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Advances in Applied Science Research, 2017, 8(3):23-31

ISSN : 0976-8610
CODEN (USA): AASRFC

Resistance Genotyping Profile of Extended-Spectrum Beta-Lactamase (ESBLs)-, AmpC Beta-Lactamase (ESBL/AmpC) and Carbapenemase (ESBL/Carbapenemase)-Producing *Escherichia coli* Isolated from Clinical, Food and Environmental Lake Water in Bangladesh

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ABSTRACT

The purpose of this current study was molecular typing of extended-spectrum β-lactamases (ESBLs) encoded by ESBLs genes of 56 Escherichia coli isolated from environment lake water (n=15), food samples (n=20) and from clinical specimens (n=21). The prevalence of CTX-M type ESBL-producing Escherichia coli was 76.5% (39 of 51 Escherichia coli) in contrast 5 Escherichia coli were found as non ESBL by molecular method. Most prevalent ESBLs type was ESBL Ditype containing both blaCTX-M and blaTEM genes (43.13%, 22/51 ESBL Escherichia coli), in addition blaVEB found negative. 1 ESBL Escherichia coli from Environment lake water and 2 ESBL Escherichia coli from clinical sample harbored blaSHV gene as ESBL Tritype (contained TEM, CTX-M and SHV type ESBL) rather ESBL Monotype (only SHV type ESBL) or ESBL Ditype (SHV type and CTX-M or TEM type ESBL). Prevalence of Plasmidic AmpC was more in ESBL Escherichia coli isolated from Food (45.5%, 5/11 ESBL Escherichia coli) than Environment lake water and clinical specimens. Plasmidic AmpC were DHA type, CMY-2 was found negative. blaOXA ESBLs gene as (ESBLM-D) ESBL miscellaneous D-type was confirmed in 6 ESBL Escherichia coli lacking carbepenemase activity, 2 from Environment lake water and 4 from clinical samples, indeed blaNDM was not found. Chromosomal mediated constitutive cefoxitin resistant ESBL Escherichia coli (n=15) and also plasmidic AmpC harboring ESBL Escherichia coli (n=9) showed resistance to Cefoxitin (Amoxy-cephalosphorin) confirmed by AmpC disk test. 100% resistance encountered against Ampcillin, Cloxacillin and Erythromycin and most sensitive to imipenam except 3 clinical ESBL Escherichia coli. Almost all Escherichia coli showed resistance against cephalosporin group antibiotics more precisely against third generation cephalosporin group antibiotics (e.g. Ceftazidime and Ceftrioxone). Furthermore, ESBL genotyping revealed that Escherichia coli strains producing multiple β-lactamases and co-resistance may be a common phenomenon in Bangladesh.

Keywords: Extended-spectrum β-lactamases, ESBL monotype, ESBL ditype, ESBL tritype

INTRODUCTION

Escherichia coli, Gram-negative bacteria that frequently found in the intestinal tract of animals and humans as normal commensals flora, though sometimes attributed as important intestinal and extra intestinal pathogens while causing a spectrum of illnesses ranging from self-limiting gastrointestinal infections to bacteremia [1]. β-lactam antibiotics are expansively used as both human and veterinary medicine for the treatment of these infections caused by human and animal pathogenic *Escherichia coli* [2,3]. Penicillins, cephalosporins, carbapenems, cephamycins, monobactams and β-lactamase inhibitors are six different groups of β-lactam antibiotics which turn out to be resistant against *Escherichia coli* due to production of β-lactamases. These β-lactamases termed as extended-spectrum β-lactamases (ESBLs) encoded by ESBLs genes are chromosomal or plasmids mediated and nullify β-lactams activity by hydrolyzing the

four membered β-lactam rings. ESBLs are categorized into four classes (classes A, B, C and D) on the based on their primary structure [4,5]. Moreover, based on with hydrolytic activity against extended-spectrum cephalosporins and/or carbapenems of acquired b-lactamases could be assigned into three categories, class A ESBLs ($ESBL_A$), miscellaneous ESBLs ($ESBL_M$) and ESBLs with hydrolytic activity against carbapenems ($ESBL_{CARBA}$). $ESBL_M$ category further subdivided into two categories termed as $ESBL_{M-C}$ (plasmid-mediated AmpC; class C) and $ESBL_{M-D}$ (OXA-ESBLs; class D). High prevalent $ESBL_A$ are CTX-M, TEM-ESBLs, SHV-ESBLs and VEB whereas $ESBL_{M-C}$ (Plasmid mediated AmpC) are CMY-2 and DHA, moreover NDM-1 (New Delhi Metallozyme) and OXAcarbapenemases with carbapenem hydrolytic activity respectively placed into $ESBL_{CARBA-A}$ and $ESBL_{CARBA-D}$ [6]. Although ESBL phenotypes have been reported from Bangladesh, there is not much information on their molecular types. Hence the present study was undertaken to characterize the Beta-lactamases in multidrug resistant clinical, environmental and food isolates of Enterobacteriaceae by molecular techniques. The proposed current study is designed to characterize isolated ESBLs *Escherichia coli* from Dhaka city Lake water as environment niche, clinical and food samples in molecular level and ESBL genotypes have been compared among those isolates.

