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Detection of *bla*$_{CTX-M}$ Gene Variants among Multidrug Resistant *Klebsiella Species* and *Escherichia coli* from Clinical Isolates at a Tertiary Care Hospital in Bangladesh

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Abstract

Background: There are more than 10 families of genes associated with ESBL, among them the highest number of variants corresponds to the CTX-M family. Gene encoding CTX-M *(bla*$_{CTX-M}$*)* has several allelic variants, which has different geographical distributions. **Objective:** The aim of the study was to detect distribution of *bla*$_{CTX-M}$ gene among multidrug resistant Escherichia coli and Klebsiella species along with it's subtypes among different clinical samples. **Methodology:** Isolation and identification of *Escherichia coli* and *Klebsiella species* was done from clinical isolates sent for culture and sensitivity test at Department of Microbiology and Immunology in Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh by using Standard Operating Procedure. Antibiotic susceptibility testing was done by Kirby Bauer disk diffusion method. Then multidrug resistant *Escherichia coli* and *Klebsiella species* were identified and phenotypic detection of ESBL was done by Double Disc Synergy Test. After that, *bla*$_{CTX-M}$ gene among the multidrug resistant organisms was identified using polymerase chain reaction (PCR). Then variants of *bla*$_{CTX-M}$ gene were identified by Sanger sequencing. **Results:** In this study, total 60 multidrug resistant laboratory isolates were collected and most of the multidrug resistant organisms were isolated from urine 30(50%), followed by wound swab 11(18.3%), sputum 10(16.7%), blood 7(11.7%) and pus 2(3.3%). Amoxicillin, cefuroxime, cefotaxime and ceftriaxone were resistant in all isolates. No resistance was found in meropenem. Twenty-three(38.3%) isolates were positive in phenotypic ESBL confirmatory test by DDST. But PCR assay showed 50(83.3%) isolates have *bla*$_{CTX-M}$ gene. Sanger sequencing of the *bla*$_{CTX-M}$ positive isolates showed that most common variant of CTX-M is CTX-M-15, which is 39(78.0%), followed by CTX-M-14, 6(12.0%) and CTX-M-3, 5(10.0%) in number. **Conclusion:** In conclusion, high prevalence of *bla*$_{CTX-M}$ gene was found among MDR *Escherichia coli* and *Klebsiella species* with different variations.

Keywords: Multidrug Resistant; *Klebsiella*; *Escherichia coli*; *bla*$_{CTX-M}$ Gene Variants

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Introduction

Multidrug resistant (MDR) organisms are the organisms which are non-susceptible to at least one antimicrobial in three or more classes, based on in vitro antibiotic susceptibility testing[1]. The rate of MDR pathogens in Bangladesh climbed significantly by two-fold between 2016 and 2018, reaching a peak of roughly 62.0% in 2019 and in 2015, 30.0% to 33.0% of MDR pathogens were identified in Bangladesh[2].

Bacterial antimicrobial resistance has many causes and mechanisms. β-lactamases are by far the most significant enzyme mediated resistance mechanisms among Gram-negative bacilli[3-4]. When it comes to *Escherichia coli* and *Klebsiella species* in particular, it is the most significant pathway for cephalosporin resistance in the Enterobacteriaceae family. As

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molecular techniques have become more widely used, more of these enzymes have been identified, each with a unique amino acid sequence and hydrolytic activity against β-lactam antibiotics[4]. Among the β-lactamases, Extended-spectrum β-lactamase is one of the major mechanisms of drug resistance.

Extended spectrum β-lactamases confer resistance to penicillin, broad-spectrum cephalosporins with an oxyimino side chain (cefotaxime, ceftriaxone and ceftazidime) and the oxyimino-monobactam aztreonam, but can be inhibited by β-lactamase inhibitors as sulbactam, clavulanate and tazobactam[5]. In a clinical isolate of *Klebsiella ozaenae* from Germany, SHV-2, the first ESBL, was discovered. Over ten families, including CTX-M, SHV, TEM, PER, VEB, BES, GES, TLA, SFO, and OXA, have been identified as being connected to ESBLs so far. Among them, the majority of the variations worldwide in last few years correspond to CTX-M family[6].

