Jagannath University Journal of Life and Earth Sciences, **8**(1) pISSN 2414-1402, eISSN 2791-1845

JnUJLES

Research Article

EXTENDED-SPECTRUM-BETA-LACTAMASES PRODUCING *ESCHERICHIA COLI* ISOLATES FROM DHAKA, BANGLADESH: MOLECULAR CHARACTERIZATION AND ANTIOXIDANT SUSCEPTIBILITY

Halima Islam[1], Kohinoor Akhter[1, 3], Shams Uddin[1], Md Jahid Hasan Dewan[1], Zahid Hassan[2], Shafinaz Khan[3], Farhana Afroze[1] and SyedaTasneem Towhid[1*]

[1]*Department of Microbiology, Jagannath University, Dhaka-1100, Bangladesh*

[2]*Department of Genetic Engineering and Biotechnology, Jagannath University, Dhaka-1100, Bangladesh*

[3]*Pathology Department, Dr. Sirajul Islam Medical College, Dhaka-1217, Bangladesh*

Received: 11 September 2022, Accepted: 28 December 2022

ABSTRACT

Resistance against antibiotics among members of Family Enterobacteriaceae is primarily due to the production of extended-spectrum beta-lactamase (ESBL) enzymes. This study presents the characterization and antioxidant susceptibility of ESBL-producing *Escherichia coli (E. coli)* from hospitalized patients in Bangladesh. Twelve specimen of ESBL *E. coli* isolates were obtained from outpatients with active urinary tract infection from hospitals in Dhaka city. Antibiotic susceptibility pattern of these isolates were determined using second and third generation β-lactam antibiotics. Conventional PCR from genomic DNA showed detection of *Lt1* and *Stx2* genes for virulence potential and bla_{CTX-M} gene variant. The effectiveness of Indian Gooseberry (*Phyllanthus emblica*) extract on the isolates used in this investigation was finally determined using droplet assay and tube-dilution method. *E. coli* ATCC strain 25922 was used as a negative control.We found 12 *E. coli* isolates with the ESBL phenotype, meaning they were resistant to ceftriaxone, cefotaxime, ceftazidime, and aztreonam, to a lesser extent ciprofloxacin. 37.5% isolates harbor bla_{CTX-M} genes. We identified presence of labile toxin 1 (*Lt1*) and Shiga-like heat stable toxin (*Stx2*). Undiluted anti-oxidant (i.e. *Phyllanthus emblica*) extract on ESBL *E. coli* isolates *in vitro* and which is significantly less effective compared to the activity of therapeutic antibiotics such as ciprofloxacin, imipenem and cefoxitim. The study indicates that there is a high incidence of ESBL-producing, multidrug-resistant, toxigenic *E. coli* isolates, and about a third contain *bla* gene variants, making them more dangerous. Strong antioxidants have potency to inhibit ESBL *E. coli* in vitro. However the efficacy of *P. emblica in vivo* remains to be explored.

Keywords: *ESBL, Antioxidant, Escherichia coli, Enterobacteriaceae*

Introduction

Extended-spectrum β-lactamases (ESBLs), a class of enzymes that increases resistance to frequently used antibiotics, are produced by a variety of Gram-negative bacteria (Nathisuwan,

[*]**Correspondence:** *towhidst@mib.jnu.ac.bd*

2001). Since infections brought on by these enzyme-producing microbes are linked to higher rates of morbidity and mortality, they are a global public health issue. These enzymes pose a clear and present hazard to the public's health due to their rising prevalence rates over the world and the ever-dwindling availability of antibiotics (Bertrand, 2012). Global reports on the prevalence of ESBL-producing bacteria come from North America, South America, Europe, Africa, and Asia (Mehrgan, 2008; Bertrand, 2012). According to data from the Tigecycline Evaluation and Surveillance Trial (TEST) worldwide surveillance database, Latin American isolates of *K. pneumoniae* produced the most ESBLs, followed by isolates from Asia/Pacific Rim, Europe, and North America (at rates of 44.0%, 22.4%, 13.3%, and 7.5%) (Bush, 2001; Jarlier *et al*., 1988 and Madhavi*et al*., 2021). ESBLs impart resistance to penicillins, cephalosporins, and aztreonam. They are also linked to resistance to other non-penicillin antibiotic families, such as aminoglycosides, fluoroquinolones, trimethoprim-sulfamethoxazole, and combinations of ß-lactam/ß-lactamase inhibitors (Yousefimashouf *et al*., 2013). As a result, multidrug resistance is a common trait among ESBL-producing organisms. The detection of ESBL-producing *E. coli* and the findings of its susceptibility testing are crucial for treating infections brought on by this pathogen as well as for limiting the spread of ESBLs. The main mechanism of bacterial resistance to β-lactam antibiotics, including penicillin and cephalosporins, is the hydrolytic enzymes known as β-lactamases that cleave the β-lactam ring (Guelmbaye, 2022.). These enzymes may be carried on bacterial chromosomes, making them part of the organism itself, or they may be transported via plasmids and have the ability to switch between bacterial populations.

