The Status of Extended-spectrum β-lactamase and AmpC β-lactamase Production in Uropathogenic Isolates of *Escherichia coli* and *Klebsiella pneumonia* in Bangladesh

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Abstract

Background: The rapid dissemination of antibiotic resistance as extended-spectrum beta-lactamases (ESBLs) and AmpC β-lactamases in bacteria became a major public health concern worldwide. Hence, this study was carried out to evaluate the status of uropathogenic isolates of Bangladesh especially *Escherichia coli* and *Klebsiella pneumoniae* in terms of ESBL and AmpC β-lactamases enzymes production. **Methods:** A laboratory-based cross-sectional survey was conducted with 553 urine samples at the Department of Microbiology, Popular Diagnostic Centre Ltd. Dhaka, Bangladesh for a period of 2.5 years from July 2017 to December 2019. Pathogens were obtained upon culture, and screened for ESBL production following the cephalosporin disk diffusion method and cephalosporin-clavulanate combination disc test (confirmatory) method. For AmpC β-lactamases enzymes, cefoxitin disc diffusion, and AmpC disc test (confirmatory) methods were performed. The positive isolates were further genotyped for the presence of *bla*TEM, *bla*SHV, *bla*OXA, and *bla*CTX-M β-lactamases genes in case of ESBL and *bla*FOX, *bla*CMY, and *bla*DHA genes for AmpC β-lactamases by polymerase chain reaction. **Results:** Of all urine samples, uropathogens were detected among 28.57% (158, n=553) samples. Among them, 46.20% (73) were *E. coli* and 20.25% (32) were *K. pneumoniae*. The prevalence of ESBL and AmpC β-lactamases production in *E. coli* was 34.24% (25) and 17.80% (13) respectively whereas it was 43.75% (14) and 21.87% (7) in *Klebsiella pneumoniae* isolates respectively. These resistance properties were further confirmed by phenotypic disc test. In case of ESBLs, most typical isolates possessed two or more resistance genes. Overall, the most prevalent gene was *bla*CTX-M (84.61%) followed by *bla*TEM (64.10%), *bla*OXA (46.15%) and *bla*SHV (25.64%). In case of AmpC β-lactamases FOX gene was most prevalent (45%) followed by CMY (20%) and DHA 3 (15%). **Conclusion:** The high prevalence of ESBL and AmpC β-lactamases production in our study emphasizes the judicious use of antibiotics in controlling urinary tract infections in Bangladesh.

Keywords: Extended-spectrum β-lactamases, AmpC β-lactamases, gram-negative isolates, antibiotic susceptibility testing.

Significance | Status of antibiotic resistance among uropathogens

Introduction

Urinary tract infection (UTI) is one of the most common infect-

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-ions and the most common causative agent of UTI in both community and healthcare settings is *Escherichia coli* (Auer et al., 2010). The choice of antibiotics for the treatment of UTI is limited due to the increasing emergence of antibiotic resistance. β-lactamase, a novel class of enzymes conveying resistance against β-lactam antibiotics was subsequently followed by extended-spectrum β-lactamases (ESBLs) produced by the organisms of the Enterobacteriaceae family which spread worldwide since the first report in 1983 (Bradford, 2001).

Extended-spectrum β-lactamases (ESBLs) and AmpC β-lactamases are of increasing clinical concern. ESBLs are produced by gram-negative bacilli which have the potential to hydrolyze β-lactam antibiotics containing an oxyimino group (e.g., third-generation cephalosporins- 3GCs, and aztreonam) and are inhibited by β-lactamase inhibitors such as clavulanic acid, sulbactam, and tazobactam. However, cephamycin (e.g., cefoxitin) or carbapenems (e.g., imipenem, meropenem, and ertapenem) are not affected by these enzymes (Altoparlak et al., 2004). AmpC β-lactamases are chromosomally encoded enzymes of certain genera of Enterobacteriaceae and mediate resistance to all penicillin and all cephalosporins except cefepime (Tenover et al., 1999). These are not inhibited by β-lactamase inhibitors and it is difficult to detect the AmpC β-lactamase producers by phenotypic confirmatory methods only. Nevertheless, the prevalence of bacterial pathogens producing both ESBLs and AmpC β-lactamases should be determined to establish the existence of these enzymes in clinical isolates (Khan et al., 2008).