Currently, class A ESBLs from the CTX-M group are the most prevalent ESBLs everywhere, particularly in developing countries[7]. The CTX-M-type β-lactamases belong in a quite heterogeneous lineage of molecular class-A active site β-lactamases, which includes more than 160 allelic variants clustered into at least six sub-lineages or groups (CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, CTX-M-25 and KLUC). Each group has a number of minor allelic variations that differ from one another by one or a few substitutions of an amino acid[8]. Since then, quite pronounced variations have been seen throughout the world, especially in respect to CTX-M family.

Highly prevalent ESBL gene in Enterobacteriaceae is *bla*$_{CTX-M}$, notably in *Escherichia coli* and *Klebsiella pneumonia*. According to a study conducted in London, strains that have *bla*$_{CTX-M}$ gene make up 1.7% of all ESBL-producing strains, which is higher than other ESBL-producing strains (0.6%) and high-level AmpC-producing strains (0.4%) among 1215 isolates of *Escherichia coli* and *Klebsiella isolates*. In particular, the CTX-M producing strains made up 50.9% and 81.9%, respectively, of the resistant isolates of *Escherichia coli* and *Klebsiella* species[9]. Although *bla*$_{CTX-M}$ type ESBLs have been found in a number of Gram-negative bacteria, the main clinical burden is borne by Escherichia coli and Klebsiella speciesthat produce CTX-M most commonly in nosocomial situations[10].

There aren't many molecular investigations in Bangladesh that focus on *bla*$_{CTX-M}$ variations. One study conducted by icddr,b shows th*a*t bla$_{CTX-M-1}$, *bla*$_{CTX-M-2}$ and *bla*$_{CTX-M-9}$ clusters were frequently found in the isolates. The most frequent one encountered was *bla*$_{CTX-M-1}$, which was found in 91%, then *bla*$_{CTX-M-9}$ in 11.9% and *bla*$_{CTX-M-2}$ in 4.5% were found in the study isolates[11]. A quick, simple to use and effective ESBL detection approach is still a major challenge due to its high prevalence and imposing clinical challenge, especially in developing nations with few resources. In general, there are two ways to find ESBLs. The double-disk synergy test (DDST)-a phenotypic approach and another is molecular methods. ESBL is phenotypically detected in most of the laboratory settings, which does not reflect the actual situation with regard to ESBL status. Only 57.8% of *Escherichia coli* and 53.42% of *Klebsiella isolates* tested positive for ESBL production using the CLSI phenotypic confirmatory test, while 88.67% of cases tested positive for *bla*$_{CTX-M12}$. Genetic detection of ESBLs is a useful tool because it is independent of gene expression and relatively quick in comparison to results from susceptibility testing and culture. Many molecular methods for *bla*$_{CTX-M}$ *ESBL* detection have been published. The simplest and most accurate way to determine whether an ESBL gene is present is using PCR using oligonucleotide primers that are specific for the *bla*$_{CTX-M}$ gene[12]. Also, there are several molecular methods for analysis of the CTX-M gene like DNA-DNA hybridization, DNA fingerprinting, and DNA sequencing. Among them, sequencing determines the DNA sequence of a targeted bacterial gene in a single sequence run, and from these data, information on variants, resistance and virulence is obtained, which is useful for outbreak investigation[13].

In Bangladesh, antibiotic resistant and multidrug-resistant microorganisms are major health care burden. Hospital patients are a high-risk population and *bla*$_{CTX-M}$ ESBLs are one of the major culprits causing these infections. In this study, multidrug resistant *Escherichia coli* and *Klebsiella* isolates were studied from patients admitted to BSMMU for the dissemination of *bla*$_{CTX-M}$ ESBL-encoding gene because there is a lack of information on the molecular characterization of ESBL generating organisms.

Methodology

Study Settings and Population: Study Design & Settings: This cross-sectional study was conducted from September 2022 to August 2023. Samples were collected and laboratory works were performed in the Department of Microbiology & Immunology,

Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh. Multidrug resistant Escherichia coli and Klebsiella isolated in the microbiology laboratory of BSMMU were included in this study. Isolates of Escherichia coli and Klebsiella from different samples which were multidrug resistant detected by Kirby-Bauer disc diffusion method. Calculated sample size was 47. However, 60 isolates of multidrug resistant Escherichia coli and Klebsiella were studied. Grossly contaminated samples were excluded.