MATERIALS AND METHODS

10 retailer juices, 5 poultry and 5 cattle meat were collected from local vendor for detection of *Escherichia coli*. Presence of Escherichia coli was confirmed by 3 tubes (Most Probable Number) MPN method followed by sub culturing into EMB (Eosine Methylene Blue) (Oxoid, Unipath Ltd., Basingstoke, Hampshire, UK). Metallic green sheen characteristics colonies are confirmed as *Escherichia coli* and further confirmed by biochemical reaction in Kligler Iron Agar (KIA), Citrate utilization test and Motility Indole Urease (MIU). 15 *E. coli* were further characterized from previous study for the presence of ESBL genes isolated from Hatirjhil lake water, recreational lake water, situated in the heart of Dhaka city [7]. Meanwhile also 21 *Escherichia coli* were collected isolated from UTI patient's urine (n=12) and from diarrheagenic patient's stool samples (n=9) with regardless patient history from Dhaka Central Hospital Lab. Midstream urine was collected for avoiding contamination and 1 µL urine was cultured into Blood agar media and MacKonkey agar. Stool samples are diluted and cultured into MacKonkey agar. Further sub-cultured directly into MacKonkey agar (Oxoid, Unipath Ltd., Basingstoke and Hampshire, UK). Pinkish characteristics colonies are further confirmed by biochemical reaction in KIA, Citrate utilization test and MIU.

Antimicrobial susceptibility tests

Susceptibility to antimicrobials was determined by an agar diffusion test using antimicrobial agents impregnated paper discs (Oxoid) as described by the Clinical Laboratory Standards Institute (CLSI) guidelines [8]. The antibiotics used in this study were ampicillin (10 µg), ceftriaxone (30 µg), ciprofloxacin (5 µg), trimethoprim sulfamethoxazole (25 µg), nalidixic acid (30 µg),imipenem (10 µg),erythromycin (15 µg), cefotaxime (30 µg), cefixime (5 µg), aztreonam (30 µg), ceftazidime (30 µg), cefoxitin (30 µg). *Escherichia coli* ATCC 25922 were used as negative control. CLSI breakpoints were used to interpret the results [8]. Isolates that showed resistance or inter mediate susceptibility to cephalosporins were tested for the presence of ESBL by doing double disc synergy test (DDST).

Phenotypic ESBL activity testing by DDST

The test inoculums (0.5 McFarland's turbidity) were spread onto Mueller-Hinton agar (MHA) by using a sterile cotton swab. A disc of augmentin (20 µg amoxycillin+10 µg clavulanate) was placed on the surface of the MHA; then, discs of cefotaxime (30 µg) and ceftazidime (30 µg) were kept 16 to 20 mm apart from the augmentin disc (centre to centre). The plate was incubated at 37°C overnight. The enhancement of the zone of inhibition of the cephalosporin disc towards the clavulanic acid disc was inferred as synergy and the strain was considered as an ESBL producer [9]. Plasmidic AmpC or chromosomally constitutive AmpC was detected by AmpC disk test of those cephalosporin resistant *Escherichia coli* which shown negative synergy by clavulanate as ESBL/AmpC not inhibited by clavulanate.

AmpC disk test

The test is based on use of Tris-EDTA which turns out bacterial cells into permeable, releasing β-lactamases into the external environment. AmpC disks were prepared in-house by applying 20 µl of a 1:1 mixture of saline and 100XTris-EDTA (0.5 M EDTA, Promega Corporation, USA, were used to prepare 100X Tris-EDTA) to sterile blank disks (Oxoid Ltd., Basingstoke, Hampshire, England), allowing the disks to dry, and storing them at 2 to 8°C. Cefoxitin susceptible *Escherichia coli* ATCC 25922 was inoculated with a lawn by a sterile cotton swab on the surface of a Mueller-Hinton agar plate (Oxoid Ltd., Basingstoke, Hampshire, England) according to the standard disk diffusion method [8]. AmpC disk test was performed for Cefoxitin resistant *E. coli* described elsewhere [10].