At present, a majority of clinical laboratories test for the production of ESBLs whereas the production of plasmid-mediated AmpC β-lactamases is usually ignored. Like ESBLs, AmpC β-lactamases have a wide-substrate contour as well as penicillins, cephalosporins, and monobactams. Additionally, it hydrolyzes cephamycins and is not inhibited by commercially available β-lactamase inhibitors.

Several ESBL detection tests that have been proposed are based on Clinical Microbiology Techniques. Screening for ESBL, NCCLS phenotypic confirmatory method, Double disc synergy/Disk approximation method, E-test ESBL strips, three-dimensional test, Vitek system, and several other molecular methods including PCR, PCR-RFLP, PCR-SSCP, LCR, Pulsed-field gel electrophoresis (PFGE), etc. have also been proposed for their detection. So far, there is no ideal test for the identification of ESBLs. NCCLS recommended the phenotypic method as a confirmatory test (Weinstein et al., 2018).

With this background, the current study is undertaken to detect ESBL and AmpC β-lactamases in uropathogenic *E. coli* and *Klebsiella pneumoniae*, as not many such studies have been done on this topic, especially in Dhaka, Bangladesh. The study is designed to confirm the detection of ESBL & AmpC-producing bacteria from uropathogenic isolates, which will reduce the treatment cost of both patient and hospital authorities and also prevent unnecessary use of antibiotics and benefit the patient by the administration of the appropriate antibiotics.

Materials and Method

Study design and sample collection

A total of 553 urine samples were collected during a study period from January 2018 to December 2020 and processed in the Department of Microbiology, Popular Diagnostic Centre Ltd., Dhaka, Bangladesh. Urine samples were collected using the clean-catch midstream technique (Fatema et al., 2018). A sterile container was provided to the patient and/or any attendants who gave their consent, and they were well-instructed to collect the midstream urine.

Isolation of Bacteria

Bacterial isolates included in this study consisted of non-duplicate, consecutive gram-negative isolates (*K. pneumoniae* and *E. coli)*. Sample processing and inoculation were performed aseptically maintaining biosafety. The quality control policy was applied in sample processing at the medical microbiology laboratory of Popular diagnostic center ltd. All the isolates were identified by CLSI guidelines (Colony morphology, biochemical method, and Vitek-2 Identification system, Biomeriux) (Weinstein et al., 2018).

Screening of extended-spectrum beta-lactamase

The isolates were screened for ESBL production by showing an inhibition zone of the size of ≤22 mm with ceftazidime (30μg), <25 mm for ceftriaxone, and ≤27 mm with cefotaxime (30μg) by disc diffusion testing on Mueller-Hinton agar (MHA) using Clinical and Laboratory Standards Institute (CLSI) recommended conditions (Weinstein et al., 2018). Quality control was performed using *E. coli* ATCC 25922, and *Klebsiella pneumoniae* ATCC 700603

Phenotypic confirmatory test for extended-spectrum beta-lactamase

Double disk diffusion method (DDDT)

A phenotypic confirmatory test for ESBL production was performed using the cephalosporin/clavulanate combination disc test method for ESBLs according to CLSI guidelines (Weinstein et al., 2018). The combination disc test was performed using CTX (30 μg), and CAZ (30 μg) discs with and without clavulanate (10 μg). A ≥5-mm increase in the diameter of the zone of CTX/CAZ in the presence of clavulanic acid compared to that of the antibiotics tested alone was considered positive for an ESBL.

Modified double-disk diffusion method (MDDDT)

An antibiotic disc that consists of amoxicillin-clavulanate (20/10 μg) was placed in the center of the plate. The discs of CTX/CAZ

(30µg) were placed 15mm and 20mm apart respectively, center to centre to that of the amoxicillin-clavulanate disc. Any distortion or increase in the zone near the disc of amoxicillin-clavulanate was treated positively for ESBL production.

Screening of AmpC beta-lactamase

The isolates were screened for AmpC beta-lactamase production. A 30µg cefoxitin disk was placed on inoculated Mueller-Hinton agar media. According to CLSI, isolates with zone diameters <14 mm were selected for confirmation of AmpC production (Weinstein et al., 2018).