Data Collection: A structured data collection sheet was used for demographic data for each isolate obtained from laboratory record book. The data collection sheet included the demographic information such as name, age, sex, and laboratory data information such as type of samples, results of antimicrobial sensitivity tests, results of molecular test (conventional PCR) and results of Sanger Sequencing. All the demographic and laboratory data, samples collection date was recorded and stored in a password protected Excel file.

Isolation of Organism & Antimicrobial Susceptibility Testing: Escherichia coli and Klebsiella species were identified from different laboratory samples by Gram staining, colony morphology on MacConkey agar media and blood agar media and were confirmed by using conventional biochemical tests (catalase test, oxidase test, carbohydrate utilization test, urease production, indole test, motility test, citrate utilization test, gas production). Antimicrobial susceptibility test was done by Kirby Bauer disc diffusion method. Antibiotic discs were collected from BioMaxima, Poland and zone of inhibition were interpreted as per recommendation of the Clinical Laboratory Standard Institute guideline. Escherichia coli ATCC 25922 was used as control strain to assess the performance of the method.

Phenotypic detection of ESBL producers: Double disc synergy test (DDST) was performed on Mueller Hinton agar media. Escherichia coli ATCC 25922 was used as control strain to assess the performance of the method.

Molecular Study for CTX-M Gene Detection: Molecular analysis was done by conventional polymerase chain reaction (PCR). PCR was carried out with the specific primer to determine CTX-M gene from 60 *Escherichia coli* and *Klebsiella isolates*. Two colonies of overnight growth of *Escherichia coli* and *Klebsiella* on MacConkey agar were used for DNA extraction. The extracted DNA was stored at -20 C for further analysis. The concentration of DNA was measured by spectrophotometric assay performed using a Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) according to manufacturer's instructions.

Concentration Calculation: A modification of the Beer-Lambert equation is used to
calculate sample concentrations. Nucleic acid sample concentrations were based on the absorbance at 260 nm, the selected analysis constant and a baseline correction. Concentrations determined by absorbance measurements with spectrophotometer are reported in terms of mass units (ng/Ml). Primer of conventional PCR for CTX-M was purchased from Orbit Trade, Japan.

Gene Sequencing: 50 samples which gave clear concise DNA bands at targeted base pair level without smearing and multiple bands were selected for sequencing. As Microbiology department of BSMMU lacks the setup for DNA sequencing, this procedure was carried out in DNA Solutions Ltd., Shaymoli, Dhaka, Bangladesh. The PCR products were carried in the PCR tube taken in a Cold box filled with frozen ice packs. Sequencing was determined from final PCR product by Sanger dideoxy method. First the PCR products were purified by enzymatic cleanup of amplified PCR product where excess primers and nucleotides were hydrolyzed in a single step. Then cycle sequencing PCR was done. After that second PCR products were purified using magnetic beads. Finally capillary electrophoresis was done by Genetic Analyzer.

Sequencing Data Analysis: After having results of Sanger sequencing in AB1 format through email, the data were analyzed using Chromas and Mega editing software. Sequences from 50 samples were edited by Chromas software. The sequences were converted to FASTA format in Chromas software. Reverse sequences were converted to complementary sequences and combined to complete the gene sequence. Sequence homology was determined using the NCBI nucleotide BLAST program. Multiple sequence alignment was conducted in MEGA ver. 11 program using the ClustalW Multiple Alignment algorithm. Then variant of CTX-M was determined with the help of Multiple Sequence Alignment.

Phylogenetic tree construction: By using the BLAST (Basic Local Alignment SearchTool), available at GenBank (http:/www.ncbi.nlm.nih. gov. blast/), FASTA sequence of different CTX-M variants from different countries were collected. Phylogenetic

dendrogram was constructed by maximum-likelihood method with MEGA ver. 11 program.

