Extended-Spectrum Beta-Lactamase-Producing *Escherichia coli* in Drinking Water Samples From a Forcibly Displaced, Densely Populated Community Setting in Bangladesh

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**Introduction:** Community-acquired infections due to extended-spectrum beta-lactamase (ESBL) producing *Escherichia coli* are rising worldwide, resulting in increased morbidity, mortality, and healthcare costs, especially where poor sanitation and inadequate hygienic practices are very common.

**Objective:** This study was conducted to investigate the prevalence and characterization of multidrug-resistant (MDR) and ESBL-producing *E. coli* in drinking water samples collected from Rohingya camps, Bangladesh.

**Methods:** A total of 384 *E. coli* isolates were analyzed in this study, of which 203 were from household or point-of-use (POU) water samples, and 181 were from source water samples. The isolates were tested for virulence genes, ESBL-producing genes, antimicrobial susceptibility by VITEK 2 assay, plasmid profiling, and conjugal transfer of AMR genes.

**Results:** Of the 384 *E. coli* isolates tested, 17% (66/384) were found to be ESBL producers. The abundance of ESBL-producers in source water contaminated with *E. coli* was observed to be 14% (27/181), whereas, 19% (39/203) ESBL producers was found in household POU water samples contaminated with *E. coli*. We detected 71% (47/66) ESBL-*E. coli* to be MDR. Among these 47 MDR isolates, 20 were resistant to three classes, and 27 were resistant to four different classes of antibiotics. Sixty-four percent (42/66) of the ESBL producing *E. coli* carried 1 to 7 plasmids ranging from 1 to 103 MDa. Only large plasmids with antibiotic resistance properties were found transferrable via conjugation. Moreover, around 7% (29/384) of *E. coli* isolates harbored at least one of 10 virulence factors belonging to different *E. coli* pathotypes.
Conclusions: The findings of this study suggest that the drinking water samples analyzed herein could serve as an important source for exposure and dissemination of MDR, ESBL-producing and pathogenic E. coli lineages, which therewith pose a health risk to the displaced Rohingya people residing in the densely populated camps of Bangladesh.

Keywords: ESBL-producing E. coli, multidrug-resistant, drinking water, Rohingya camps, Bangladesh

INTRODUCTION

Extended-spectrum beta-lactamase (ESBL)-producing E. coli have been recognized as a major multidrug-resistant bacteria implicated in serious hospital and community-acquired infections worldwide, especially in places where poor sanitation, and inadequate hygienic practices are very common (1–4). Infections caused by MDR-E. coli incur huge medical costs and limit treatment options (5–7).

Multidrug-resistant E. coli has been detected in different ecological niches in the community and environment (8, 9). For instance, ESBLs and New Delhi Metallo beta-lactamase 1 (NDM-1) producing E. coli were detected in drinking water and retail meat, respectively (10, 11). In Bangladesh, ESBL-producing E. coli were reported from drinking water as well as from river water samples (12, 13). Though E. coli has had a significant role in water microbiology as an indicator of fecal pollution, it is of greater public health concern when these E. coli isolates turn out to be multidrug-resistant pathogens (14). Detection of ESBL-producing E. coli in drinking water samples is important to recognize the risk of transmission of antimicrobial resistance (AMR) and gastrointestinal diseases. Transmission of ESBL-encoding genes among bacteria is often plasmid-mediated (15), and aquatic environments provide ideal settings for horizontal transfer of AMR genes encoded on various forms of mobile genetic elements (16).

Though the majority of E. coli are typically innocuous, some E. coli variants are virulent and may inflict varying severity of enteric infections. Currently, there are six different E. coli pathotypes that have been documented to cause intestinal infections, they include, enterotoxigenic E. coli (ETEC), enteroinvasive E. coli (EIEC), enteropathogenic E. coli (EPEC), shiga toxin-producing E. coli (STEC), enteraggregative E. coli (EAEC), and diffusely adhering E. coli (DAEC) (17). In Bangladesh, following rotavirus, the second most leading cause of diarrheal infections are caused by pathogenic E. coli (18). Several virulence genes such as st, lt (ETEC); bfp, eae (EPEC); aat, aai (EAEC) are associated with diarrheagenic E. coli pathotypes (19), which can be used to detect these pathotypes using PCR based gene amplification. Watery diarrhea is caused by the secretion of heat-labile (LT) and/or heat-stable (ST) enterotoxins from ETEC. Shiga toxin (Stx) expression is the unique feature of EHEC where systemic absorption of this toxin leads to possibly life-threatening complications. Multiple putative virulence factors expression for typical EAEC strains, containing the aggregative adherence fimbriae (AAF), dispersin, the dispersin translocator Aat, and the Aai type VI secretion system directs to adherence and triggering diarrhea. EPEC adhesion is associated with attaching and effacing adhesion and intestinal colonization, which also include bundle-forming pili (BFP), EspA filaments and intimin (19, 20).

