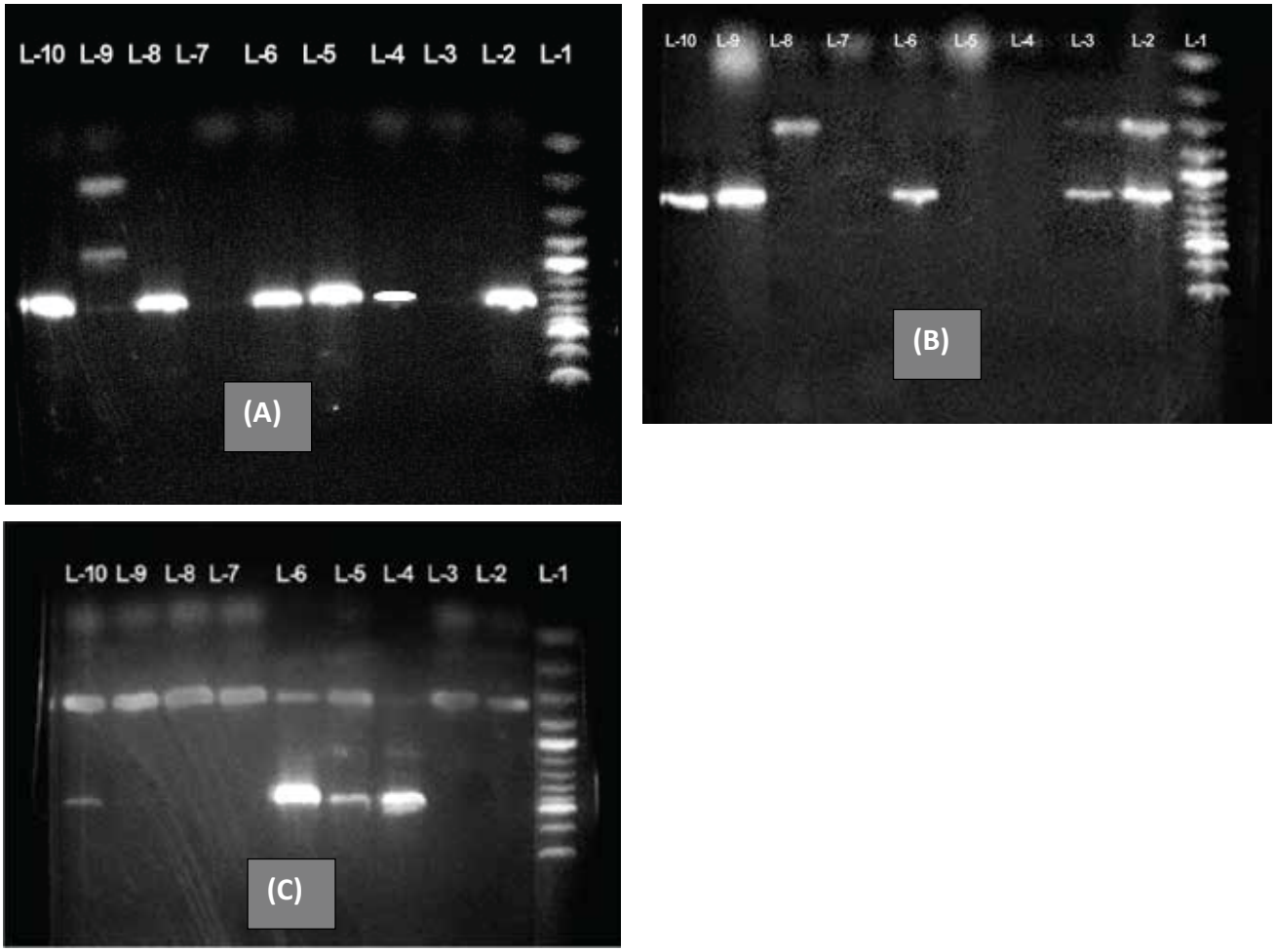

Figure II: Gel electrophoresis picture showing the results for PCR amplification of $bla_{\text{CTX-M}}$ gene, bla_{SHV} and bla_{TEM}. Picture

(A) Analysis for $bla_{\text{CTX-M}}$ (857bp); Lane L1-100bp ladder; L2, L5, L6, L8, L10 = samples positive for $bla_{\text{CTX-M}}$; L7, L9 = samples negative for $bla_{\text{CTX-M}}$. Picture; L3 = NC; L4=PC

(B) Analysis for bla_{SHV} (615bp); Lane L-100bp ladder; L2,L6, L9, L10 = samples positive for bla_{SHV}; L5, L7, L8 = samples negative for bla_{SHV}; L4 =NC; L3 = PC; Picture **(C)** Analysis for bla_{TEM} (972bp); Lane L-100bp ladder; L4, L6, L10 = samples positive for bla_{TEM}; L2, L3, L9 = samples negative for bla_{TEM}; L7= NC; L5 = PC; NC = negative control; PC = positive control.