Statistical Analysis: All the data were rechecked, coded and analyzed by using IBM SPSS statistics processor (version-27). Descriptive analysis of all relevant variables was done by using frequency, percentage, table and figure. To see the association Chi-square test was done. P value of <0.05 was considered as statistically significant.

Ethical Consideration: The study was ethically approved by Institutional Review Board (IRB) of Bangabandhu Sheikh Mujib Medical University. All procedures of the present study were carried out in accordance with the principles for human investigations (i.e., Helsinki Declaration 2013) and also with the ethical guidelines of the Institutional research ethics. 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 the data collection periods. All data were collected anonymously and were analyzed using the coding system.

Results

In this study, total sixty multidrug resistant laboratory isolates of Escherichia coli and Klebsiella spp. were collected from the microbiology laboratory, BSMMU. Out of sixty isolates, 28(46.7%) were Escherichia coli and 32(53.3%) were Klebsiella. Among twenty-eight Escherichia coli isolates, 18(64.3%) were isolated from urine, 5(17.9%) from wound swab, 3(10.7%) from blood and 2(7.1%) from pus. Out of thirty-two Klebsiella isolates, 12(37.5%) were isolated from urine, 6(18.8%) from wound swab, 10(31.3%) from sputum and 4(12.5%) from blood (Table 1).

Table 1: Distribution of Multidrug Resistant *Escherichia coli* and *Klebsiella species* in different Laboratory

Sample Type	Culture	
	***E. coli* (N=28)**	***Klebsiella* (n=32)**
Urine (n=30)	18(64.4%)	12(37.5%)
Wound swab (n=11)	5(17.9%)	6(18.8%)
Sputum (n=10)	0(0.0%)	10(31.3%)
Blood (n=7)	3(10.7%)	4(12.5%)
Pus (n=2)	2(7.1%)	0(0.0%)

Among 60 multidrug resistant isolates, 60(100%) were resistant to amoxicillin, ciprofloxacin, nalidixic acid, cefotaxime, cefuroxime and ceftriaxone followed by 41 (68.3%) isolates were resistant to cotrimoxazole, 33(55%) were resistant to gentamicin, 31(51.7%) were resistant to amikacin, 58(96.7%) were resistant to ceftazidime, cefixime and aztreonam and 46(76.7%) were resistant to tazobactam-piperacillin. Present study shows that among 28 multidrug resistant *Escherichia coli*, 9 (32.1%) are phenotypically ESBL positive. Among 32 multidrug resistant *Klebsiella*, 14 (43.8%) are phenotypically ESBL positive (Table 2).

Table 2: Antimicrobial Resistances Pattern of Multidrug Resistant Escherichia coli and Klebsiella species (n=60)

Antimicrobial Drug	***E. coli* n=28**	***Klebsiella spp.* n=32**
Amoxicillin	28(100%)	32(100%)
Cefotaxime	28(100%)	32(100%)
Ceftazidime	27(96.4%)	31(96.9%)
Cefuroxime	28(100%)	32(100%)
Ceftriaxone	28(100%)	32(100%)
Cefixim	27(96.4%)	31(96.9%)
Aztreonam	26(93%)	32(100%)
Gentamicin	15(53.6%)	18(56.3%)
Amikacin	14(50%)	17(53.1%)
Netilmicin	14(50%)	17(53.1%)
Co-trimoxazole	18(64.3%)	23(72%)
Ciprofloxacin	28(100%)	32(100%)
Nalidixic Acid	28(100%)	32(100%)
Meropenem	0(0)	0(0)
Piperacillin-Tazobactam	23(82%)	23(72%)

Among 60 multidrug resistant Escherichia coli and klebsiella spp., 50(83%) isolates were positive and 10(17%) isolates were negative for *bla*$_{CTX-M}$ gene detected by PCR method (Figure I).