Phenotypic confirmatory tests for AmpC beta-lactamase

The isolates which were positive by screening test were further confirmed for AmpC beta-lactamase by AmpC disk test method, and disk approximation method was performed (Black et al., 2005; Shahid et al., 2004).

Detection of ESBLs and AmpC β-lactamases by the polymerase chain reaction

Screening or phenotypically confirmed strains were used to perform Polymerase Chain Reaction (PCR) by using specific primers (Table 1) and cycling conditions to detect the genes encoding ESBLs (*bla*TEM, *bla*SHV, *bla*OXA, and *bla*CTX-M) and AmpC (*bla*FOX, *bla*CMY, and *bla*DHA) β-lactamases, as previously described (Edelstein et al., 2003; Govindaswamy et al., 2018; Stürenburg et al., 2004).

The products were separated by electrophoresis in 1.0% agarose gel with 1× TBE (Tris/ borate/EDTA) buffer, stained with safe stain load dye, and visualized by trans-illuminator.

Preparation of reaction mixture

For PCR amplification, 2.0 µl of template DNA was added to prepare 25 µl of reaction mixture containing 10× PCR buffer 2.5 µl (Ex Taq), 2.0 µl of dNTPs mixture (2.5 mM of each of dATP, dTTP, dGTP and dCTP), 0.5 µl of *Taq* polymerase (250 U), 2.5 µl of each primer (10 pmol/ µl), and remaining 13.0 µl with nuclease free water. PCR was performed in a thermal cycler (Eppendorf, USA) and the amplification was carried out following the conditions given below.

Initial denaturation at 94 °C for 3 minutes, 35 cycles comprised of denaturation at 94 °C for 30 sec, annealing at 50 °C for 30 sec for TEM, SHV, and OXA gene and 40 sec for CTX-M, DHA, FOX and CMY genes, and extension at 72 °C for 2 mins and a final extension at 72 °C for 5 minutes.

Statistical analysis

Statistical analysis was performed by using the computer-based program Excel and SPSS version 21.0 statistical software.

Results

Prevalence and distribution of UTI causative agents

Out of 553 urine samples, 158 (28.57%) culture-positive ($\geq 10^5$ CFU/ml) samples were found (Fig. 1). In these culture-positive samples (n=158), the most prevalent causative agent was *Escherichia coli* (46.20%), followed by *Klebsiella pneumonia* (20.25%), *Enterococcus spp.* (11.39%), *Staphylococcus spp.* (8.86%), *Pseudomonas spp.* (5.69%), *Proteus spp.* (3.26%), *Acinetobacter spp.* (2.53%), and *Candida spp.* 3 (1.89%).

Escherichia coli and *Klebsiella pneumonia* (a total of 105 isolates) were further checked for ESBL and AmpC activity.

Detection of ESBL and AmpC activity among *E. coli and Klebsiella pneumoniae* isolates

Out of 105 isolates, Extended-spectrum β-lactamase (ESBL) activity was found in 37.14% of cases. Almost half of the tested *Klebsiella pneumonia* (43.75%, n=32) were ESBL producers whereas one-third of the tested *E. coli* (34.24%, n=73) possessed this property (Fig. 2) (Table 2).

On the contrary, out of 105 gram-negative (*E. coli* & *Klebsiella pneumoniae)* isolates, AmpC β-lactamases were found in 5 (19.05%) cases. The highest occurrence of AmpC β-lactamases was observed in *Klebsiella pneumoniae* (21.87%, n=32) whereas 17.80% *E. coli* (n=73) were detected positive (Fig. 2)(Table 4).

Genotypic characterization of extended-spectrum beta-lactamase and AmpC-producing *Escherichia coli* isolates

The isolates which tested positive for ESBL production by screening and confirmatory tests were further genotyped for the presence of *bla*CTX-M, *bla*TEM, *bla*OXA, and *bla*SHV β-lactamases genes in the case of ESBL and AmpC β-lactamases *FOXM, CMY,* and *DHA-type* genes were detected by PCR (Fig. 3). The genotypic prevalence of these genes is shown in Table 3.