The contaminated drinking water was found to be responsible for a number of waterborne gastroenteritis outbreaks due to diarrheagenic E. coli (21–23). Therefore, it is pertinent to analyze the prevalence and properties of ESBL-E. coli from drinking waters in community settings, particularly, from human habitations that are projected to pose exceptionally high risks of waterborne diseases due to overcrowding, scarcity of safe drinking water, and unhygienic living conditions. In Bangladesh, the displaced Rohingya people are one such community with a population of ~1.16 million who are living in 32 congested camps in a challenging hilly landscape of Cox’s Bazar district (290,000 persons per square kilometer). This displacement, of a large population are facing complicating problems, particularly related to water, sanitation, hygiene and health care (24–27). Water from hand-pumped tube wells is the primary water supply for the people in Rohingya camps. Around 6057 water points and 50087 emergency latrines have been built (during the study in 2018). Moreover, in the absence of efficient community sanitation, insufficient sewage disposal, and treatment facilities, the risks of transmission of enteric pathogens become extremely high, and the community as a whole face serious public health concerns (28–30).

In our previous study, we analyzed source water (tubewell) samples, as well as POU drinking water samples, from Rohingya camps and found 10.5% source water and 34.7% POU water samples were contaminated with E. coli, which could cause waterborne diseases in the camps (26). An outbreak of ESBL-producing E. coli might create a medical emergency in a large congested habitation like Rohingya camps because of limited treatment options. The AMR surveillance, especially with regard to ESBL-producing E. coli has never been carried out in these camps. Therefore, this study aims to determine the prevalence of ESBL-producing, MDR, and virulent E. coli in drinking water samples. Furthermore, plasmid profiling and horizontal transfer of resistant gene analyses of isolated ESBL-producing E. coli will provide important insights in understanding the dissemination of resistance determinants.

MATERIALS AND METHODS

Bacterial Isolates

We employed drinking water samples from Rohingya camps collected in our previous study to culture E. coli isolates (26). From a total of 2512 E. coli contaminated water samples, 421
water samples were randomly selected for the present study. One random \textit{E. coli} isolate was taken as a representative from each sample, which was further tested using the API-20E test kit (Biomerieux SA, Marcy-l’Etoile, France), and 384 API-20E confirmed \textit{E. coli} were stored at −70°C in 30% LB glycerol-broth for downstream analysis. Out of 384 \textit{E. coli} isolates, 203 isolates were from the household water samples and 181 from source water samples. In brief, for each sample, 100 ml water was filtered through a 0.22 µm membrane filter (Sartorius Stedim, Goettingen, Germany), the membrane filter paper was then firmly laid on the mTEC agar plate. Later, the culture plate was incubated for 2 h at 35 ± 0.5°C, followed by further incubation for 22 ± 2 h at 44.5 ± 0.2°C. After incubation, red to magenta-colored colonies, typical of \textit{E. coli} colony was picked and subcultured on MacConkey agar plate and incubated at 35 ± 2°C for 18 h to 24 h. After incubation, the characteristics of 35 dark pink colonies typical of \textit{E. coli} were obtained and confirmed using API-20E kit.

**Isolation and Confirmation of ESBL-Producing and Carbapenem-Resistant \textit{E. coli} Using Chromagar**

All 384 confirmed \textit{E. coli} isolates were cultured on CHROMagar ESBL and CHROMagar KPC media at 37°C for 18–24 h. The production of extended-spectrum beta-lactamases and carbapenemase was confirmed by observing the growth and characteristic colony morphology on respective culture media. Dark pink to reddish colonies on CHROMagar ESBL plate indicate ESBL producing \textit{E. coli} whereas pink to reddish colonies on CHROMagar KPC media suggest carbapenem-resistant \textit{E. coli}.

**Confirmation of \textit{E. coli} by VITEK 2**

ESBL positive \textit{E. coli} isolated from the ESBL CHROMagar plate were further confirmed by the VITEK 2 system (bioMérieux, Marcy l’Etoile, France) using VITEK 2 GN ID card. \textit{Enterobacter hormacchi} (ATCC-700323) was used as a positive control for the identification in this system. For VITEK 2 assays, pure isolates were streaked on MacConkey agar plates and incubated at 35°C overnight. One to three isolates were selected from each MacConkey agar plate and suspended in saline for preparation of inoculum to obtain an absorbency of ~0.5 McFarland Units before being subjected to VITEK 2 analysis.

**Detection of Diarrheagenic and ExPEC Associated Virulence Genes**

Several virulence genes such as \textit{st, lt} (ETEC); \textit{bfp, eae} (EPEC); \textit{aat, aai} (EAEC) are associated with diarrheagenic \textit{E. coli} pathotypes (19) are used to detect the pathotypes using PCR based gene amplification. In the present study, PCR based screening of diarrheagenic virulence genes was carried out for all 384 \textit{E. coli} isolates. Gene-specific primers entailing heat-labile (\textit{lt}), heat-stable (\textit{st}), attaching and effacing (\textit{eae}), anti-aggregation protein transporter (\textit{aat}), bundle forming pilus (\textit{bfp}) and aggR-activated island (\textit{aaiC}) were used to detect the respective genes employing a multiplex PCR setup (31–34). The boiling lysis method was used to obtain the DNA template (35). A 3 µl template DNA was taken for a 25 µl PCR reaction containing 12.5 µl of 2X GoTaq G2 Green Master Mix (Promega, USA) with 0.44 µl of eae primer, the primers \textit{lt, st, aaiC, aat, bfp} were taken in 0.4 µl volume each. The PCR was carried out at standard cycling conditions with an annealing temperature of 57°C for 20 s. A separate PCR was performed for Shiga toxin genes (\textit{stx1} and \textit{stx2}), which was described previously (36, 37). PCR for invasion plasmid antigen \textit{H} (\textit{ipaH}) and the invasion associated locus (\textit{ial}) was performed according to the procedure described elsewhere (38–41). Primer details are tabulated in Supplementary Table 1.