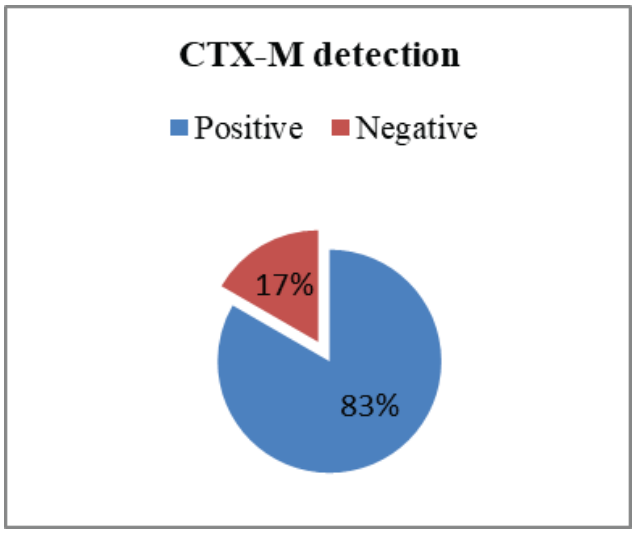

Figure I: *bla*$_{CTX-M}$ Positive *Escherichia coli* and *Klebsiella* in 60 isolates

Table 3 shows by DDST method 23(38.3%) *Escherichia coli* and *Klebsiella* isolates were positive for ESBL phenotypically and *bla$_{CTX-M}$* gene was positive in 50(83.3%) isolates.

Table 3: Rate of ESBL Positivity of Isolated Organisms by DDST and PCR Method (n=60)

Bacteria	Total	DDST Method	PCR method	P value
		ESBL +ve	CTX-M gene +ve	
E. coli	28	9(32.1%)	22(78.6%)	0.014
Klebsiella	32	14(43.8%)	28(87.5%)	
Total	60	23(38.3%)	50(83.3%)	

Table 4 shows that among 50 *bla*$_{CTX-M}$ positive *Escherichia coli* and *Klebsiella* isolates, 39(78%) were bla$_{CTX-M-15}$, 6(12%) were *bla*$_{CTX-M-14}$ and 5(10%) were *bla*$_{CTX-M-3}$ variants.The nucleotide sequence was identical to coding regions of *bla*$_{CTX-M-15}$ genes from GenBank accession no. AY013478, *bla*$_{CTX-M-14}$ genes from GenBank accession no.KT459694 and *bla*$_{CTX-M-3}$ genes from GenBank accession no. EF077620.

Table 4: Distribution of bla$_{CTX-M}$ Gene Variants in Different Isolates by Sanger Sequencing (n=50)

Bacteria	CTX-M Gene Variants		
	CTX-M-15(%)	CTX-M-14(%)	CTX-M-3(%)
E. coli	18(81.8%)	2(9.1%)	2(9.1%)
Klebsiella	21(75%)	4(14.3%)	3(10.7%)
Total	39(78%)	6(12%)	5(10%)

The unrooted tree was constructed using the maximum likelihood method in MEGA 11.0. Scale bar shows the number of nucleotide substitutions per site. The bar 0.2 represent the changes per site. The accession numbers of 4 *bla*$_{CTX-M-15}$ from different countries were recorded (Figure II).

The unrooted tree was constructed using the maximum likelihood method in MEGA 11.0. Scale bar shows the number of nucleotide substitutions per site. The bar 0.2 represent the changes per site.