Molecular detection of $ESBL_A$, $ESBL_{M-C}$, $ESBL_{M-D}$, $ESBL_{CARBA-A}$ and $ESBL_{CARBA-D}$ resistance genes in ESBL *Escherichia coli*

(*bla*TEM, *bla*SHV, *bla*CTX-M, *bla*OXA, *bla*VEB, *bla*DHA, *bla*NDM-1 and *bla*CMY-2) were detected by PCR

(Verity 96 well Thermal Cycler, Applied Biosystem) using reverse and forward primer pairs listed in Table 1. Boiled suspension of bacterial cells was used as DNA template and cycling parameters were as previously described with minor modifications [11-15] (Table 1).

RESULTS

Prevalent ESBL genotype is ESBL ditype consisting blaTEM and blaCTX-M in *E. coli* isolated from environmental lake water and clinical specimens. From Figure 1 Almost 50% clinical ESBL *E. coli* and 40% Environmental lake water ESBL *E. coli* posed ESBL ditype. Yet 40% *E. coli* (n=4) have no *bla* genes in *E. coli* termed non ESBL *E.*

Table 1: PCR primers used in this study

	Oligonucleotide sequence (5' to 3')	PCR conditions	References	Expected size (bp)
TEM-F	ATGAGTATTCAACATTTCCG	1 cycle of 5 min at 96°C; 35 cycles of 1 min at 96°C, 1 min at 58°C, 1 min at 72°C; 1 cycle of 10 min at 72°C	[11]	867
TEM-R	CTGACAGTTACCAATGCTTA			
SHV-F	GGTTATGCGTTATATTCGCC	1 cycle of 5 min at 96°C; 35 cycles of 1 min at 96°C, 1 min at 60°C, 1min at 72°C; 1 cycle of 10 min at 72°C	[11]	867
SHV-R	TTAGCGTTGCCAGTGCTC			
OXA-F	ACACAATACATATCAACTTCGC	1 cycle of 5 min at 96°C; 35 cycles of 1 min at 96°C, 1 min at 60°C, 2 min at 72°C; 1 cycle of 10 min at 72°C	[11]	885
OXA-R	AGTGTGTTTAGAATGGTGATC			
CTX-MU1	ATGTGCAGYACCAGTAARGT	1 cycle of 7 min at 94°C; 35 cycles of 50 s at 94°C, 40 s at 50°C, 1 min at 72°C; 1 cycle of 5 min at 72°C	[12]	593
CTX-MU2	TGGGTRAARTARGTSACCAGA			
DHA-1U	CACACGGAAGGTTAATTCTGA	1 cycle of 5 min at 94°C; 35 cycles of 30 s at 94°C, 45 s at 50°C, 1 min at 72°C; 1 cycle of 8 min at 72°C	[13]	970
DHA-1L	CGGTTATACGGCTGAACCTG			
VEB-1A	CGACTTCCATTTCCCGATGC	1 cycle of 5 min at 96°C; 30 cycles of 1 min at 96°C, 1 min at 55°C, 2 min at 72°C; 1 cycle of 10 min at 72°C	[14]	1014
VEB-1B	GGACTCTGCAACAAATACGC			
NDM-1F	CTTCCAACGGTTTGATCGTC	1 cycle of 5 min at 96°C; 30 cycles of 1 min at 96°C, 1 min at 56°C, 2 min at 72°C; 1 cycle of 10 min at 72°C	[16]	465
NDM-1R	TAGTGCTCAGTGTCGGCATC			
CMY-2F	GACAGCCTCTTTCTCCACA	1 cycle of 5 min at 96°C; 30 cycles of 1 min at 96°C, 1 min at 50°C, 2 min at 72°C; 1 cycle of 10 min at 72°C	[17]	1143
CMY-2R	TGGAACGAAGGCTACGTA			

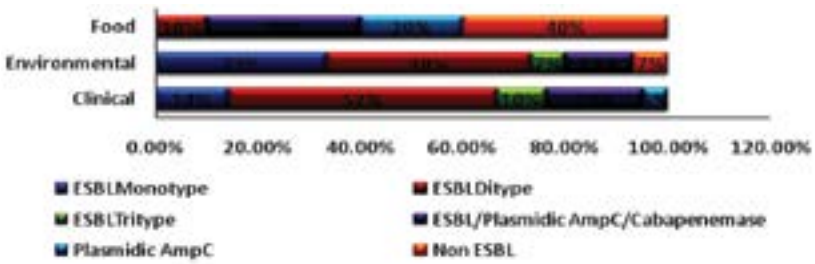

Figure 1: Occurrence of ESBL genotypes E. coli isolated from among food, environmental lake and clinical sources

