Original Article

Detection and antibiotic resistance of diarrheagenic *Escherichia coli* from patients with diarrhea in Ulaanbaatar, Mongolia

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Abstract

Introduction: Diarrheal diseases are common with worldwide distribution, and diarrheagenic *Escherichia coli* (DEC) strains are the main causative agents. The present study aimed to define the association of various pathotypes of *E. coli* from diarrheal patients in Mongolia.

Methodology: A total of 341 *E. coli* strains were isolated from the stool of diarrheal patients. Bacterial susceptibility to antimicrobial agents was determined by the Kirby Bauer disk diffusion method. DEC isolates were identified by HEp-2 cell adherence assay and multiplex polymerase chain reaction (PCR).

Results: DEC pathogens were detected in 53.7% of 341 *E. coli* isolates. Enteroaggregative *E. coli* (EAEC) was the most common DEC pathotype identified by HEp-2 adherence assay and multiplex PCR methods in 97 samples (28.4%), followed by atypical enteropathogenic *E. coli* (EPEC) in 50 samples (14.7%), diffusely adherent *E. coli* (DAEC) in 25 samples (7.3%), enterohaemorrhagic *E. coli* (EHEC) in 6 samples (1.8%), enterotoxigenic *E. coli* (ETEC) in 4 samples (1.2%), and enteroinvasive *E. coli* (EIEC) in 1 sample (0.3%). DEC strains had > 50% antibiotic resistance against cephalothin, ampicillin, and trimethoprim/sulfamethoxazole. All tested DEC strains were susceptible to imipenem. Among the 183 DEC strains, 27 (14.8%) were extended spectrum beta-lactamase producing isolates, and 125 (68.3%) isolates were multiple drug resistant.

Conclusions: We have identified six pathotypes of DEC from the clinical isolates tested and concluded that a high prevalence of antimicrobial resistance was observed in these pathotypes. EAEC was the most common pathotype identified and this is the first report of EHEC identification in Mongolia.

Key words: Mongolia; diarrheagenic *Escherichia coli*; HEp-2 cell adherence assay; multiplex PCR.


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Introduction

Diarrheal diseases are one of the most serious public health problems in the world [1]. Globally, nearly 1.7 billion cases of childhood diarrheal disease are reported annually. In 2019, it accounted for approximately 9% of all deaths worldwide among children under the age of 5 years [2,3]. Diarrheagenic *Escherichia coli* (DEC) strains are known to be one of most common diarrheal disease-causing agents which are commonly spread in low and middle-income countries [4].

The rates of DEC-related infections might be underestimated due to limitations in the ability to identify with the traditional diagnostic methods for DEC identification such as microbiological, biochemical and serotyping tests [5]. In clinical practice, DEC strains are isolated and identified from nonpathogenic flora to reveal the authentic causative agent for diarrheal diseases [6]. Therefore, virulence gene-targeted genotyping assay and bacterial adhesion-based phenotyping assay are the most acceptable methods to identify DEC from nonpathogenic flora [7].

DEC can be identified by polymerase chain reaction (PCR) amplification of virulence genes. In this case, multiplex PCR is a rapid and economical method which reduces the cost and time for screening and identification of DEC [8].

DEC can be classified into six major pathotypes based on their specific virulence properties: enterohaemorrhagic *E. coli* (EHEC), enteropathogenic...
E. coli (EPEC), enteroaggregative E. coli (EAEC), enterotoxigenic E. coli (ETEC), enteroinvasive E. coli (EIEC), and diffusely adherent E. coli (DAEC).[9] Each pathotype possesses specific virulence genes associated with the disease symptoms. For example, stII or lt encoding heat stable enterotoxin (ST) and heat labile enterotoxin (LT) for ETEC, eae encoding intimin for atypical EPEC, bfpA encoding bundle forming pilus for typical EPEC, ipaH encoding invasive plasmid antigen H (IpaH) for EIEC, stx1 or stx2 encoding shiga toxin I and shiga toxin II for EHEC, daaE for DAEC, and aap and aggR encoding the dispersin surface protein, and transcriptional activator for EAEC [10,11]. Besides that, the plasmid-borne aggR gene is an important gene for the pathogenesis and adherence properties of EAEC, where strains possessing the aggR gene are known as “typical EAEC strains” [7].

One of the most powerful phenotypic assays for the diagnosis of DEC is the HEp-2 cell adherence assay [12]. This assay has been recognized as the golden standard for the identification of EAEC and DAEC [7]. According to the assay, EAEC strains are bound in an aggregative adherence (AA) pattern, which is characterized by a stacked brick-like arrangement on the surfaces of the HEp-2 cells as well as on the glass surface between cells [12]. DAEC strains are defined by a pattern of diffuse adherence (DA), in which the bacteria uniformly cover the entire cell surface [13].

In Mongolia, few studies on the prevalence of DEC strains and their role in diarrhea have been reported. Therefore, we have carried out HEp-2 cell adherence assay and PCR with clinical isolates from diarrheal patients in Mongolia to identify DEC-specific virulence factors and their antibiotic resistance, and identified the association of various pathotypes of isolated E. coli.