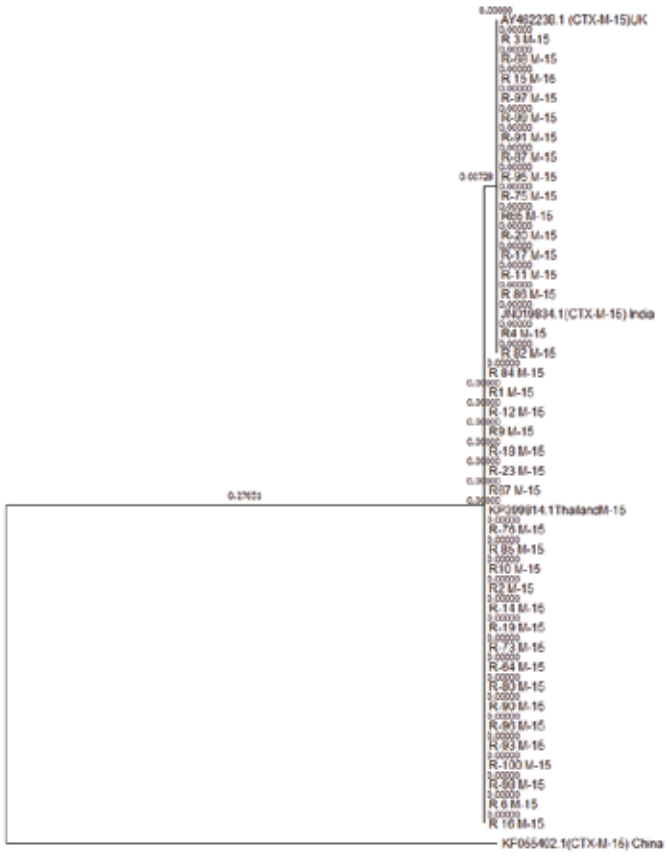

Figure II: Phylogenetic Tree Based on 39 *bla*$_{CTX-M-15}$ Sequences (544 bp)

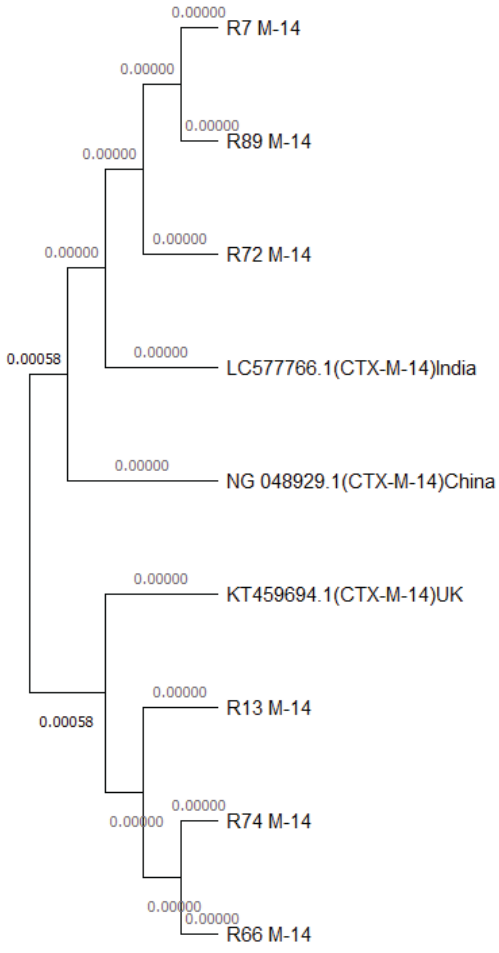

Figure III: Phylogenetic Tree Based on 6 *bla$_{CTX-M-14}$* Sequences (544 bp)