Discussion

The increasing incidence of antimicrobial resistance is a major global public health issue in Bangladesh. This led to prolonged hospital stays for the patients, limited antibiotic options to treat, and a financial crisis that increased the morbidity and mortality of patients. The study was designed to assess the level of antibiotic resistance that is commonly used in urinary tract infections and also to see the prevalence of ESBL and AmpC β -lactamase-producing *E. coli* and *K. pneumoniae* in UTIs. The prevalence of ESBL and Amp-C β-lactamases production varies from one geographical region to another.

In the present study, the different strains among urine samples showed the maximum rate in *E. coli*, 73 (46.20%) followed by *K. pneumoniae* 32 (20.25%). This study was supported by other studies where *E. coli, and Klebsiella* spp. were the main pathogens of urinary tract infection (Ejaz et al., 2006; Humayun & Iqbal, 2012; Moyo et al., 2010; Qureshi, 2005; Rahman et al., 2004).

In our study, the prevalence of ESBLs was 39 (37.14%) which was lower in comparison to reports from different parts of the country

Table 1: Primer sequences used for ESBL and AmpC beta-lactamase detection

Target gene	Primers	Sequences	Amplicon size
TEM	TEM F	5'-CTTCCTGTTTTTGCTCACCCA-3'	857 bp
	TEM R	5'-TACGATACGGGAGGGCTTAC-3'	
SHV	SHV F	5'-TCAGCGAAAAACACCTTG-3'	471 bp
	SHV R	5'-TCCCGCAGATAAATCACC-3'	
OXA	OXA F	5'-AACAAGCGCTATTTTTATTTCAG-3'	641 bp
	OXA R	5'-AGCGCTATTTTTATTTCAGAACA-3'	
CTX-M	CTX-MR	5'-ATGTGCAGYACCAGTAARGT-3'	593 bp
	CTX-MF	5'-GCAGYACCAGTAARGTATGT-3'	
DHA	DHAMF	5'-AACTTTCACAGGTGTGCTGGGT-3'	405 bp
	DHAMR	5'-CCGTACGCATACTGGCTTTGC-3'	
FOX	FOXMF	5'-AACATGGGGTATCAGGGAGATG-3'	190 bp
	FOXMR	5'-CAAAGCGCGTAACCGGATTGG-3'	
MOX, CMY	MOXMF	5'-GCTGCTCAAGGAGCACAGGAT-3'	520 bp
	MOXMR	5'-CACATTGACATAGGTGTGGTGC-3'	

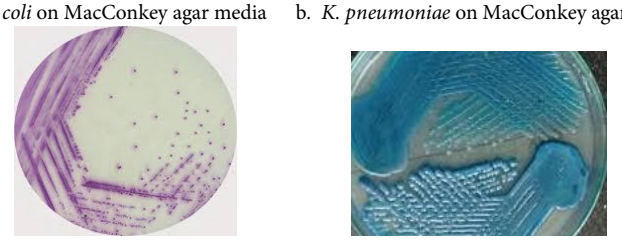
Table 2: Prevalence of ESBL Beta-lactamase type by Phenotypic Screening method

Beta-lactamase type	Name of strain	Phenotypic Screening method Number of isolates (%)
ESBL type	*E. coli* (n=25)	25 (100.00%)
	Klebsiella pneumoniae (n=14)	14 (100.00%)
AmpC type	*E. coli* (n=13)	13 (100.00%)
	Klebsiella pneumoniae (n=7)	7 (100.00%)

Table 3: Genotypes of extended-spectrum beta-lactamase and AmpC-producing isolates

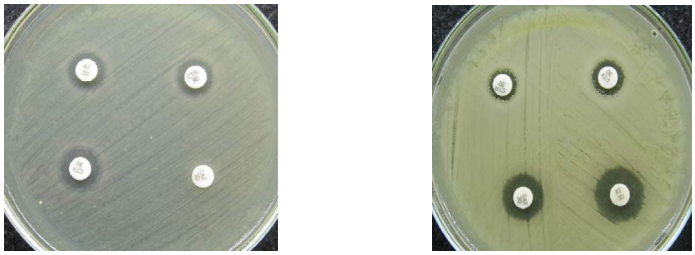
β -lactamase type		Specific gene	Number of isolates	Prevalence (%)
ESBL type		TEM	25	64.10
		SHV	10	25.64
		CTX-M	33	84.61
		OXA	18	46.15
AmpC type		FOX	9	45.00
		CMY	5	20.00
		DHA	3	15.00