To examine the presence of seven ExPEC associated virulence factors, we performed two multiplex PCRs on 55 non diarrheagenic ESBL-\textit{E. coli} isolates (42). Among these, the first one was done to screen the presence of \textit{KpsMII} (group II capsule), \textit{papA} (pilus-associated protein A), \textit{sfaS} (S-fimbrial adhesine), and \textit{fogC} (F1C fimbriae protein) genes; whereas the second multiplex was performed to detect \textit{hlyD} (haemolysin D), \textit{afa} (afimbrial adhesine), and \textit{iuA} genes (aerobacter siderophore ferric receptor protein).

**Antibiotic Susceptibility by VITEK 2**

Antibiotic susceptibility testing (AST) was performed using VITEK 2 system with VITEK 2 cards (AST-N280) for 19 antimicrobial agents according to the CLSI guidelines and manufacturer’s recommendations; two additional antimicrobial agents (cefixime and ceftazidime) were also incorporated. \textit{E. coli} ATCC 25922, susceptible to all drugs, was used for AST in each VITEK testing step as quality control. The 21 antibiotics tested included amikacin, amoxicillin/clavulanic acid, ampicillin, cefepime, cefixime, cefoperazone/sulbactam, ceftazidime, ceftriaxone, cefuroxime, cefuroxime axetil, ciprofloxacin, colistin, ertapenem, gentamicin, imipenem, meropenem, nalidixic acid, nitrofurantoin, piperacillin, tigecycline, and trimethoprim/sulfamethoxazole. Minimum inhibitory concentrations (MIC) were determined, and the isolates were classified into resistant, intermediate and susceptible as per CLSI criteria. The raw MIC data from the VITEK 2 assay are shown in Supplementary Table 2.

**Detection of ESBL, Quinolone, and Carbapenemase Resistance Genes by PCR**

All CHROMagar confirmed ESBL-producing \textit{E. coli} isolates were screened for molecular determinants of ESBL and carbapenemase resistance comprising of \textit{blaSHV}, \textit{blaTEM}, \textit{blaCTX-M–15} and all CTX-M-groups (\textit{blaCTX-M–1–group, blaCTX-M–2–group, blaCTX-M–8–group, blaCTX-M–9–group}) including, \textit{blaOXA–1–group, blaOXA–47}, and \textit{blaNDM–1} were screened (34, 43–48). The gene \textit{blaCMY–2} encoding for AmpC β-lactamase was also screened by PCR as per a published protocol (12, 49). Besides, all 66 isolates were tested for the three \textit{qnr} (quinolone) resistance genes; \textit{qnrA, qnrB}, and \textit{qnrS} according to the methods described by others (50–52).

**Plasmid Profiling and Conjugal Transfer**

Plasmids from ESBL-\textit{E. coli} isolates were extracted employing the Kado alkaline lysis method (53) and visualized after separation in low percent agarose gel (0.7%) electrophoresis. The size of
extracted plasmids was determined comparing with size standard plasmids ran alongside. The following plasmids were used as size standards: Sa (23 MDa), RP4 (34 MDa), R1 (62 MDa), pDK9 (140 MDa), and E. coli V517 plasmids (1.4, 1.8, 2.0, 2.6, 3.4, 3.7, 4.8, and 35.8 MDa) (54). ESBL-producing E. coli were used as donors, and the sodium azide resistant strain E. coli J53 was employed as a recipient for conjugation using broth mating assays at 30°C for 19 ± 1 h. Transconjugants obtained were plated on MacConkey plates prepared with cefotaxime (20 μg/L) and sodium azide (100 mg/L), transconjugants were selected observing their growth and colony morphology. Transconjugants were analyzed for antibiotic susceptibility tests using VITEK 2 assay, plasmid profiling and presence of ESBL genes (53).

**Results**

**ESBL-Positive but Carbapenem Sensitive E. coli Recovered From Drinking Water**

To screen for ESBL-producing E. coli, 384 isolates were cultured on ESBL CHROMagar. The typical growth of pink colonies on the CHROMagar plate was considered as ESBL positive E. coli. Of the 384 isolates tested, 17.2% (n = 66) were found to be ESBL-producing E. coli (Table 1). About 15% (27/181), and 19% (39/203) ESBL producing E. coli originated from E. coli contaminated source water and POU water samples, respectively. Further, the ESBL producing E. coli was investigated for carbapenem resistance on CHROMagar KPC media, and none of the isolates was able to grow on CHROMagar KPC media. Therefore, we did not detect any carbapenem-resistance in our collection of ESBL-E. coli isolates.