Table 2: Antibiotic resistance pattern corresponding to ESBL genotypes

	$ESBL_A$			$ESBL_A$+AmpC	$ESBL_{A+}ESBL_{M-D}$	$ESBL_A$+AmpC+$ESBL_{M-D}$	AmpC	
Antibiotic Resistance n (%)	**$ESBL_{Monotype}$ (n=14)**	**$ESBL_{Ditype}$ (n=22)**	**$ESBL_{Tritype}$ (n=3)**	**$ESBL_A$+$ESBL_{M-C}$ (n=3)**	**$ESBL_A$+$ESBL_{M-D}$ (n=3)**	**$ESBL_A$+$ESBL_{M-C}$+$ESBL_{M-D}$(n=3)**	**$ESBL_{M-C}$ (n=3)**	**Non ESBL (n=5)**
Azetronam	7 (50%)	9 (41%)	2 (66%)	0 (0%)	2 (66%)	3 (100%)	1 (33%)	1 (20%)
Cefixime	7 (50%)	12 (55%)	2 (66%)	1 (33%)	3 (100%)	3 (100%)	2 (66%)	2 (40%)
Cotrimoxazole	9 (64%)	14 (64%)	1 (33%)	1 (33%)	3 (100%)	1 (33%)	2 (66%)	1 (20%)
Ampicillin	14 (100%)	22 (100%)	3 (100%)	3 (100%)	3 (100%)	3 (100%)	3 (100%)	5 (100%)
Cefipime	7 (50%)	12 (55%)	2 (66%)	1 (33%)	2 (66%)	3 (100%)	1 (33%)	2 (40%)
Cephalexin	7 (50%)	12 (55%)	2 (66%)	1 (33%)	3 (100%)	3 (100%)	2 (66%)	2 (40%)
Ceftrioxione	7 (50%)	12 (55%)	2 (66%)	1 (33%)	2 (66%)	3 (100%)	1 (33%)	2 (40%)
Ciprofloxacin	11 (79%)	13 (59%)	1 (33%)	2 (66%)	3 (100%)	2 (66%)	2 (66%)	2 (40%)
Cloxacillin	14 (100%)	22 (100%)	3 (100%)	3 (100%)	3 (100%)	3 (100%)	3 (100%)	5 (100%)
Erythromycin	14 (100%)	22 (100%)	3 (100%)	3 (100%)	3 (100%)	3 (100%)	3 (100%)	5 (100%)
Cefoxitin	5 (36%)	7 (32%)	2 (66%)	3 (100%)	3 (100%)	3 (100%)	3 (100%)	1 (20%)
Imipenum	0 (0%)	3 (14%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Nalidixic acid	9 (64%)	18 (82%)	3 (100%)	3 (100%)	3 (100%)	2 (66%)	2 (66%)	3 (60%)
Levofloxacin	7 (50%)	15 (68%)	1 (33%)	1 (33%)	3 (100%)	2 (66%)	1 (33%)	1 (20%)
Cefotaxime	10 (71%)	19 (86%)	3 (100%)	3 (100%)	2 (66%)	3 (100%)	3 (100%)	5 (100%)
Ceftazidime	12 (86%)	20 (91%)	3 (100%)	3 (100%)	3 (100%)	3 (100%)	3 (100%)	4 (80%)

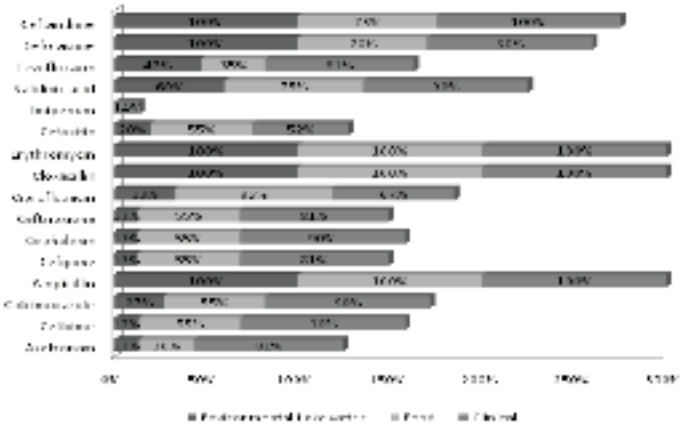

Figure 2: Antibiotic resistance percentage among *E. coli* isolated from environmental lake water, food and from clinical specimens

coli isolated from retailer juices and meanwhile one from Environmental lake water. 36% and less than 20% ESBL monotype (single ESBL carrying gene) *E. coli* attributed consecutively from environment lake water and in clinical ESBL *E. coli*. ESBL monotype genotype was found negative in ESBL *E. coli* isolated from food sample (Table 2).