Numerous Gram-negative microbes may be found in clinical settings, and each one is capable of causing a particular kind of antibiotic resistance. There are four main kinds of antibiotic resistance, which can either be innate or acquired:

1. Enzymatic degradation occurs when enzymes hydrolyze or inactivate antibiotic compounds. For instance, the distinctive lactam ring of beta-lactam antibiotics is broken down by a group of enzymes called beta-lactamases, rendering the medicine inert.

2. A change in the binding site occurs when the target where an antibiotic binds and acts to kill bacteria does.

3. The capacity of an antibiotic to enter a bacterial cell is decreased by the loss of porin channels. For *Acinetobacter baumanii*, a Gram-negative bacterium that can cause serious hospital-acquired infections and for which isolates that are resistant to almost all clinically useful antibiotics have been recovered, loss of porin transport channels has recently resulted in clinically significant resistance to imipenem (Miller, 2016).

4. Boosted efflux pumps lessen the antibiotic's ability to stay in the bacterial cell (Webber, 2002).

Due to its antibacterial and antioxidant characteristics, Indian gooseberry has been employed in Ayurvedic treatment for thousands of years. It has a variety of tannins, alkaloids, and phenolic chemicals that can all snuff out a variety of reactive oxygen species (Ruangchakpet, 2007). The

active molecules founded in *Phyllanthus emblica* are gallic acid, ellagic acid, quercetin, chebulinic acid, chebulagic acid, emblicanin A, emblicanin B, punigluconin, pedunculagin, citric acid, ellagotannin, trigallayl glucose, pectin, 1-Ogalloyl-b-D-glucose, 3,6-di-O-galloyl-D-glucose, chebulagic acid, corilagin, 1,6-di-O-galloyl-b-D-glucose, 3 ethylgallic acid and isostrictiniin, kaempferol 3 O-a-L rhamnopyranoside and kaempferol 3 O-a-L rhamnopyranosid (Dhanani, 2014) e. With the help of these powerful active ingredients, *P. emblica* is endowed with the potency to eradicate a wide range of microorganisms, and regular ingestion of the plant gives the user a robust immune system. Aflatoxin B1, a strong toxin, as well as heavy metal toxicity from cadmium, aluminum, cesium chloride, and excessive chromium are all prevented by *P. emblica* from causing DNA damage (Thilakchand, 2013). As a result, the goals and objectives of the current study were to find ESBL genes from our isolates, to check for the presence of virulence factors like labile toxin 1 (*Lt1*) and Shiga Like heat-stable toxin (*Stx1*), and finally to find out how well an extract from the *P. emblica* affected ESBL *Escherichia coli* isolates *in vitro*.

Materials and Methods

Selection of Study Population

All the samples were collected from UTI (Urinary Tract Infection) patients admitted at Sirajul Islam Medical College, Mogbazar, Dhaka 1217. The ethical committee of the medical college provided their approval, to collect first urine sample from patients adhering to biosafety level 2 practices appropriate for clinical sample.

Isolation and Identification

50 mL of urine samples were collected in a universal sterile container were inoculated using an inoculating loop calibrated at a 10 μL volume on cysteine lactose electrolyte deficient (CLED) agar plates (Hi-Media, India). The MacConkey agar plates and blood agar plates were inoculated with additional liquid specimens via an inoculating loop (Product Material Safety Datasheet) (Dortet *et al*. 2015).

Sample Collection and Validation

Since the BD CHROMagar Orientation Medium is a nonselective medium for the isolation, direct identification, differentiation, and enumeration of urinary tract pathogens, all of the samples were sub-cultured on Chrome agar (*E. coli*). The colorless chromogenic conjugate is broken down by the enzyme of the target organism, releasing the chromophore.

Inclusion-exclusion Criteria

Samples that did not produce Mauve colonies on Chrome agar or did not show resistance against fourth-generation β-lactam antibiotics were excluded from the study.