**High Prevalence of MDR in ESBL-Producing E. coli**

Antimicrobial susceptibility of 66 ESBL-E. coli isolates (Supplementary Table 3) were tested against 21 different antibiotics (Supplementary Table 4) using VITEK 2 assay. All 66 CHROMagar confirmed ESBL positive E. coli isolates demonstrated resistance against ampicillin, ceftriaxone, cefazidime, cefoxime, and cefuroxime axetil (Figure 1). About 70% (46/66) of the isolates were found to be resistant to nalidixic acid, 37.9% (25/66) isolates were resistant to trimethoprim/sulfamethoxazole, whereas 22.7% (15/66) and 19.7% (13/66) isolates were resistant for cefepime and ciprofloxacin, respectively. However, no resistance was detected in any of the tested strains to the antibiotics used of aminoglycosides (amikacin and gentamicin), cefoperazone/sulbactam, glycylcycline, carbapenem, and polymyxins groups. It was found that 71% (47/66) of E. coli isolates were MDR that were resistant to at least three classes of antibiotics. Among the 47 MDR isolates, 20 were resistant to three different classes, and 27 were resistant to four different classes of antibiotics.

**bla<sub>CTX-M-1</sub> Group Is the Predominant ESBL Gene Detected**

The presence of molecular determinants of ESBLs was tested on all ESBL-producing E. coli isolates. Out of the 66 ESBL-E. coli isolates, 59% (39/66) isolates harbored both bla<sub>CTX-M-1</sub>-group and bla<sub>CTX-M-15</sub> gene. However, 4.5% (3/66) isolates harbored either bla<sub>CTX-M-1</sub>-group or bla<sub>CTX-M-15</sub> gene. The bla<sub>TEM</sub> β-lactamase gene was present in 35% (23/66) of isolates, and none of the isolates harbored other β-lactamase genes such as bla<sub>SHV</sub>, bla<sub>CTX-M-2</sub>-group, bla<sub>CTX-M-8</sub>-group, and bla<sub>CTX-M-9</sub>-group. The two genes, bla<sub>OXA-1</sub>-group and bla<sub>OXA-47</sub> were screened among 66 ESBL- E. coli isolates, but none of the isolates was found to be positive. In addition, the New Delhi metallo-β-lactamase gene, bla<sub>NDM-1</sub> as well as plasmid-mediated ampC-type β-lactamase gene the bla<sub>CMY-2</sub> was not present in any of the isolates. The quinolone resistance gene; qnrS, and qnrB were found in 34% (22/66) and 5% (3/66) of the ESBL-E. coli isolates, respectively (Figure 2, Supplementary Table 5).

**Distribution of E. coli Pathotypes**

Screening for the presence of virulence factors demonstrated that 7% (29/384) of E. coli isolates were positive for at least one virulence factor out of the 10 E. coli pathotype-specific virulence genes tested. Ten isolates were positive for only aaiC gene and five isolates were positive for both aaiC and aat; whereas four isolates were positive for both bfp and eae genes. Heat labile (li) gene was present in 7 isolates, whereas the heat stable (st) gene was found in 2 isolates and a single isolate was found positive for stx1. None of the isolates was positive for ipaH and iaa genes. Among the 29 pathogenic E. coli isolates detected; 52% (15/29) were EAEC, 31% (9/29) were ETEC, 14% (4/29) were EPEC and 4% (1/29) were EHEC (Figure 3).

When the ExPEC virulence genes were screened only three out of the seven virulence factors were detected that comprised of KpsMII, sfaS, and tuta genes, their prevalence rates were, 21.8% (12/55), 5.4% (3/55), and 16.4 % (9/55), respectively. Most of isolates (12/18) harboring ExPEC genes were affiliated to phylogenetic group D. However, out of the 55 isolates tested, only 6 isolates qualify as ExPEC as per the inclusion criteria (strains harboring at least two ExPEC associated virulence factors) 5 out of these 6 isolates were from phylogroup D.

The potential pathogenic (diarrheagenic and ExPEC) E. coli isolates showed high resistance rates 83% (39/47) to ampicillin, followed by 74% (35/47) to nalidixic acid, 68% (32/47) to cefuroxime, cefoxime axetil, 61% (29/47) to cefixime, cefazidime, and 51% (24/47) to sulphonamides. Out of 29 pathogenic E. coli 11 were found to be ESBL producing in this study (Table 2). Of note, all the pathogenic isolates detected were found to be susceptible to carbapenems, aminoglycosides (amikacin and gentamicin), and polymyxin.
### TABLE 1 | Antibiotic resistance pattern, presence of antibiotic resistance genes and plasmid patterns of ESBL-producing Escherichia coli isolated from water sample.