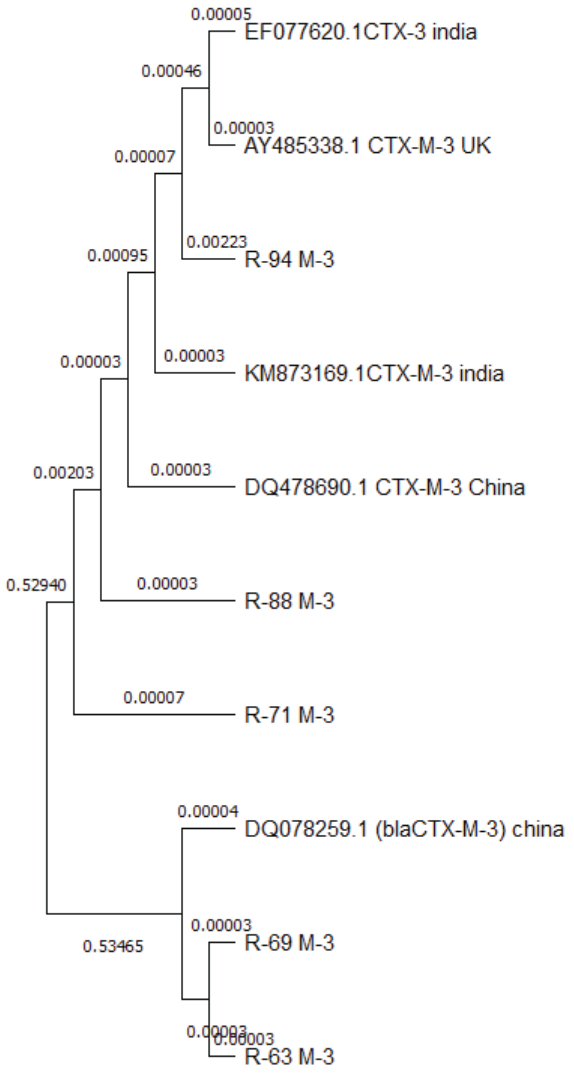

Figure IV: Phylogenetic Tree Based on 5 *bla*$_{CTX-M-3}$ Sequences (544 bp)

The accession numbers of 3 *bla*$_{CTX-M-14}$ from different countries are shown in the figure-III.

The unrooted tree was constructed using the maximum likelihood method in MEGA 11.0. Scale bar shows the number of nucleotide substitutions per site. The bar 0.2 represent the changes per site. The accession numbers of 5 *bla*$_{CTX-M-3}$ from different countries were analyzed (Figure IV).

Discussion

In this study, total 60 multidrug resistant laboratory isolates were collected from the microbiology laboratory, BSMMU. Among them, 28(46.7%) were *Escherichia coli* and 32(53.3%) were *Klebsiella* species. Resistance was observed with commonly used antimicrobials. Amoxicillin, cefuroxime, cefotaxime and ceftriaxone were resistant in all isolates. Ceftazidime, cefixime and aztreonam were resistant in 96.9% isolates. Ciprofloxacin and Nalidixic acid were 100.0% resistant. Resistance for Gentamicin and Amikacin were 55.0% and 51.7% respectively. No resistance was detected to Meropenem. These findings were similar to a study conducted in Bangladesh[14], where almost all the isolates were resistant to penicillin and cephalosporin and least resistant drug was imipenem (7.3%). Another study[12] conducted in India also showed 100.0% resistance for penicillin, a high resistance for cephalosporin (90.0%) and resistance to Imipenem was (7.0%).

Double Disc Synergy Test (DDST) was done among the isolates for phenotypic detection of ESBL producing organisms. Among 28 isolates of *Escherichia coli*, 32.1% gave positive results for ESBL. Among 32 isolates of *Klebsiella species*, 43.8% were positive for phenotypic ESBL confirmatory test. Overall, 23(38.33%) isolates were positive in phenotypic confirmatory test. The result was lower from the study conducted by Bora et al[12], where the phenotypic ESBL confirmatory found to be positive for only 57.78% for multidrug resistant *Escherichia coli* and 53.4% Multidrug resistant *Klebsiella pneumoniae* isolates. Another study in Bangladesh also showed that phenotypic ESBL positivity rate was 71.0% among multidrug resistant Escherichia coli and *Klebsiella pneumoniae*[15].

Sanger sequencing of the *bla*$_{CTX-M}$ gene positive isolates showed that most common variant of *bla*$_{CTX-M}$ is *bla*$_{CTX-M-15}$, which is 39(78.0%), followed by *bla*$_{CTX-M-14}$, 6(12.0%) and *bla*$_{CTX-M-3}$, 5(10.0%) in number. Among the 28 *Klebsiella isolates*, 21(75.0%) were positive for *bla*$_{CTX-M-15}$ gene, 4(14.3%) were positive for *bla*$_{CTX-M-14}$ gene and 3(10.7%) were positive for *bla*$_{CTX-M-3}$ gene. Among the 22 *Escherichia coli* isolates, 18(81.8%) were *bla*$_{CTX-M-15}$ gene positive, 2(9.1%) *bla*$_{CTX-M-14}$ gene positive and 2(9.1%) *bla*$_{CTX-M-3}$ gene positive. There is a study conducted in Bangladesh by Mazumder et al[7] where 84.0% of ESBL-producing Escherichia coli isolates harbored the *bla*$_{CTX-M-15}$ gene[7].