Data Gathering and Sharing Throughout the Healthcare System

ESBL detection techniques: According to the CLSI guidelines from 2012, *E. coli* was first tested phenotypically for the formation of ESBLs before a confirmatory genotyping test was conducted (Kumar, 2014; Aruna, 2012).

Phenotypic Evaluation of ESBL

Four antibiotic (Ceftazidime, aztreonam, cefotaxime, and ceftriaxone) discs from commercial manufacturers (Oxoid, UK) were used, according to CLSI 2012 guidelines, to check ESBL producers from the isolates under study.

Extended spectrum beta-lactamase was defined according to CLSI guidelines (2012):

- Ceftazidime ≤ 22 mm or
- Aztreonam ≤ 27 mm or
- Ceftriaxone ≤ 25 mm or
- Cefotaxime ≤ 27 mm

Double disk diffusion techniques were used for the confirmatory test. Double disk synergy (DDS) is a disk diffusion test in which 30 µg antibiotic disks containing ceftazidime, ceftriaxone, cefotaxime, and aztreonam are positioned on the lawn culture plate of *E. coli* on MHA, 30 mm (center to center) from the amoxicillin/clavulanic acid (20/10 µg) disk. This plate was incubated aerobically at 37°C overnight, and the edge of the antibiotic disks' zone of inhibition was watched for any movement toward the disk containing clavulanate. It is viewed as synergy, proving the existence of an ESBL (CDC Guidelines 2021). We also performed disc diffusion test with ciprofloxacin (5 µg), cefoxitin (5 µg) and imipenem (0.002 µg).

Genomic DNA Extraction and PCR

Log phase culture of *E. coli* isolates was taken from Luria Broth culture, adjusted to 10^6 CFU/ml in micro-centrifuge tubes and centrifuged at 2000 RPM for 1 minute and room temperature. The cell pellet was re-suspended at 600 µL Lysis Buffer and DNA was isolated according to the Phenol: Chloroform method (Di Pietro, 2011.). Final DNA was suspended in TE buffer and the concentration of DNA was measured with a spectrophotometer (Hettich, Germany). DNA samples that were >20 ng/µl with the 260/280 ratio of 1.8 (MultiScan High Spectrophotometer, South Korea) were chosen for PCR.

Table 1. Primers used for PCR to detect common toxin genes in *E. coli* isolates

SI	Primer Name	Type	Sequence	Amplicon Size
1	Lt250	Forward	5’-GGC-GAC-AGA-TTA-TAC-CGT-GC-3’	250bp
2	Lt250	Reverse	5’CGG-TCT-CTA-TAT-TCC-CTG-T-3’	250bp
3	STh6	Forward	5’-TAC-AAG-CAG-GAT-TAC-ACC-AC-3’	510bp
4	STh6	Reverse	5’-TCA-CCT-TTC-CCT-CAG-GAT-3’	510bp

PCR Master Mix was prepared with GoTaq (10×) mastermix, 10 µM of forward primer, 10 µM of reverse primer, 5U/µl of Taq polymerase in a final volume of 20 µl for 2 µl of template DNA. The thermal cycling condition consisted of an initial denaturation of 95^{o} C for 2 minutes, cyclic denaturation of 94^{o}C for 15 seconds, annealing for 30 seconds at 47.35^{o}C, cyclic elongation for 1 minute at 72^{o} C and a final elongation at 72^{o} C for 5 minutes (Joseph *et al*. 2020). The sequences of primers for molecular confirmation of *E. coli* are given in Table 1.

The PCR for Stx2 gene was done using Stx2 primer pair (F 5'-CCATGACAACGGACAGCAGTT-3') and R (5'- CCTGTCAACTGAGCAGCACTTTG-3'). Temperature conditions consisted of an initial 95°C denaturation step for 3min followed by 35 cycles of 95°C for 20 s, 58°C for 40 s, and 72°C for 90 s. The final cycle was followed by a 72°C for 5 min. Amplicon fragments were resolved by agarose gel electrophoresis using 1% (w/v) agarose (Fagan *et al*. 1999).

Detection of *Bla* gene Variants with PCR

20ng/µl DNA template was used in GoTaq (10×) mastermix, 10 µM of forward primer CTX-MU1 (5′- ATG TGC ACC AGT AAR GT-3′), 10 µM of reverse primer CTX-MU2 (5′-TGG GTR AAR TAR GTS ACC AGA-3′), 5U/µl of Taq polymerase in a final volume of 20 µl for 2 µl of template DNA. The thermal cycling condition consisted of an initial denaturation of 95^{o} C for 5 minutes, cyclic denaturation of 94^{o}C for 15 seconds, annealing for 30 seconds at 50^{o}C, cyclic elongation for 1 minute at 72^{o} C and a final elongation at 72^{o} C for 5 minutes (Moghaddam *et al*. 2013).