TTable 7: Patterns of ESBL Encoding Genes in ESBL Producing Enterobacetriaceae

Genes	ESBL Genes			Total (52)
	***E. coli* (44)**	***Klebsiella spp.* (5)**	***Enterobacter spp.* (3)**	
$bla_{CTX\text{-}M}$	38 (86.3%)	5 (100%)	3 (100%)	46 (88.5%)
bla_{SHV}	10 (22.7%)	0 (0.0%)	0 (0.0%)	10 (19.2%)
bla_{TEM}	8 (18.2%)	1 (20.0%)	1 (33.3%)	10 (19.2%)
Single Genes/ Multiple Genes				
$bla_{CTX\text{-}M}$	23 (52.3%)	4 (80.0%)	2 (66.7%)	29 (55.8%)
bla_{TEM}	1 (2.3%)	0 (0.0%)	0 (0.0%)	1 (1.9%)
bla_{SHV}	1 (2.3%)	0 (0.0%)	0 (0.0%)	1 (1.9%)
$bla_{CTX\text{-}M+TEM}$	6 (13.6%)	1 (20.0%)	1 (33.3%)	8 (15.4%)
$bla_{CTX\text{-}M+SHV}$	8 (18.2%)	0 (0.0%)	0 (0.0%)	8 (15.4%)
$bla_{CTXM+SHV+TEM}$	1 (2.3%)	0 (0.0%)	0 (0.0%)	1 (1.9%)

Discussion

Frequency of use of antibiotics and even dosages and period of administration vary significantly from country to country, region to region even in a locality[25]. This has resulted in emergence of resistance among Enterobacteriaceae causing UTI to many available antibiotics. The present study clearly brings forth that *E. coli* is the causative organism in 80.2% of UTI cases and there is increasing prevalence of ESBLs uropathogens in different districts of Bangladesh. Of 171 Enterobacteriaceae, 52 (30.4%) were found to be ESBL producing isolates supported by other studies in Bangladesh, India and Saudi Arabia[26,27,28]. It was observed that rate of detection of ESBL isolates was higher in Satkhira (44.44%) and Faridpur (40.83%) and lower in Brahmanbaria (11.3%) and Kishoreganj (17.4%). This may be due to samples were collected from laboratories situated in rural area of Brahmanbaria and Kishoreganj. ESBLs producers were more resistant to cefepime (48.1%), gentamicin (15.4%), ciprofloxacin (57.7%), co-trimoxazole (59.6%) and less resistant to piperacillin/tazobactum (5.8%), amikacin (7.6%), nitrofurantoin (1.9%), in addition, no resistance to imipenem (0.0), colistin (0.0) in comparison with non-ESBLs producers. These findings correspond with the findings of previous studies in Bangladesh, South Mumbai and Norway[8,29,30]. Many factors responsible for increasing antibiotic resistance which includes enormous use and misuse of antibiotics by health practitioners in addition to self-prescription by community. None of ESBLs producers was resistant to colistin in this study but a little higher rate of colistin resistant ESBLs uropathogens (3.9%) was reported in India[20]. Fosfomycin retains its activity against ESBLs isolates which corresponds with the results reported in Spain and Thailand[31,32]. In perspective of such analysis, use of nitrofurantoin and fosfomycin as oral preparations and piperacillin-tazobactam, amikacin, imipenem and colistin as injectable preparations constitute the most effective treatment option against ESBL producers.

MIC of CAZ alone did not detect nine isolates which were detected as ESBLs producers by DDST. Similar findings were also reported by several studies[33,14]. MIC reduction test as confirmatory test by agar dilution using CAZ-CA detected 51 (98.1%) ESBLs producers with ≥ 2 doubling dilution reduction criteria. One *Enterobacter spp*. was detected later by PCR which confirmed in DDST but did not show recommended MIC reduction criteria. This study recommended sensitivity of MIC reduction test can be increased by using ≥ 2 doubling dilution reduction[14] than CLSI[12] recommended ≥ 3 doubling dilution reduction criteria. DDST is easier to perform in laboratories than MIC reduction test and this study found DDST better than MIC reduction test (agar dilution). Earlier report mentioned similar interpretation for MIC reduction test and DDST from their study[14].