a. *E. coli* on MacConkey agar media b. *K. pneumoniae* on MacConkey agar medi

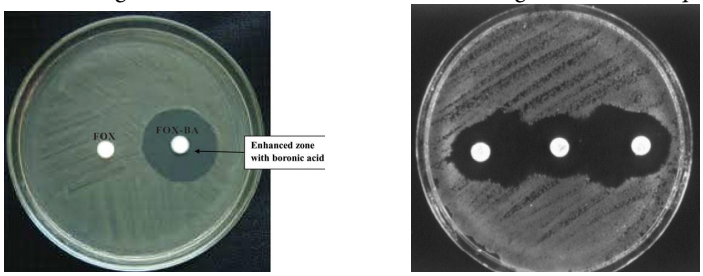


c. *E. coli* on hi-chrome (UTI) agar media d. *K. pneumoniae* on hi-chrome (UTI) media

Figure 1: Colony morphology of *E. coli* and *K. pneumoniae* in MacConkey agar and hi-chrome (UTI) agar media.

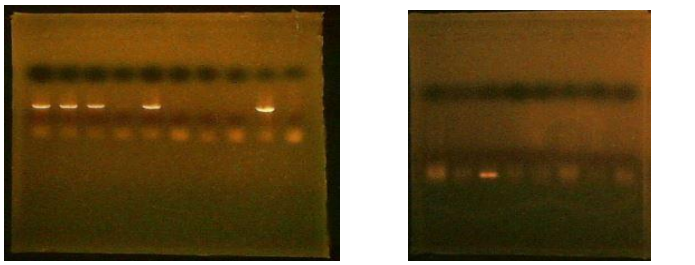


a. Screening for ESBL in *E. coli* b. Screening for ESBL in *K. pneu*

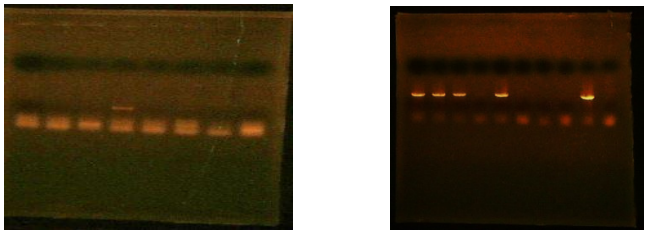


c. Phenotype of AmpC β -lactamase d. Phenotype of ESBL

Figure 2: Screening of ESBL- & AmpC-producing *E. coli* and *K. pneumoniae* by double-disk diffusion method.



a. *bla*TEM, Product size: 857 bp b. *bla*OXA Product size: 198 bp



c. *bla*SHV, Product size: 768 bp d. *bla*CTX-M, Product size: 593 bp

Figure-3: PCR detection of ESBL and AmpC related genes in the *E. coli* and *K. pneumoniae* isolates.

Table 4: Comparison of ESBLs & AmpC beta-lactamase positive strain by Phenotypic method and Genotypic method

Beta-lactamase type	Name of strain	Screening method Number of isolates (%)	confirmatory Method Number of isolates (%)	Genotypic Method Number of isolates (%)
ESBL type	*E. coli* (n=73)	25 (100.00%)	22 (88.00%)	21 (84.00%)
	Klebsiella pneumoniae (n=32)	14 (100.00%)	12 (85.71%)	12 (85.71%)
AmpC type	*E. coli* (n=73)	13 (100.00%)	10 (76.92%)	8 (61.53%)
	Klebsiella pneumoniae (n=32)	7 (100.00%)	5 (71.42%)	5 (71.42%)