Ampicillin: β-lactum Antibiotic, Cefipime: 4th Genaration Cephalosporin, Cephalexin: 1st Generation Cephalosporin, Ceftrioxione: 3rd Generation Cephalosporin, Cloxacillin: β-lactum Antibiotic, Cefoxitin: 2nd Generation Cephalosporin, Imipenum: Carbapenem, Cefotaxim: 3rd Generation Cephalosporin, Ceftazidime: 3rd Generation Cephalosporin, Aztronam: Monobactum

ESBL *E. coli* found to be resistant towards diversified groups of β-lactam antibiotics include Penicillin, Cephalosporine, Cephamycin, Monobactum, Amoxy-Cephalosporin and even Carbapenem as harboring $ESBL_A$ genes along with AmpC and $ESBL_{M-D}$ genes (Figure 2).

Isolated *E. coli* showed 100% resistance against ampicillin, cloxacillin and erythromycin. Lowest resistance observed

against imipenem. All isolated ESBL *E. coli* from environment lake water and food samples sensitive to imipenem. 14% resistance showed against imipenem in clinical ESBL *E. coli*. 50% *E. coli* showed resistant to three tested 3rd generation cephalosporin antibiotics (Cefixime, Ceftazidime and Ceftrioxione). Moreover 55.4%, 44.6% and 53.6% resistance shown against 1st, 2nd and 3rd generation cephalosporin consecutively. 30.4% *E. coli* resistance to all cephalosporin including amoxy-cephalosporin resistance. 51 *E. coli* were confirmed molecularly as ESBL *E. coli* among 56 *E. coli* since contained one or more than one β-lactamases genes (Table 3).