Another study conducted in icddr,b by Mahmud et al16 in 2022 found that *bla*$_{CTX-M-1}$(91%) and *bla*$_{CTX-M-9}$ (11.9%) in the isolates collected from clinical and environmental samples. As the study conducted by icddr,b included both patient and environmental samples, so it may vary with this study[14]. According to Mazumder et al[7], another study conducted in Bangladesh showed 52.0% *Escherichia coli* were *bla*$_{CTX-M-15}$ positive.

In this study, *bla*$_{CTX-M}$ positive *Escherichia coli* and *Klebsiella pneumoniae* isolates showed high antimicrobial resistance rates, except for meropenem. Positive isolates for *bla*$_{CTX-M-14}$ and *bla*$_{CTX-M-15}$ showed almost similar antimicrobial resistance rates. However, *bla*$_{CTX-M-15}$ positive isolates showed a higher resistance rate of ceftazidime than *bla*$_{CTX-M-14}$ positive isolates (100.0% vs. 66.3%; p=0.001). On the other hand, the gentamicin and amikacin resistance rate were higher in *bla*$_{CTX-M-14}$ positive isolates (83.0% and 66.7% respectively) than in *bla*$_{CTX-M-15}$ positive isolates (51.3% for both). *bla*$_{CTX-M-15}$ positive isolates showed higher resistance rates of piperacillin-tazobactam than *bla*$_{CTX-M-14}$ positive isolates (79% and 66.7% respectively). *bla*$_{CTX-M-15}$ positive isolates are more resistant to co-trimoxazole than *bla*$_{CTX-M-14}$ positive isolates (71.8% and 66.7% respectively). For the other antimicrobial agents used in this study, there were no significant differences of resistance rates between *blaC*$_{TX-M-14}$ and *bla*$_{CTX-M-15}$ positive isolates. No meropenem resistant isolates were identified among *bla*$_{CTX-M}$ producing isolates which is similar to the study conducted by Lina et al[17]. So, overall result shows that *bla*$_{CTX-M-15}$ positive isolates are more resistant to Ceftazidime than *bla*$_{CTX-M-14}$ positive isolates.

According to Zhao and Hu[18], *bla*$_{CTX-M-15}$ and *bla*$_{CTX-M-14}$ are the most common variants detected worldwide in clinically important pathogens, followed by *bla*$_{CTX-M-2}$ and *bla*$_{CTX-M-3}$. A study conducted in India by Siddaramppa et al[19], showed that their all-study isolates were *bla*$_{CTX-M-15}$ positive. In Europe, *bla*$_{CTX-M}$ is also predominant and among them *bla*$_{CTX-M-3}$ and *bla*$_{CTX-M-14}$ are most frequently detected[15].

In Bangladesh, majority of tertiary care hospitals are now facing the problem of treating infections with Multidrug resistant *Escherichia coli* and *Klebsiella species*. In this study, high prevalence of Multidrug resistant *Escherichia coli* and *Klebsiella* isolates in various clinical samples was observed. High prevalence of *bla*$_{CTX-M}$ gene among MDR *Escherichia coli* and *Klebsiella species* with different variations having different ancestors from different part of the world suggests it's capacity to spread throughout the world very rapidly with enhanced antibiotic resistance.

Conclusion

In conclusion, most of the multidrug resistant *Escherichia coli* and *Klebsiella species* were positive for *bla*$_{CTX-M}$ genes whereas few of them showed positive result in DDST for ESBL. So, regular screening and national surveillance characterizing the *bla*$_{CTX-M}$ genes needs to be instituted at different geographical locations and healthcare settings to monitor the transmission and spread of ESBL mediated resistance, as other variants of *bla*$_{CTX-M}$ genes may exist elsewhere in Bangladesh.

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None

Conflict of Interest
All authors declared no conflict of interests.

Financial Disclosure
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Authors' contributions
Mahnaz Tabassum Raisa conceived and designed the study, analyzed the data, interpreted the results, and wrote up the draft manuscript, contributed to the analysis of the data. Sanjida Khondakar Setu helped in data collection. Abu Naser Ibne Sattar critically reviewed and edited the manuscript. Quazi Mehranuddin Ahmed involved in the manuscript review. 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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