Collection of Indian Gooseberry (*Phyllanthus emblica*) Extract

Indian gooseberry (scientific name *P. emblica*) grows in tropical regions such as Bangladesh and especially in some south-east Asian countries. At first, all the fruits were washed with distilled water. The flesh was separated from the seeds and the seeds were discarded. The flesh was smashed into smooth paste without any water by using a grinding machine. The juice was then separated by using sterile cheese cloths. Then the juice was filtered and sterilized with Whatmann filter paper by slow percolation method. Then the juice was stored at 4^{o}C.

MIC Assay with Micro-dilution Technique

This is an automated method to test antimicrobial susceptibility quickly and accurately where serial dilution of various antimicrobial agents is made in a microtiter plate and the test organism is inoculated simultaneously and the appearance of no growth in a well after incubation shows that the test microbes are sensitive to the antibiotic at that concentration. A button of growth in a well indicates no effect of antimicrobial compounds on the organism.

Step 1: preparation of the plate map

Step 2: transferring 250µl bacterial suspension in each well

Step 3: Addition of inhibitory agent IC_{50} concentration

Step 4: Incubation for 16 hours

Step 5: Observation for growth of microorganisms

Step 6: Dropping the entire volume of the MIC wells into EMB agar

After the MIC assay, each sample was further assayed with a drop plate experiment for their visible decline in growth. EMB agar plate was used for the drop plate assay. Each EMB plate was divided into 8 sections with a marker. In every section 50µl of the sample was inoculated with a micropipette. 8 section inoculated with NC, PC, 1 F/L concentration, 0.5ml F/L concentration, 0.25ml F/L Concentration. Two hours were given after inoculation to allow the inoculum to settle. The plates were then incubated for 24 hours in order to observe antimicrobial potential.

In addition, we performed a micro-dilution assay with ciprofloxacin (5 µg/ml), cefoxitin (5 µg/ml) and imipenem (0.002 µg/ml) as well as 1:2 v/v dilution of *P. emblica* extract to compare inhibitory effect of the extract to those of designated antibiotics.

Results and Discussion

Results

E. coli isolates were found in clinical samples sent to the bacteriology lab at the department of microbiology of Sirajul Islam Medical College and Hospital, located in the Mogbazar area of Dhaka. All the safety procedures were followed while collecting those samples. Universal precautions for collecting and handling all specimens were strictly followed. Contamination with indigenous flora was avoided by use of semi-selective media.

Antibiogram was done on Muller Hinton Agar (MHA) medium, followed by incubation with ESBL panel disks. Isolates that show a zone of inhibition greater than 19.5mm were regarded as β-lactum sensitive. Isolate less than or equal to 16mm zone of inhibition was considered ESBL (CLSI Guidelines). *E. coli* isolates were sourced from healthcare facilities that performed antibiograms on a routine diagnostic basis. The observations from the isolates under study are listed in Table 2.

We cultured the isolates on blood agar to show the hemolytic activity, all the fourteen isolates were α-hemolytic.

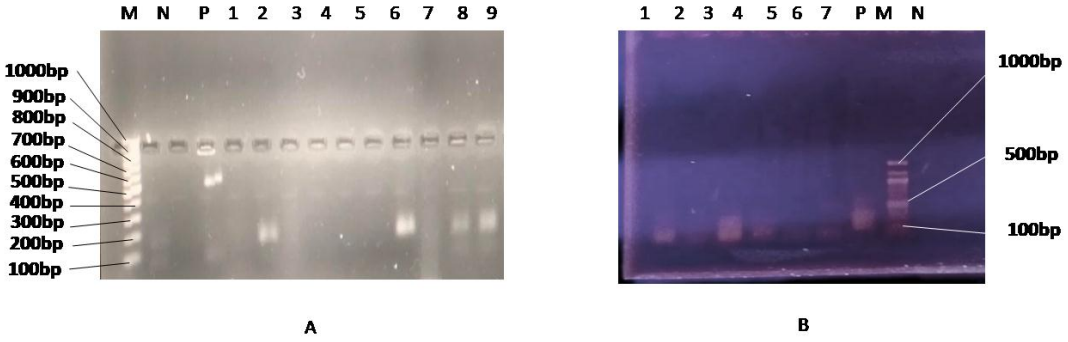

Fig. 1. PCR amplification of ESBL *E coli* isolates under study (A) *Lt1* toxin gene (B) *Stx2* toxin gene.