| Serial no | Isolates ID | Antibiotic resistance Pattern* | Presence of antibiotic resistant genes | Plasmid size in MDa |
|-----------|-------------|---------------------------------|----------------------------------------|---------------------|
| 1         | 05095B      | Amp, Cro, Cxm, Cfa, Czm, NA     | bla<sub>CTX-M-1</sub>, bla<sub>CTX-M-15</sub>, bla<sub>TEM</sub>, qnrS | 75, 54, 4.5, 2.8, 2.6, 2 |
| 2         | 09036H2     | Amp, Cro, Cxm, Cfa, Czm, NA     | bla<sub>CTX-M-1</sub>, bla<sub>CTX-M-15</sub>, qnrS | No plasmid |
| 3         | 34022A      | Amp, Fep, Cro, Cxm, Cfa, NA, Sxt, Czm | bla<sub>CTX-M-1</sub>, bla<sub>CTX-M-15</sub>, bla<sub>TEM</sub> | 90 |
| 4         | 34008B      | Amp, Fep, Cro, Cxm, Cfa, NA, Czm | bla<sub>CTX-M-1</sub>, bla<sub>CTX-M-15</sub>, qnrS | No plasmid |
| 5         | 34012H2     | Amp, Fep, Cro, Cxm, Cfa, NA, Czm | bla<sub>CTX-M-1</sub>, bla<sub>CTX-M-15</sub>, qnrS | No plasmid |
| 6         | 05080H2     | Amp, Fep, Cro, Cxm, Cfa, NA, Sxt, Czm | bla<sub>CTX-M-1</sub>, bla<sub>TEM</sub> | 94 |
| 7         | 11023H2     | Amp, Cro, Cxm, Cfa, Czm | bla<sub>CTX-M-1</sub>, bla<sub>CTX-M-15</sub>, qnrS | No plasmid |
| 8         | 34022H1     | Amp, Cro, Cxm, Cfa, NA, Czm | bla<sub>CTX-M-1</sub>, bla<sub>CTX-M-15</sub>, qnrS | 22 |
| 9         | 5375B       | Amp, Cro, Cxm, Cfa, NA, Tzp, Czm | bla<sub>CTX-M-1</sub>, bla<sub>CTX-M-15</sub>, qnrS | No plasmid |
| 10        | 5095H2      | Amp, Cro, Cxm, Cfa, NA, Czm | bla<sub>CTX-M-1</sub>, bla<sub>CTX-M-15</sub>, qnrS | No plasmid |
| 11        | 1109H1      | Amp, Cro, Cxm, Cfa, Czm | bla<sub>CTX-M-1</sub>, bla<sub>CTX-M-15</sub>, qnrS | 6.5, 4.6, 4.3, 3.4, 2.7 |
| 12        | 8E756H2     | Amp, Cro, Cxm, Cfa, NA, Czm | bla<sub>CTX-M-1</sub>, bla<sub>CTX-M-15</sub> | 77, 56, 6.5, 4.6, 4.3, 3.4, 2.8 |
| 13        | 9125B       | Amp, Cro, Cxm, Cfa, Sxt, Czm | bla<sub>TEM</sub> | 85, 57, 49, 37 |
| 14        | 8E285B      | Amp, Cro, Cxm, Cfa, NA, Czm | bla<sub>TEM</sub> | 29, 25 |
| 15        | 11269H1     | Amp, Cro, Cxm, Cfa, Czm | bla<sub>TEM</sub> | 37, 3.3 |
| 16        | 9736H2      | Amp, Cro, Cxm, Cfa, NA, Tzp, Czm | bla<sub>CTX-M-1</sub>, bla<sub>CTX-M-15</sub> | 68 |
| 17        | 11597A      | Amp, Cro, Cxm, Cfa, NA, Sxt, Czm | bla<sub>CTX-M-1</sub>, bla<sub>CTX-M-15</sub> | 65 |
| 18        | 1161H1      | Amp, Cro, Cxm, Cfa, NA, Sxt, Czm | bla<sub>CTX-M-1</sub>, bla<sub>CTX-M-15</sub>, qnrS | No plasmid |
| 19        | 04584H2     | Amp, Cro, Cxm, Cfa, Czm | bla<sub>CTX-M-1</sub>, bla<sub>CTX-M-15</sub>, qnrS | No plasmid |
| 20        | 8W645H2     | Amp, Cro, Cxm, Cfa, Czm | bla<sub>CTX-M-1</sub>, bla<sub>CTX-M-15</sub>, qnrS | No plasmid |
| 21        | 8W390H1     | Amp, Cro, Cxm, Cfa, Czm | bla<sub>CTX-M-1</sub>, bla<sub>CTX-M-15</sub>, qnrS | 42, 3.2, 2.6 |
| 22        | 8W803H2     | Amp, Cro, Cxm, Cfa, NA, Sxt, Czm | bla<sub>CTX-M-1</sub>, bla<sub>CTX-M-15</sub> | 103, 4.9, 2.9, 2.6 |
| 23        | 8W454H1     | Amp, Fep, Cro, Cxm, Cfa, NA, Sxt, Czm | bla<sub>CTX-M-1</sub>, bla<sub>CTX-M-15</sub> | No plasmid |
| 24        | 18544A      | Amp, Cro, Cxm, Cfa, NA, Fd, Czm | bla<sub>CTX-M-1</sub>, bla<sub>CTX-M-15</sub> | No plasmid |
| 25        | 18162H2     | Amp, Cro, Cxm, Cfa, NA, Fd, Czm | bla<sub>CTX-M-1</sub>, bla<sub>CTX-M-15</sub> | No plasmid |
| 26        | 18544B      | Amp, Cro, Cxm, Cfa, NA, Fd, Czm | bla<sub>CTX-M-1</sub>, bla<sub>CTX-M-15</sub> | No plasmid |
| 27        | 1224H1      | Amp, Cro, Cxm, Cfa, NA, Czm | bla<sub>CTX-M-1</sub>, bla<sub>CTX-M-15</sub>, qnrS | No plasmid |
| 28        | 1144H1      | Amp, Cro, Cxm, Cfa, NA, Czm | bla<sub>CTX-M-1</sub>, bla<sub>CTX-M-15</sub>, qnrS | 97, 39, 2 |
| 29        | 9414H2      | Amp, Cro, Cxm, Cfa, NA, Czm | bla<sub>CTX-M-1</sub>, bla<sub>CTX-M-15</sub>, qnrS | 34, 2.3 |
| 30        | 1E181H2     | Amp, Fep, Cro, Cxm, Cfa, NA, Sxt, Czm | bla<sub>CTX-M-1</sub>, bla<sub>CTX-M-15</sub> | No plasmid |
| 31        | 2W242H2     | Amp, Cro, Cxm, Cfa, NA, Sxt, Czm | bla<sub>CTX-M-1</sub>, bla<sub>CTX-M-15</sub>, bla<sub>TEM</sub> | 63, 6.4 |
| 32        | 2W246A      | Amp, Cro, Cxm, Cfa, NA, Sxt, Czm | bla<sub>CTX-M-1</sub>, bla<sub>CTX-M-15</sub>, bla<sub>TEM</sub> | 89, 4.4 |
| 33        | 1E86H5      | Amp, Cro, Cxm, Cfa, NA, Czm | bla<sub>CTX-M-1</sub>, bla<sub>CTX-M-15</sub>, qnrS | No plasmid |
| 34        | 1E07H2      | Amp, Cro, Cxm, Cfa, NA, Czm | bla<sub>TEM</sub> | 71, 33, 30 |
| 35        | 2W150H2     | Amp, Cro, Cxm, Cfa, NA, Czm | bla<sub>TEM</sub> | No plasmid |
| 36        | 2W047H2     | Amp, Cro, Cxm, Cfa, NA, Czm | bla<sub>TEM</sub>, bla<sub>CTX-M-15</sub>, qnrS | No plasmid |
| 37        | 2W246B      | Amp, Cro, Cxm, Cfa, NA, Czm | bla<sub>CTX-M-1</sub>, bla<sub>CTX-M-15</sub>, qnrS | 92, 74, 55 |
| 38        | 1E391A      | Amp, Cro, Cxm, Cfa, NA, Czm | bla<sub>CTX-M-15</sub>, qnrS | 92, 74, 56 |
| 39        | 1E424H2     | Amp, Fep, Cro, Cxm, Cfa, Czm | bla<sub>CTX-M-15</sub>, qnrS | No plasmid |
| 40        | 2E218H1     | Amp, Cro, Cxm, Cfa, Czm | qnrS | 83 |
| 41        | 2E219A      | Amp, Cro, Cxm, Cfa, Czm | bla<sub>TEM</sub>, bla<sub>CTX-M-15</sub>, qnrS | No plasmid |
| 42        | 2E179H2     | Amp, Fep, Cro, Cxm, Cfa, Czm | bla<sub>TEM</sub>, bla<sub>CTX-M-15</sub>, qnrS | No plasmid |