The $bla_{CTX\text{-}M}$ gene among *E. coli* was observed in 86.3% isolates followed by bla_{SHV} and bla_{TEM} genes in 22.7% and 18.2% isolates respectively. The $bla_{CTX\text{-}M}$ gene was also found highest in urinary isolates in other study of Bangladesh, Morocco, Jordan and Lebanon but they reported bla_{TEM} following CTX-M[34,35,36]. The prevalence of bla_{SHV} was observed higher than bla_{TEM} among *E. coli* in this study explains incidence of bla_{SHV} in increasing after CTX-M in our country. A study from Qatra reported $bla_{\text{CTX-M}}$ (66.1%) followed by bla_{SHV} (53.2%) and bla_{TEM} (40.4%) in different sample of ICU37. So, further study is needed in Bangladesh to eslablish these finding. The high

distribution of $bla_{\text{CTX-M}}$ among these isolates explains the high rate of resistance to cephalosporin such as cefotaxime, ceftriaxone, and ceftazidime in this study. Several studies[10,38,39] reported bla_{TEM} as predominant ESBL genes in uropathogenic isolates which is not in agreement with this study. Four isolates were not detected for any of three genes but were positive in both phenotypic tests which might be due to the presence of other variants of ESBL genes in the studied isolates.

Combined genes were concurrently detected in *E. coli* than *Klebsiella spp*. and *Enterobacter spp*.. These results indicate genetic diversity of ESBL producing Enterobacteriaceae that might be due to high transfer of genes among these bacteria. Several studies[40,41] reported both bla_{SHV} and bla_{TEM} encoded as multiple genes that supported this study. Single bla_{TEM} and bla_{SHV} were reported individually only in 1.92% isolates. Coexistance of $bla_{\text{CTX-M}}$ and bla_{TEM} (15.4%) isolated in this study was observed much more lower than the report of 87% and 50% from uropathogenic isolates in other studies in India and Iraq but coexistance of $bla_{\text{CTX-M+TEM+SHV}}$ was observed in only one isolates (1.9%) which corresponds with them[40,20]. Coexistance of $bla_{\text{CTX-M+SHV}}$(15.4%) was found also supported by a study where they reported CTX-M+SHV (58.3%) in uropathogenic Enterobacteriaceae in Nigeria[41].

Conclusion

ESBL type of drug resistance was detected in high rate among isolated gram-negative bacteria. The frequency was found to be higher in Faridpur, Satkhira and lower in Kishoreganj and Brahmanbaria from the selected districts of Bangladesh. Lower rate from these regions may be due to the sample collected from the laboratories situated in rural area. A combination therapy is recommended for use guided by antimicrobial susceptibility testing and we should furthermore exercise restraint in using or prescribing imipenem or colistin to prevent selection of resistant isolates. Fosfomycin can be an alternative to nitrofuratoin and constitute the treatment option for ESBLs producing Enterobacteriaceae in UTI from community. Double disc synergy test (DDST) is easier method to perform in laboratories than MIC reduction test. The sensitivity of detection rate of ESBL producers was increased in MIC reduction test with ≥ 2 doubling dilution than with ≥ 3 doubling dilution. Although the bla_{CTX-M} predominates among all ESBLs encoding genes in the uropathogenic Enterobacteriaceae, the bla_{SHV} is found to be increasing also.

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Conflict of Interest
All authors declared no conflict of interests.

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Authors' contributions
Ferdous S, Islam KMS conceived and designed the study, analyzed the data, interpreted the results, and wrote up the draft manuscript. Rahman A, Jalil RA contributed to the analysis of the data, interpretation of the results and critically reviewing the manuscript. Khandakar A, Sharmin R involved in the manuscript review and editing. All authors read and approved the final manuscript.

Data Availability
Any inquiries regarding supporting data availability of this study should be directed to the corresponding author and are available from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate
Ethical approval for the study was obtained from local ethics committee. All methods were performed in accordance with the relevant guidelines and regulations.

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ORCID
Saieda Ferdous: https://orcid.org/0009-0005-8448-9169
KM Shahidul Islam: https://orcid.org/0009-0006-4001-7407
Arifur Rahman: https://orcid.org/0009-0004-0274-2048
Rafia Afreen Jalil: https://orcid.org/0000-0002-9823-3261
Adeeba Khanduker: https://orcid.org/0000-0001-6442-7095
Robayet Sharmin: https://orcid.org/0009-0004-7170-9655

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