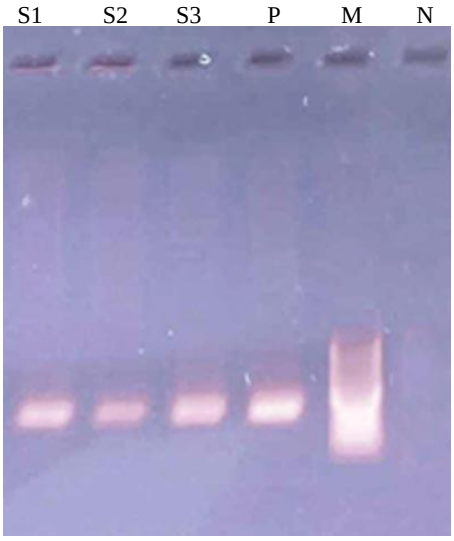

Fig 2: PCR for *bla*$_{CTX-M}$ gene from ESBL *E. coli* isolates (S = sample, P = positive control, M= marker, N = negative control.).

Table 2: Antibiogram of isolates against Extended Spectrum β-lactam antibiotics

Isolate ID	Observation								Resistance pattern
	Ceftazidime		Aztreonam		Ceftriaxone		Cefotaxime		
	Zone-of-inhibition Diameter (mm)	type	Zone-of-inhibition Diameter (mm)	type	Zone-of-inhibition Diameter (mm)	type	Zone-of-inhibition Diameter (mm)	type	
E1	21	I	27	R	26	R	27	R	Resistant
E2	21	I	26	I	26	R	27	R	Resistant
E3	21	I	24	I	24	I	25	I	Intermediate
E4	21	I	26	I	26	R	27	R	Resistant
E5	21	I	25	I	25	I	26	I	Intermediate
E6	20	I	25	I	24	I	27	I	Intermediate
E7	21	I	26	I	26	R	27	R	Resistant
E8	20	I	24	I	25	I	26	I	Intermediate
E9	23	R	26	I	26	R	28	R	Resistant

Isolate ID	Observation								Resistance pattern
	Ceftazidime		Aztreonam		Ceftriaxone		Cefotaxime		
	Zone-of-inhibition Diameter (mm)	type	Zone-of-inhibition Diameter (mm)	type	Zone-of-inhibition Diameter (mm)	type	Zone-of-inhibition Diameter (mm)	type	
E10	22	R	27	R	25	I	27	R	Resistant
E11	23	R	26	I	26	R	27	R	Resistant
E12	22	R	26	I	25	R	27	R	Resistant
E13	21	I	28	R	26	R	28	R	Resistant
E14	23	R	27	R	25	R	26	I	Resistant

The PCR of Lt1 is showed amplicons of 250bp from some of the isolates (Fig. 1A).

The PCR of the *Stx1* gene didn't show amplification from any isolate under study (result not shown), indicating potential of these clinical isolates to cause hemorrhagic diarrhea. However *Stx2* gene was found in some of the isolates capable of causing hemorrhagic uremic syndrome (Fig. 1B). Five of the isolates expressed $Bla_{CTX\text{-}M}$ gene corresponding to production of a specific sub-group of beta-lactamases imparting resistance against cefotixin, imipenem and ciprofloxacin (Fig. 2) (Table 3).

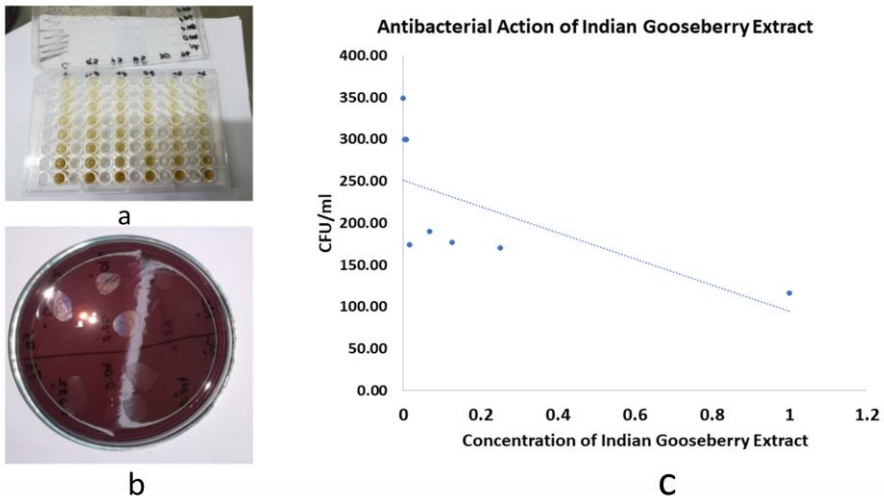

Fig. 3. Bactericidal activity of Indian Gooseberry extract on log-phase cultures of ESBL *E. coli in vitro.*