Table 3: ESBL genotyping profile

Strain ID	Isolation Source	ESBL Genotypes	ESBL Group [a]	Antibiotic Resistance traits [b]
E7	Environment lake water	bla_{TEM} bla_{CTX-M}	$ESBL_{Ditype}$	AMP,OB,E,CTX,CAZ
E6	Environment lake water	bla_{TEM} bla_{CTX-M} bla_{SHV}	$ESBL_{Tritype}$	AMP,OB,E,NA,LE,CTX,CAZ
E10	Environment lake water	bla_{TEM} bla_{CTX-M}	$ESBL_{Ditype}$	AMP,OB,SXT,E,NA,CTX,CAZ
E11	Environment lake water	bla_{CTX-M}	$ESBL_{Monotype}$	AMP,OB,CIP,E,NA,LE,CTX,CAZ,
E5	Environment lake water	bla_{TEM}	$ESBL_{Monotype}$	SXT, CIP,AMP,OB,E,CX,NA,LE,CTX,CAZ
E1	Environment lake water	bla_{TEM} bla_{CTX-M}	$ESBL_{Ditype}$	AMP,OB,E,CTX,CAZ
E3	Environment lake water	ESBL Negative	Non ESBL	SXT,AMP,OB,E,CTX,CAZ
E5R1	Environment lake water	bla_{CTX-M}	$ESBL_{Monotype}$	AMP,OB,E,CTX,CAZ
E5R2	Environment lake water	bla_{TEM} bla_{CTX-M} bla_{DHA} bla_{OXA}	ESBL+AmpC+$ESBL_{M-D}$	AT,CFM,AMP,CPM,CN,CTR,CIP,OB,E,NA,LE,CTX,CAZ
E1R1	Environment lake water	bla_{CTX-M}	$ESBL_{Monotype}$	AMP,OB,E,CTX,CAZ
E6R3	Environment lake water	bla_{TEM} bla_{CTX-M} bla_{DHA} bla_{OXA}	ESBL+AmpC+$ESBL_{M-D}$	AT,CFM,AMP,CPM,CN,CTR,OB,E,CTX,CAZ
E7R3	Environment lake water	bla_{TEM} bla_{CTX-M}	$ESBL_{Ditype}$	AMP,OB,E,NA,LE,CTX,CAZ
E11R3	Environment lake water	bla_{TEM} bla_{CTX-M}	$ESBL_{Ditype}$	AMP,CIP,OB,E,NA,LE,CTX,CAZ
E2	Environment lake water	bla_{TEM}	$ESBL_{Monotype}$	SXT,CIP,AMP,OB,ENA,LE,CTX,CAZ
E4	Environment lake water	$bla_{TEM}$$bla_{CTX-M}$	$ESBL_{Ditype}$	AMP,OB,E,NA,LE,CTX,CAZ
U1	Urine	bla_{CTX-M}	$ESBL_{Monotype}$	AT,CFM,COT,AMP,CPM,CN,CTR,CIP,OB,E,NA,LE,CTX,CAZ
U2	Urine	bla_{TEM} bla_{SHV} bla_{CTX-M}	$ESBL_{Tritype}$	AT,CFM,COT,AMP,CPM,CN,CTR,CIP,OB,E,CX,NA,LE,CTX,CAZ
U6	Urine	bla_{TEM} bla_{CTX-M}	$ESBL_{Ditype}$	AT,CFM,COT,AMP,CPM,CN,CTR,CIP,OB,E,CX,IMP,NA,LE,CTX,CAZ
U7	Urine	bla_{TEM} bla_{CTX-M}	$ESBL_{Ditype}$	AT,CFM,AMP,CPM,CN,CTR,OB,E,CX,CTX,CAZ
U8	Urine	bla_{TEM} bla_{SHV} bla_{CTX-M}	$ESBL_{Tritype}$	AT,CFM,AMP,CPM,CN,CTR,OB,E,CX,NA,CTX,CAZ
U9	Urine	bla_{CTX-M}	$ESBL_{Monotype}$	AT,CFM,COT,AMP,CPM,CN,CTR,CIP,OB,E,NA,LE,CTX,CAZ
S3	Stool	bla_{TEM} bla_{CTX-M}	$ESBL_{Ditype}$	AT,CFM,COT,AMP,CPM,CN,CTR,CIP,OB,E,CX,IMP,NA,LE,CTX,CAZ
S4	Stool	bla_{TEM} bla_{CTX-M}	$ESBL_{Ditype}$	AT,CFM,COT,AMP,CPM,CN,CTR,OB,E,NA,LE,CTX,CAZ
S5	Stool	bla_{TEM} bla_{CTX-M}	$ESBL_{Ditype}$	AT,CFM,COT,AMP,CPM,CN,CTR,CIP,OB,E,CX,IMP,NA,LE,CTX,CAZ
S10	Stool	bla_{TEM} bla_{CTX-M}	$ESBL_{Ditype}$	COT,AMP,OB,E,NA,LE,CTX,CAZ
U4	Urine	bla_{TEM} bla_{CTX-M}	$ESBL_{Ditype}$	AT,CFM,COT,AMP,CPM,CN,CTR,CIP,OB,E,CX,NA,LE,CTX,CAZ
U5	Urine	bla_{TEM} bla_{CTX-M}	$ESBL_{Ditype}$	CFM,COT,AMP,CPM,CN,CTR,CIP,OB,E,NA,LE,CTX,CAZ
U6"	Urine	bla_{TEM} bla_{CTX-M} bla_{OXA}	ESBL+$ESBL_{M-D}$	AT,CFM,COT,AMP,CPM,CN,CTR,CIP,OB,E,CX,NA,LE,CTX,CAZ