Table 5: Resistant pattern of ESBL and non-ESBL isolates

Antibiotic	ESBL (n=39) No. (%)	Non-ESBLs (n=66) No. (%)	AmpC (n=20) No. (%)	Non-AmpC (n=85) No. (%)
Cephradine	39 (100%)	60 (90.90%)	20 (100.0%)	18 (90.0%)
Cefoxitin	0 (0.00%)	41 (62.12%)	20 (100.0%)	41 (48.23%)
Cefixime	39 (100%)	41 (62.12%)	18 (90.00%)	58 (68.23%)
Ceftriaxone	39 (100%)	32 (48.48%)	15 (75.00%)	42 (49.00%)
Ceftazidime	37 (94.87%)	26 (39.39%)	14 (70.00%)	31 (36.47%)
Cefepime	35 (89.74%)	22 (33.33%)	12 (60.00%)	27 (31.76%)
Aztreonam	39 (100%)	28 (42.42%)	14 (70.00%)	31 (36.47%)
Imipenem	1 (2.56%)	0 (0.00%)	0 (0.00%)	3 (3.52%)
Meropenem	1 (2.56%)	1 (5.00%)	1 (5.00%)	4 (4.70%)
Ciprofloxacin	26 (66.66%)	37 (56.06%)	11 (55.00%)	52 (61.17%)
Levofloxacin	23 (58.97%)	34 (51.51%)	10 (50.00%)	48 (56.47%)
Doxycycline	33 (84.61%)	58 (87.87%)	17 (85.00%)	74 (87.05%)
Tetracycline	33 (84.61%)	58 (87.87%)	17 (85.00%)	74 (87.05%)
Gentamicin	13 (33.3 3%)	18 (27.27%)	6 (30.00%)	23 (27.05%)
Netilmicin	11 (28.20%)	14 (21.21%)	5 (25.00%)	21 (24.70%)
Amikacin	8 (20.51%)	10 (15.15%)	3 (15.00%)	12 (14.11%)
Co-trimoxazole	34 (87.17%)	41 (62.12%)	15 (75.00%)	59 (69.41%)
Nitrofurantoin	15 (38.46%)	15 (22.72%)	6 (30.00%)	22 (25.88%)

(from 17% to 70%) (Chandel et al., 2011; Sharma et al., 2008; Wadekar et al., 2013). In 2004, a study in Bangladesh showed that a high prevalence (43.2%) of ESBL-producing *E. coli* was found in an Urban Hospital in Dhaka that was relatively higher than this study. The present study correlates with the previous studies (Nasir et al., 2015).

It has been analyzed that the prevalence of ESBLs among the clinical isolates varies from country to country and institution to institution within the same country. This might be due to the judicious usage of cephalosporins and the adoption of appropriate infection-control measures in our country.

In the present study, AmpC production was found to be 19.04%. In various other studies, the AmpC production rate varied from 8% to 50%. Since multidrug-resistant strains were used in the study, a moderately higher prevalence of AmpC-producing organisms was observed here. Different geographic areas and sample variations can also be a cause (Bandekar et al., 2011; Nagarathnamma & Rudresh, 2011; Polsfuss et al., 2011; Rattan et al., 2005; Sasirekha, 2013; Singh & Khunti, 2020; Taneja et al., 2012). However, the rate of AmpC β-lactamase production (19.04%) was relatively higher and lower than the various reports of the neighboring country, India (Hemalatha et al., 2007; Patel et al., 2010; Rattan et al., 2005; Sharma et al., 2008).

The current study was done to understand the prevalence of ESBL and AmpC-producing *E. coli,* and *K. pneumoniae* which varied from one geographical region to another (Govindaswamy et al., 2018).

Most of the typeable *E. coli,* and *K. pneumoniae* isolates possessed two or more ESBL genes (53.83%) compared with similar studies (Govindaswamy et al., 2018). Overall, the commonest genotype was *bla*CTX-M-group genes (84.61%, n=39) followed by *bla*TEM (64.10%, n=39), *bla*OXA (46.15%, n=39) and *bla*SHV (25.64%, n=39) which is similar to other studies (Goyal et al., 2009; Manoharan et al., 2012; Parveen et al., 2011). It was also found that all clinical isolates were positive for CTX-M genes (Mugnaioli et al., 2006; Prelog et al., 2008). A high prevalence of the CTX-M group in ESBL-producing *E. coli* isolated from household tap water and pigeons was observed in the previous studies performed in Bangladesh (Hasan et al., 2014; Talukdar et al., 2013).