Table 3. Antibiogram of isolates against cephoxitin, imipenem and ciprofloxacin

	Cephoxitin		Imipenem		Ciprofloxacin		Conclusion
ID	Zone-of-inhibition Diameter (mm)	type	Zone-of-inhibition Diameter (mm)	type	Zone-of-inhibition Diameter (mm)	type	
E1	18	S	13	I	16	S	S
E2	17	S	13	I	26	S	S
E3	12	R	12	R	14	I	R
E4	14	I	16	S	16	S	S
E5	13	I	14	I	15	I	I
E6	17	S	13	I	14	I	I
E7	14	I	14	I	16	S	I
E8	18	S	14	I	15	I	I
E9	17	S	14	I	16	S	S
E10	16	S	15	S	15	I	S
E11	19	S	17	S	16	S	S
E12	17	S	14	I	15	I	I
E13	18	S	17	S	16	S	S
E14	17	S	18	S	15	I	S

Log-phase cultures of ESBL *E. coli* isolates showed decrease in CFU/ml after overnight exposure to raw cold-pressed extract of Indian Gooseberry (Amloki) (Fig. 3), though the reduction was not statistically significant due to high standard deviation among replicates of the same isolate as well as variation between isolates. However, the micro-dilution assay with antibiotics did show significant difference between inhibitory effects of antibiotics and *P. emblica* extract (Table 4), implying though the extract had inhibitory effects on ESBL-*E. coli* isolates, the effects is not of therapeutic value (Farrel *et al*., 2014).

Table 4. Comparison between inhibitory effects of *P. emblica* and cephoxitin, imipenem and ciprofloxacin on ESBL *E. coli* isolates (CFU/mL)

	Negative control	Cephoxitin	Imipenem	Ciprofloxacin	*P. emblica* extract
E1	476	197	233	159	349
E2	390	281	154	138	384
E3	513	354	403	336	397
E4	462	169	251	154	373
E5	359	208	187	121	266
E6	537	246	275	297	390
E7	493	272	206	163	306
E8	388	215	190	129	174
E9	448	186	255	191	222
E10	592	286	269	187	371
E11	405	168	237	202	286
E12	569	217	274	199	353
E13	458	228	243	176	302
E14	346	216	259	116	277

Discussion

This study presents basic findings of an experiment demonstrating antimicrobial potency of natural compounds derived from *P. emblica* (amloki) extract. The richness of vitamin C and other antioxidant has been proven by other groups (Ruangchakpet *et al*., 2007) as well as against other pathogens. We report the potency of amloki extract against clinically pathogenic ESBL E. coli in order to add antimicrobials to the list of natural sources. We previously reported antibacterial activity of *Syzygium cumini* (kalojam) against methicillin-resistant *Staphylococcus aureus* (MRSA) from nosocomial strains (Towhid *et al*., 2021).

The isolates were identified culturally by virtue of growth on Chromagar *E. coli* and Chromagar orientation medium as well as from PCR detection of *lt250* with conventional PCR (Fig 1A). These isolates showed various levels of sensitivity against Ceftazidime, Cefotaxime, Ceftriaxone and Aztreonam. Table 4 shows a categorical classification of the resistance status of the isolates, resistance against at least 2 antibiotics is defined as ESBL. Fig. 2 shows both ESBL resistant and intermediate isolates are equally sensitive to *P. emblica* extract, implying an inherent sensitivity against antioxidants. The concentration of the extracts used was within physiologic conditions (amount of active components abosorbed into bloodstream from intestine when consumed).