U7"	Urine	bla_{TEM} bla_{CTX-M}	$ESBL_{Ditype}$	AT,CFM,COT,AMP,CPM,CN,CTR,CIP,OB,E,NA,LE,CTX,CAZ
U10	Urine	bla_{TEM} bla_{CTX-M} bla_{OXA}	ESBL+$ESBL_{M-D}$	AT,CFM,COT,AMP,CPM,CN,CTR,CIP,OB,E,NA,LE,CTX,CAZ
U11	Urine	bla_{TEM} bla_{OXA}	ESBL+$ESBL_{M-D}$	CFM,COT,AMP,CN,CIP,OB,E,NA,LE,CAZ
S1	Stool	bla_{TEM} bla_{CTX-M}	$ESBL_{Ditype}$	COT,AMP,OB,E,NA,CAZ
S2	Stool	bla_{CTX-M}	$ESBL_{Monotype}$	AT,CFM,COT,AMP,CPM,CN,CTR,OB,E,NA,AMC,CTX,CAZ
S3"	Stool	bla_{DHA}	AmpC	AT,CFM,COT,AMP,CN,OB,E,CX,LE,AMC,CTX,CAZ
S8	Stool	bla_{CTX-M} bla_{DHA} bla_{OXA}	ESBL+AmpC+ $ESBL_{M-D}$	AT,CFM,COT,AMP,CPM,CN,CTR,CIP,OB,E,CX,IMP,NA,LE,CTX,CAZ
S9	Stool	bla_{TEM} bla_{CTX-M}	$ESBL_{Ditype}$	AT,CFM,COT,AMP,CPM,CN,CTR,CIP,OB,E,CX,IMP,NA,LE,CTX,CAZ
F1	Beef	bla_{TEM}	$ESBL_{Monotype}$	AMP,CIP,OB,E,NA
F2	Beef	bla_{TEM}	$ESBL_{Monotype}$	AMP,CIP,OB,E,NA
F3	Beef	bla_{TEM}	$ESBL_{Monotype}$	AMP,CIP,OB,E,NA
F4	Beef	bla_{TEM}	$ESBL_{Monotype}$	AMP,CIP,OB,E,NA,LE
F5	Beef	bla_{TEM}	$ESBL_{Monotype}$	AMP,CIP,OB,E,NA,LE
P6	Poultry meat	bla_{TEM} bla_{CTX-M}	$ESBL_{Ditype}$	AMP,OB,E,CX,NA
P7	Poultry meat	bla_{TEM} bla_{CTX-M}	$ESBL_{Ditype}$	AMP,OB,E,NA
P8	Poultry meat	bla_{TEM} bla_{CTX-M}	$ESBL_{Ditype}$	COT,AMP,CIP,OB,E,NA
P9	Poultry meat	bla_{TEM} bla_{CTX-M}	$ESBL_{Ditype}$	COT,AMP,CIP,OB,E,NA
P10	Poultry meat	bla_{TEM}	$ESBL_{Monotype}$	AMP,OB,E,CX,NA
J1	Juice	bla_{DHA}	AmpC	AMP,OB,E
J2	Juice	ESBL Negative	Non ESBL	AMP,OB,E
J3	Juice	bla_{TEM} bla_{CTX-M}	$ESBL_{Ditype}$	AMP,OB,E
J4	Juice	ESBL Negative	Non ESBL	AMP,OB,E
J5	Juice	bla_{CTX-M} bla_{DHA}	ESBL+AmpC	AMP,OB,E,CX
J6	Juice	ESBL Negative	Non ESBL	AMP,OB,E
J7	Juice	bla_{CTX-M} bla_{DHA}	ESBL+AmpC	AMP,OB,E,CX
J8	Juice	bla_{CTX-M} bla_{DHA}	ESBL+AmpC	AMP,OB,E,CX
J9	Juice	bla_{DHA}	AmpC	AMP,OB,E,CX
J10	Juice	ESBL Negative	Non ESBL	AMP,OB,E

[b]ESBLmonotype; harbor one β-lactamase (ESBL-A genotype) gene, ESBLditype; harbor two β-lactamase (ESBL-A genotype) genes, ESBLtritype; harbor three β-lactamase (ESBL-A genotype) genes, ESBL+AmpC; contain one ESBL-A or more than one ESBL-A genotypes along with plasmidic AmpC (blaDHA), ESBL+AmpC+$ESBL_{M-D}$; contain simultaneously ESBL-A, AmpC and blaOXA type β-lactamase (lacking carbapenem hydrolytic capability), ESBL+ $ESBL_{M-D}$; contain ESBL-A genotypes and blaOXA type β-lactamase (lacking carbapenem hydrolytic capability). [a]AT (Azetronam), CFM (Cefixime), COT (Cotrimoxazole), AMP (Ampicillin), CPM (Cefipime), CN (Cephalexin), CTR (Ceftrioxione), CIP (Ciprofloxacin), OB (Cloxacillin), E (Erythromycin), CX (Cefoxitin), IMP (Imipenum), NA (Nalidixic acid), LE (Levofloxacin), CTX (Cefotaxim), CAZ (Ceftazidime)

DISCUSSION AND CONCLUSION

ESBL *E. coli* frequently reported from various sources in Bangladesh including environmental surface water, household supply water, poultry farms, coastal line of Bay of Bengal and hospital settings from urine and wound infection [18-28]. Most reported predominant ESBL genotype in this study is CTX-M type in Bangladesh. Co-resistance, harboring multiples ESBLs genes, is very common in isolated *E. coli* in that region. Other than blaCTX-M, most of the ESBL *E. coli* harbored blaTEM isolated from Dhaka city tap water (21). Support our study result that 60% (9 ESBL *E. coli* in 15 Environmental *E. coli*) *E. coli* attributed more than one ESBL-A genes which were termed as ESBL ditype and ESBL tritype in this study. Moreover, two *E. coli* harbored both blaOXA as ESBLM-D and blaDHA as AmpC along with blaCTX-M and blaTEM as ESBL-A leaving resistant against 1st, 2nd, 3rd and 4th generation Cephalosporin and also monobactum.