(Continued)
Phylogrouping
Among the ESBL-E. coli isolates, all phylogenetic groups were represented except for phylogroup F. The predominant phylogenetic group identified was B1 (23/66; 34.8%), followed by D (22/66; 33.3%), E (17/66; 25.7%), B2 and C (2/66; each 3%). Among the 47 multidrug resistant ESBL-E. coli (20/47) were of phylogroup D followed by A (13/47), B1 (12/47), C (2/47). Majority of isolates carrying diarheagenic virulence genes were from B1(14/29; 48%) followed by A(6/29; 20.6%), B2, C, and unknown groups (2/29; each 7%) (Figure 4).

Plasmid Analysis of ESBL-Producing E. coli
Plasmid profiling and conjugation analysis were performed to see whether the antibiotic-resistance genes of the 66 ESBL producing isolates were plasmid-mediated and can they be horizontally transferred. Plasmid number and size were determined using conventional lysis and agarose gel electrophoresis. About 63% (n = 42) isolates carried 1 to 7 plasmids ranging in size from ~1 to 103 MDa (Figure 5), and the distribution of plasmids was heterogeneous (Table 1). Further, the plasmid containing isolates that showed the ESBL phenotype were tested for their ability to transfer the ESBL determinant by conjugation experiments. Nine isolates were able to transfer the cefotaxime resistance marker to a susceptible E. coli recipient with transfer rates ranging from 4.75 × 10^{-6} to 1.19 × 10^{-4} per donor cell (Table 3). Large plasmids (30–103 MDa) were transferred to the sodium azide resistant E. coli-J53 recipient. Whereas, the smaller plasmids (<30 MDa) were not seen to be transferred during conjugation. Among the nine donor isolates, two were able to transfer two plasmids each whereas seven isolates transferred single plasmids (Table 3).

DISCUSSION
In our previous study, we investigated the occurrence of E. coli and fecal coliforms in source and household drinking water samples in Rohingya camps, wherein 10.5% tubewell water and
34.7% POU water samples were found to be contaminated with *E. coli* (26). In the current study, the ESBL-producing *E. coli* isolates from the contaminated drinking water samples of our previous study were characterized concerning antimicrobial susceptibility, dissemination of drug resistance, and pathogenic potential to comprehend the extent of public health threat due to the exposure of contaminated drinking water in Rohingya camps of Bangladesh.

ESBL-producing *E. coli* has been increasingly reported globally, it is not only restricted to clinical settings but also recovered from environmental niches like livestock, wildlife and particularly water (56–58). The pandemic spread of ESBL-producing Gram-negative bacteria is a serious health concern. Human habitation and the surrounding environment in Bangladesh are reportedly contaminated by antimicrobial-resistant bacteria (59, 60). Reports emanating from developing countries like Bangladesh indicated a high prevalence of ESBL-producing *E. coli* in hospital and community drinking water samples (34, 61).