In the case of AmpC β-lactamases, the *bla*FOX gene was predominant 9 (45.00%) followed by *bla*CMY 5 (20.00%) and *bla*DHA 3 (15.00%). A similar study done by Manoharan *et al.* also showed the *bla*FOXM (43.7%) gene to be commonest among *E. coli* isolates (Manoharan et al., 2012). Similar other studies related to AmpC β-lactamases were reported with the *bla*FOX gene as the predominant (21.9%) one followed by the *bla*CMY group gene (9.19%) (Govindaswamy et al., 2018).

Thus, the combination of phenotypic and molecular identification methods could serve as the best detection method of ESBL and AmpC-mediated resistance in diagnostic laboratories. Several phenotypic and genotypic tests have been proposed for the detection of ESBL and AmpC production. However, phenotypic methods are less expensive and easy to perform and interpret which include screening and confirmatory tests (Weinstein et al., 2018).

The cefoxitin disk used for screening of AmpC β-lactamases has good sensitivity and negative predictive value making it a reliable marker for AmpC production compared to the molecular detection method (PCR). The PCR method used in our study was an important technique for the identification of plasmid-mediated AmpC β-lactamase genes in *E. coli* and *K. pneumonia* (Govindaswamy et al., 2018).

Multidrug-resistant strains are expected to be more common among organisms harboring genes for ESBL and AmpC β-lactamases (Bradford, 2001). This study also reveals that the incidence of multidrug-resistant strains is higher in ESBL and AmpC β-lactamase producers than in non-ESBL and non-AmpC producers. It is evident from Table 5 that most of the ESBLs-producing strains were resistant to 3GC and 4GC. High resistance to other antibiotics such as cotrimoxazole, gentamicin, netilmicin, amikacin, ciprofloxacin, levofloxacin, doxycycline, and tetracycline was also observed in this study, which implied that ESBL and AmpC beta-lactamase-producing organisms are multidrug-resistant. Genes that code for ESBL and AmpC beta-lactamase are also linked to other resistance genes (Iftikhar & Abdus, 2002).

In this study, 95-100% of ESBL-producing organisms were sensitive to imipenem and meropenem. However, one crucial finding of his preliminary study was the prevalence of meropenem resistance among ESBL organisms (5%). Although the literature on carbapenem-resistant ESBL producers is limited, available regional studies demonstrate substantially lower rates of carbapenem resistance. No carbapenem resistance has been documented in India, Bangladesh, and Pakistan (Alipourfard & Nili, 1970; Khan et al., 2008; Shaikh et al., 2015). The carbapenem (imipenem, meropenem) is still the first choice of treatment for serious infections with ESBL-producing *E. coli* and *K. pneumoniae.* It has been reported that more than 98% of the ESBL-producing *E. coli* and *K. pneumonia* are still susceptible to these drugs (Rahman et al., 2004).

However, all the ESBL and AmpC-producing isolates were 95-100% sensitive to Imipenem, thereby repeatedly making the continued efficacy of carbapenems as the first-line agents for the treatment of infections caused by *Enterobacteriaceae* with ESBL and AmpC beta-lactamases. However, the use of third-generation cephalosporins should be limited for the prevention of ESBLs and AmpC resistance. This may reduce the indiscriminate and inappropriate use of antibiotics in Bangladesh where these drugs

are often sold over the counter without a physician's prescription (Rahman et al., 2004). All ESBL-producing *Escherichia coli* isolates were susceptible to carbapenems which indicates the functionality of treatment of patients infected with multidrug-resistant ESBL-producing bacteria. In the current study, 92.85% of *Escherichia coli* and *Klebsiella pneumoniae* with ESBL were sensitive to cephamycin (Cefoxitin).

However, all the ESBL and AmpC-producing isolates were sensitive to imipenem, indicating the potential for continued efficacy of carbapenems as the first-line agents for the treatment of organisms producing ESBL and AmpC β-lactamases. A clinical microbiology laboratory needs to implement one or more methods to detect ESBLs (Bradford, 2001). Early detection and prompt contamination can limit the spread of these multidrug-resistant pathogens (Medeiros, 1993).

Author Contributions

SB, MA, AI, and HR conceptualized and designed the study. HR and MA prepared the protocol, did the experiments, collected data and drafted the manuscript. HR, MA, and SB analyzed and interpreted the results. HR, MA, and SB did meticulous revision of the manuscript and all authors approved the final version of the manuscript.

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Competing financial interests

The author has no conflict of interest.

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