Taken together, our study shows presence of ESBL-*E. coli* from Bangladeshi patients in out-door healthcare facility in Dhaka City, Bangladesh. There were other reports of ESBL-*E.coli* from Bangladeshi UTI patients with reference to its infectivity and impact in a dense population like Dhaka. We identified *lt250* gene that encodes labile toxin (associated with enteric diarrhea) (Table1) as well as *Stx2* gene which encodes hemolytic toxin (associated with symptoms of hemolytic uremic syndrome) in some isolates (Fig. 1) indicating genetic elements that could cause diarrhea and/ or urinary tract symptoms. We could detect $Bla_{CTX\text{-}M}$ gene variant in 5 of the isolates (Fig. 2), and could not detect any other major *Bla* variant from other isolates (data not shown). Ten out of 14 isolates in this study were ESBL, while the other 4 exhibited intermediate-

sensitivity towards ESBL group of antibiotics (Table 2) as well as some other therapeutic antibiotics (Table 3), which is alarming considering the high rate of β-lactams prescribed for infection control. We demonstrate the antibacterial activity of *P. emblica* extract on ESBL-*E. coli* isolates in this study (Fig. 3), though no concentration of the extract could practically inhibit the isolates completely to 0 CFU/ml in our study (Table 4).

Conclusion

The inhibitory function of *P. emblica* extracts on our isolates are not of statistical significance because the reduction of bacterial concentration was not lower than uninhibited negative control, while the bacterial concentration between negative control and micro-dilution with antibiotics were significantly different. The isolates micro-diluted with *P. emblica* extract were significantly higher than those micro-diluted with antibiotics, meaning *P. emblica* extract was not able to inhibit the ESBL-*E. coli* isolates to a level important in clinical cases (Guet-Revillet *et al*., 2014, Fournier *et al*., 2013).

Acknowledgement

This study was funded by the Jagannath University Annual Research Allocation 2019-2020.

References

Aruna, K., and Mobashshera, T. (2012). Prevalence of extended spectrum beta-lactamase production among uropathogens in South Mumbai and its antibiogram pattern. *EXCLI Journal*, *11*: 363.

Bertrand, X. and Dowzicky, M. (2012). Antimicrobial Susceptibility Among Gram-Negative Isolates Collected From Intensive Care Units in North America, Europe, the Asia-Pacific Rim, Latin America, the Middle East, and Africa Between 2004 and 2009 as Part of the Tigecycline Evaluation and Surveillance Trial. *Clinical Therapeutics*, 34(1): 124–137.

Bush, K. (2001). New beta-lactamases in gram-negative bacteria: diversity and impact on the selection of antimicrobial therapy. *Clinical Infectious Diseases*, 32(7): 1085–1089.

Dhanani, T., Shah, S. and Kumar, S. (2014). A Validated High-Performance Liquid Chromatography Method for Determination of Tannin-Related Marker Constituents Gallic Acid, Corilagin, Chebulagic Acid, Ellagic Acid and Chebulinic Acid in Four Terminalia Species from India. *Journal of Chromatographic Science*, 53(4): 625–632.

Di Pietro, F., Ortenzi, F., Tilio, M., Concetti, F., and Napolioni, V. (2011). Genomic DNA extraction from whole blood stored from 15-to 30-years at−20°C by rapid phenol–chloroform protocol: A useful tool for genetic epidemiology studies. *Molecular and cellular probes*, 25(1): 44–48.

Dortet, L., Poirel, L., and Nordmann, P. (2015). Rapid detection of ESBL-producing Enterobacteriaceae in blood cultures. *Emerging infectious diseases*, 21(3): 504.

Fagan, P. K., Hornitzky, M. A., Bettelheim, K. A., and Djordjevic, S. P. (1999). Detection of Shiga-like toxin (*stx1* and *stx2*), intimin (*eaeA*), and enterohemorrhagic *Escherichia coli* (EHEC) hemolysin (EHEC *hlyA*) genes in animal feces by multiplex PCR. *Applied and Environmental Microbiology*, 65(2):868–872

Farrell, D. J., Mendes, R. E., Rhomberg, P. R., and Jones, R. N. (2014). Revised reference broth microdilution method for testing telavancin: effect on MIC results and correlation with other testing methodologies. *Antimicrobial agents and chemotherapy*, 58(9): 5547–5551.

Fournier, D., Chirouze, C., Leroy, J., Cholley, P., Talon, D., Plesiat, P., and Bertrand, X. (2013). Alternatives to carbapenems in ESBL-producing *Escherichia coli* infections. *Médecineet maladies infectieuses*, 43(2): 62–66.

Guelmbaye, N., Fissou, H. and Bessimbaye, N., (2015). Antimicrobial resistance in extended spectrum -lactamases (ESBL)-producing *Escherichia coli* isolated from human urinary tract infections in Ndjamena, Chad. *African Journal of Microbiology Research*, 9(11):776–780.