In the current study, we detected 17.2% (66/384) ESBL-producing *E. coli*, of which 71% (47/66) was multidrug-resistant. The high prevalence of multidrug-resistant *E. coli* among the ESBL-producing isolates implies that not only β-lactam antibiotics but resistance to other classes of antibiotics is being co-selected. In Bangladesh, cephalosporins and penicillins are the most commonly used antibiotics (62), which explain why all the 66 ESBL-producing isolates are found to be resistant to both the classes of antibiotics. Besides, the majority of ESBL-producing isolates were found to be resistant to the quinolone class of antibiotics (46 to nalidixic acid). This may reflect the overuse and misuse of antibiotics (63) as these drugs are often sold and distributed over the counter (64). The uncontrolled and unregulated use of antibiotics severely limits the therapeutic options as well as aids the rapid dissemination of resistance in such overpopulated Rohingya camps.

We found ESBL-producing *E. coli* isolates are 100% susceptible to the antibiotics tested of carbapenem, aminoglycoside (amikacin and gentamicin), glycylcycline, and polymyxin groups. This finding was similar to a study in Jordan, where all the *E. coli* isolates from drinking water were sensitive to carpenapem and glycylcycline (65). There might be several factors responsible for susceptibility, such as these drugs are rarely prescribed in Bangladesh (64) and are not readily available in the hard to reach hilly terrain like Rohingya camps.

In this study, most of the isolates were positive for bla*CTX*–M–1 group and bla*CTX*–M–15, genes that concur the previous reports from Bangladesh (59, 60, 66). All bla*CTX*–M–1
ESBL-Producing E. coli in Drinking Water

The gene qnrB has been recognized in various enterobacterial species, such as E. coli and Klebsiella spp. (72–74). Plasmid-mediated quinolone resistance is intervened by the genes (qnr) encoding proteins that protect DNA gyrase and topoisomerase IV against quinolone compounds (75). Among the nonclinical sources, qnr gene was reported in E. coli isolated from swine, livestock and poultry (76, 77). In the present study, 25 isolates harbored plasmid-mediated qnr genes comprising of 22 isolates positive for qnrS and 3 isolates for qnrB genes. Isolates harboring qnrS gene also demonstrated co-existence of blaCTX-M-15 and blaCTX-M-1 group gene. Additionally, they were resistant to a
TABLE 2 | ESBL-producing pathogenic E. coli.

| Serial no. | Isolates ID | Antibiotic resistance pattern | ESBL genes | Virulent genes | Pathotypes |
|------------|-------------|-------------------------------|------------|---------------|------------|
| 1          | 34012H2     | Amp, Fep, Cro, Cem, Cfa, NA, Caz, Cfm | bla<sub>CTX-M-1</sub>, bla<sub>TEM</sub> | st | ETEC       |
| 2          | 05080H2     | Amp, Fep, Cro, Cem, Cfa, Cip, NA, Sxt, Caz, Cfm | bla<sub>CTX-M-1</sub> | aacG | EAEC       |
| 3          | 8E285B      | Amp, Cro, Cem, Cfa, Caz, Cfm | bla<sub>CTX-M-1</sub> | aacG | EAEC       |
| 4          | 8W454H1     | Amp, Fep, Cro, Cem, Cfa, Cip, NA, Sxt, Caz, Cfm | bla<sub>CTX-M-1</sub>, bla<sub>TEM</sub> | aacG, aat | EAEC       |
| 5          | 18544A      | Amp, Cro, Cem, Cfa, NA, Fd, Caz, Cfm | bla<sub>CTX-M-1</sub> | aacG | EAEC       |
| 6          | 18162H2     | Amp, Cro, Cem, Cfa, NA, Fd, Caz, Cfm | bla<sub>CTX-M-1</sub> | aacG | EAEC       |
| 7          | 18544B      | Amp, Cro, Cem, Cfa, NA, Fd, Caz, Cfm | bla<sub>CTX-M-1</sub> | aacG | EAEC       |
| 8          | 1E181H2     | Amp, Fep, Cro, Cem, Cfa, NA, Sxt, Caz, Cfm | bla<sub>CTX-M-1</sub>, bla<sub>TEM</sub> | aacG | EAEC       |
| 9          | 1E370H2     | Amp, Cro, Cem, Cfa, Cip, NA, Fd, Tzp, Sxt, Caz, Cfm | bla<sub>CTX-M-1</sub>, bla<sub>TEM</sub> | aacG | EAEC       |
| 10         | 2W241H2     | Amp, Cro, Cem, Cfa, NA, Sxt, Caz, Cfm | bla<sub>CTX-M-1</sub>, bla<sub>TEM</sub> | aacG, aat | EAEC       |
| 11         | 192B        | Amp, Cro, Cem, Cfa, NA, Caz, Cfm | bla<sub>CTX-M-1</sub>, bla<sub>TEM</sub> | aacG | EAEC       |

range of 7–11 antibiotics, including ciprofloxacin and nalidixic acid. In contrast, all isolates containing qnrS gene also harbored the two ESBL genes, bla<sub>CTX-M-1</sub> group and bla<sub>CTX-M-15</sub>; they were resistant to 8 different antibiotics; most interestingly, they were susceptible to ciprofloxacin. Hence, the presence of qnrS gene alone may not be indicative of the isolate being resistant to fluoroquinolones as also been observed in a previous study (78).