ESBL *E. coli* frequently associated with Urinary Tract Infection (UTI) and diarrhea world widely since ESBL *E. coli* have reported in an erratic rate most parts of the world. ESBL ditype (TEM, CTX-M) is predominant genotype isolated from urine and stool, 3 ESBL *Escherichia coli* associated with UTI contained blaOXA and no plasmidic AmpC, moreover 2 ESBL *E. coli* had all three type ESBLs (TEM, SHV, CTX-M). Similar multiples ESBL *E. coli* genotypes also isolated from other contemporary studies in Bangladesh (24, 25, 27, 28). blaOXA gene were recurrently detected in ESBL producing uropathogenic and diarrheagenic *Escherichia coli* in southeast of Iran compared to blaPER and blaVEB were less common [29].

Other than four *E. coli* isolated from clinical specimens, two from environmental lake water out of 56 *E. coli* harbored blaOXA though sensitive to imipenem. According to Giske et al. these *E. coli* termed as $ESBL_{M-D}$ (OXA-ESBLs; class D) since lacking catalytic activity against carabapenem. In contrast, three imipenem resistant ESBL *E. coli* isolated from clinical specimens neither positive for blaOXA nor blaNDM, indeed may be contain other type of carbapenemase genes (blaKPC, blaGES not tested in this study) or acquire imipenem resistance due to amalgamation of $ESBL_A$ or $ESBL_M$ beta-lactamase activity or a combination of porin loss/alteration; similar Carbapenem resistance observed in Chilean isolates [30]. These imipenem resistant ESBL *E. coli* are ESBLditype contained blaCTX-M and blaTEM also poised same antibiogram resistant pattern but no plasmidic AmpC though resistant to cefoxitin. Hyperproduced AmpC enzyme along with porin alteration responsible for imipenem resistant nevertheless over expression of AmpC enzymes inevitably required by deregulation of the ampC chromosomal gene for carbapenem resistant trait [31].

Plasmid-encoded AmpC genes belonging to the DHA family were 16%, predominant type currently in Bangladesh ESBL *E. coli* isolated from lake water, food and clinical specimens. Juices and retailer poultry meat contain 35% and lake water have 38% DHA type AmpC *E. coli* similar DHA type predominance was observed in India [32]. In contrast, blaCMY-2 being the predominant type not in Sweden [33] also in line with reports from most other countries [34-37]. Nevertheless, blaCMY-2, acquired plasmidic AmpC was absent in this study whereas Talukdar et al. [21] reported blaCMY-2 in ESBL *E. coli* isolated from household water in Bangladesh. In the total collection, 15 *E. coli* (26.8%) had putative chromosomal AmpC (phenotypic AmpC-test positive, but negative in PCR for plasmid-mediated AmpC) also cefoxitin resistant and were not defined as ESBLM-producers in this study [6].

So, pattern of ESBL genotypes is quite distinguishable among ESBL *E. coli* isolated from food, environmental lake water and clinical settings. blaTEM and blaCTX-M as ESBL-A predominant in clinical ESBL *E. coli*. blaSHV predominant ESBL before, co-harbored with blaTEM and blaCTX-M as ESBL tritype not as ESBL monotype and ESBL ditype, characteristic feature in this study. Indicating blaSHV is replaced by other types of ESBL genotypes. blaDHA as Plasmidic AmpC is predominant in ESBL *E. coli* isolated from food sample. While consumption of ESBL/AmpC *E. coli* contaminated food, serve as ESBL/ampC genes reservoir and share this resistance trait with other bacteria in the gastro-intestinal tract by plasmid-transfer in presence of administered beta-lactam antibiotics [38]. blaDHA as ESBL/AmpC producing *E. coli* also isolated from clinical specimens indicating that ESBL/AmpC harboring *E. coli* in the gastro-intestinal tract can act as a source for infections in other parts of the body, like the urinary tract [39].

In conclusion, our results have revealed that the high prevalence of CTXM-type ESBLs within many *ESBL E. coli* in Bangladesh, moreover coexistence pattern of several bla genes emulate similar co-resistance pattern of ESBL *E. coli* in Asia-Pacific region [40] where plasmid-mediated AmpC β-lactamases such as blaDHA coexisted with ESBL-A (blaTEM, blaCTX-M and blaSHV) less frequent with carbapenemase.

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