Guet-Revillet, H., Emirian, A., Groh, M., Nebbad-Lechani, B., Weiss,B., Join Lambert,O., Bille, E., Jullien, V., and Zahar, J. R. (2014). Pharmacological study of cefoxitin as an alternative antibiotic therapy to carbapenems in treatment of urinary tract infections due to extended spectrum-β-lactamase-producing *Escherichia coli*. *Antimicrob Agents Chemotherapy*, 58(8):4899–901.

Jarlier, V., Nicolas, M., Fournier, G., and Philippon, A. (1988). Extended broad-spectrum -lactamases conferring transferable resistance to newer-lactam agents in Enterobacteriaceae: Hospital prevalence and susceptibility patterns. *Clinical Infectious Diseases*, 10(4): 867–878.

Joseph, A., Cointe, A., Mariani Kurkdjian, P., Rafat, C., and Hertig, A. (2020). Shiga toxin-associated hemolytic uremic syndrome: A narrative review. *Toxins*, 12(2): 67.

Kumar, D., Singh, A., Ali, M. and Chander, Y. (2014). Antimicrobial Susceptibility Profile of Extended Spectrum β-Lactamase (ESBL) Producing *Escherichia coli* from Various Clinical Samples. *Infectious Diseases: Research and Treatment*, 7: 13820.

Lina, T. T., Khajanchi, B. K., Azmi, I. J., Islam, M. A., Mahmood, B., Akter, M., Banik, A., Alim, R., Navarro, A., Perez, G., Cravioto, A., and Talukder, K. A. (2014). Phenotypic and molecular characterization of extended-spectrum beta-lactamase-producing *Escherichia coli* in Bangladesh. *PloS One*, 9(10): e108735.

Madhavi, R. and Hanumanthappa, A., (2021). Prevalence of Extended Spectrum Beta-Lactamase Producing Gram-Negative Bacilli causing Surgical Site Infections in a Tertiary Care Centre. *Journal of Pure and Applied Microbiology*, 15(3):1173–1179.

Mehrgan, H., and Rahbar, M. (2008). Prevalence of extended-spectrum beta lactamase producing *Escherichia coli* in a tertiary care hospital in Tehran, Iran. *International journal of antimicrobial agents*, 31 (2):147–151.

Miller, S. I. (2016). Antibiotic Resistance and Regulation of the Gram-Negative Bacterial Outer Membrane Barrier by Host Innate Immune Molecules. *mBio*, 7(5): e01541–16.

Moghaddam, M. N., Beidokhti, M. H., Jamehdar, S. A., Ghahraman, M. (2014).Genetic properties of $bla_{CTX\text{-}M}$ and bla_{PER} β-lactamase genes in clinical isolates of Enterobacteriaceae by polymerase chain reaction. Iranian Journal of Basic Medicinal Science, 17(1): 378–383

Nathisuwan, S., Burgess, D. and Lewis, J., (2001). Extended-Spectrum β-Lactamases: Epidemiology, Detection, and Treatment. *Pharmacotherapy*, 21(8): 920–928.

Ruangchakpet, A., and Sajjaanantakul, T. (2007).Effect of browning on total phenolic, flavonoid content and antioxidant activity in Indian gooseberry (*Phyllanthus emblica* Linn.). *Agriculture and Natural Resources*, 41(5): 331–337.

Thilakchand, K. R., Mathai, R. T., Simon, P., Ravi, R. T., Baliga-Rao, M. P., & Baliga, M. S. (2013). Hepatoprotective properties of the Indian gooseberry (*Emblica officinalis* Gaertn): a review. *Food & Function*, 4(10), 1431–1441.

Towhid, S. T., Alam, M. U., Asaduzzaman, M. ., Sagor, M. S., Mahmud, M. A. ., and Samad, M. A. (2022). Antimicrobial Effect of *Syzygium cumini* Extract Against Methicillin Non-Susceptible *Staphylococcus aureus*: Newborn care knowledge. *Bangladesh Medical Research Council Bulletin*, 47(1): 82–89.

Webber, M.(2002). The importance of efflux pumps in bacterial antibiotic resistance. *Journal of Antimicrobial Chemotherapy*, 51(1): 9–11.

Yousefimashouf, R. and Malekpoor, M., (2013). P111 Antibiotics resistance patterns of *Pseudomonas aeruginosa* isolated from hospital environments to some β-lactam antibiotics, cephalosporins, and aminoglycosides. *International Journal of Antimicrobial Agents*, 42: S77.