Using PCR for virulence genes, 29 (7%) was found to be pathogenic out of 384 isolates from drinking water. The most prevalent pathotype was EAEC, accountable for 52% of the pathogenic isolates; followed by ETEC, EPEC, and EHEC responsible for 31, 14, and 4% of the pathogenic isolates, respectively. In addition to the diarrheagenic E. coli around 22% of E. coli isolates were at least positive for 1 ExPEC associated virulence genes, moreover 11% (6/55) of the isolates were detected to be potential ExPEC strains. This indicates that the drinking water samples present potential risk of disease epidemic particularly, the diarrheal disease, this assumes more important as in this particular setting where the drinking water is not treated before consumption. Though the reservoir for
EAEC is still unclear, it is generally considered to be human (79–81). The transmission of EAEC is often described as waterborne or foodborne; therefore, it is assumed to be transmitted by the fecal-oral route (82). The presence of ETEC in drinking water and environmental water has been reported previously in Bangladesh; viability after long term water incubation and capacity of biofilm formation might imply that the water is an important transmission route of ETEC (83–85). From the 29 pathogenic isolates, 11 were found to be ESBL positive and surprisingly, 10 of them were of EAEC pathotype. In recent studies of Iran and China, a high prevalence of ESBL in EAEC was also reported (86, 87). The alarming rate of ESBL-producing EAEC isolates recommends strict infection control policies to prevent additional spreading of the virulent and
resistant EAEC strains. All phylogenetic groups were represented in the *E. coli* isolates indicating that they were not homogenous in their population structure instead they belonged to diverse phylogenetic backgrounds, mainly the phylogenetic groups that are associated with commensal (group B1) as well as pathogenic and antimicrobial resistant *E. coli* lineages (group D) were detected. The presence of ESBL producing *E. coli* among the phylogenetic group D strains represents major public health risks due to the spread of such strains via drinking water.

In this study, 64% of the isolates were observed to harbor plasmids ranging from 1 to 103 MDa and a negligible similarity of plasmids pattern among the isolates inferred their clonal diversity due to heterogenous people of diverse geographical origin. Conjugation experiments are important to understand the transfer potential of plasmids conferring extended-spectrum-β-lactamase resistance. It is reported that only plasmids above 35 MDa contain and transfer antibiotic resistance genes via conjugation (18, 88), and In line with other studies, we have also observed plasmid-mediated transfer of antibiotic resistance genes (18, 88). Conjugative plasmids, carrying cefotaxime resistance phenotype among different isolates, ranged from 42 to 103 MDa in size. These findings imply that horizontal gene transfer might worsen the existing antibiotic resistance scenario by speeding up the spread of antimicrobial resistance (AMR) within heterogeneous bacterial communities in environment (89).

Lack of proper sanitation and hygiene in a densely-populated area like Rohingya camps (26) might play a key role in the development and dissemination of AMR. Open defecation with poor personal hygiene, poor community sanitation and lack of controlled antibiotic usage has been reported to exacerbate the transmission of AMR infections (90). A previous study in four middle-income countries, Brazil, Indonesia, India, and Nigeria showed that improvement in water quality and sanitation alone could lead to reduction in antibiotic usage (90). The contamination of drinking water with ESBL-producing *E. coli*, as observed in this study could be due to poor sanitation and hygiene, including, open defecation, inappropriate fecal sludge management, etc. This study has shown that the environmental *E. coli* pose public health threat by being carriers of ESBL-genes. Moreover, these ESBL-producing *E. coli* were harboring virulence factors corresponding to major *E. coli* pathotypes. Limitations of this study include lack of genetic fingerprinting analysis of the antibiotic-resistant *E. coli* from drinking water, lack of exhaustive antimicrobial resistance gene, and extraintestinal pathogenic *E. coli* (ExPEC) virulence gene screening.

In conclusion, the findings of this study suggest that the drinking water samples analyzed herein could serve as an important source for exposure and dissemination of MDR, ESBL-producing and pathogenic *E. coli* variants, which also pose a health risk to the displaced Rohingya population residing in the densely populated camps in Cox’s Bazar. Based on the results of this work we recommend that the policymakers should make considerable efforts in implementing strong infection control strategies by focusing on providing good quality water and ensuring water quality monitoring programs in the Rohingya camps.

**DATA AVAILABILITY STATEMENT**

All datasets generated for this study are included in the article/Supplementary Material.

**ETHICS STATEMENT**

The Ethical Review Committee of International Centre for Diarrhoeal Disease Research, Bangladesh has approved the study.

**AUTHOR CONTRIBUTIONS**

ZM, MI, SH, MW, KI, DJ, and NA planed and organized the study. ZM, MI, SA, MK, MM, TN, SH, MW, DJ, DA, and NA were involved to implement the study. ZM, SA, MK, KI, DA, and NA carried out laboratory work. ZM, MM, MI, SH, DA, and NA were involved to interpret the data. ZM, MK, SA, MM, MI, and NA had a major contribution in writing the manuscript. All authors contributed in proofreading the manuscript.

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**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2020.00228/full#supplementary-material

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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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