**Methodology**

**Bacterial strains**

A total of 341 E. coli isolates were isolated from stool samples of diarrheal patients at the National Center for Communicable Diseases. All specimens were processed by routine microbiological and biochemical tests in bacteriological laboratories to identify Salmonella spp., Shigella spp., and Campylobacter spp., and they were also examined for Entamoeba histolytica and Giardia lamblia. All specimens used in this study were negative for the above mentioned bacterial and parasitic pathogens. Bacterial strains were stored at -20 °C in skim milk with glycerol until used.

**HEp-2 cell adherence assay**

341 E. coli isolates were tested for adherence to HEp-2 cells according to the method described by Nataro et al. HEp-2 cells were grown overnight to 50% confluent monolayers on glass coverslips in 24-well tissue culture dishes. The culture medium was discarded, and 20 µL of overnight L-broth bacterial culture mixed with 1 mL of fresh Eagle's minimal essential medium (Gibco, New York, USA) with 0.5% Table 1. Primers used in the multiplex polymerase chain reaction assay and the size of amplicon.

<table>
<thead>
<tr>
<th>Pathotypes of diarrheagenic E. coli</th>
<th>Primer</th>
<th>Sequence (5’ - 3’)</th>
<th>Amplification size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPEC</td>
<td>bfpA</td>
<td>F: GGAAGTCAAAATCTCAGGGGTAT</td>
<td>300</td>
</tr>
<tr>
<td>EPEC</td>
<td>eae</td>
<td>R: GGAATCACAGCGACGTGGATTG</td>
<td>482</td>
</tr>
<tr>
<td>ETEC</td>
<td>Lt</td>
<td>F: GCACACGAGCTCCTACGGTC</td>
<td>218</td>
</tr>
<tr>
<td>ETEC</td>
<td>stII</td>
<td>R: TCCTTCATCTTTCAATGGCCTT</td>
<td>129</td>
</tr>
<tr>
<td>EIEC</td>
<td>ipaH</td>
<td>F: CTCGCGACGTTTTAAATTAGTCTG</td>
<td>933</td>
</tr>
<tr>
<td>EHEC</td>
<td>stx1</td>
<td>R: GTGGAAGCTGAAAGTTCTCCTG</td>
<td>348</td>
</tr>
<tr>
<td>EHEC</td>
<td>stx2</td>
<td>R: GCGTCACTGATACACAGGAGC</td>
<td>584</td>
</tr>
<tr>
<td>EAEC</td>
<td>aap</td>
<td>F: GCAAAAAATTAGTTTATTC</td>
<td>378</td>
</tr>
<tr>
<td>EAEC</td>
<td>aggR</td>
<td>R: AACCCATTCCCGTGGTACAGC</td>
<td>542</td>
</tr>
<tr>
<td>DAEC</td>
<td>DaaE</td>
<td>F: CAGAATACATCAGTACAGT</td>
<td>433</td>
</tr>
</tbody>
</table>

EPEC: enteropathogenic E. coli; ETEC: enterotoxigenic E. coli; EIEC: enteroinvasive E. coli; EHEC: enterohaemorrhagic E. coli; EAEC: enteroaggregative E. coli; DAEC: diffusely adherent E. coli.
D-mannose added to each well. The dishes were incubated at 37 °C in 5% CO₂ for 3 h. After the incubation, the cells were washed twice with phosphate buffered saline, fixed with 70% methanol for 5 min, and then stained with 10% Giemsa (Sigma-Aldrich, Saint Louis, USA) for 15 min. The characteristics of stacked brick, and diffuse adhesion on HEp-2 cells were evaluated on the glass coverslip by light microscopy [14,15].

DNA extraction

*E. coli* isolates were grown in Luria Bertani agar (Difco, Franklin Lakes, USA) at 37 °C overnight. Bacteria were resuspended in sterile distilled water and boiled at 95 °C for 10 min. After centrifugation, the supernatants were stored as DNA templates at -20 °C until used in PCR tests [16].

Detection of DEC virulence genes

Multiplex PCR assays were used to detect the virulence genes of six types of DEC. The minimum criteria for determination of DEC were defined as follows: the presence of *bfpA* and/or *eae* for EPEC, the presence of *lt* or *stII* for ETEC, the presence of *ipaH* for EIEC, the presence of *stx1* and/or *stx2* for EHEC, the presence of *aap* and *aggR* for EAEC, and the presence of *daaE* for DAEC. PCR was performed with the Accupower PCR Premix (Bioneer, Daejeon, South Korea) according to the manufacturer’s instructions. The primer sequence of each virulence gene for PCR was described by Vidal *et al.*., Tokuda *et al.*, and Sarantuya *et al.* [15,17,18]. All primer sequences and amplification sizes of PCR products in our study are listed in Table 1. PCR was performed in 3 sets according to the amplification size and annealing temperature. PCR 1 assay was used to detect the *AggR* gene of EAEC. PCR 2 assay was used to identify DAEC, EPEC, and *aap* gene of EAEC. PCR 3 assay identified ETEC, EIEC, and EHEC. The cycling programs were set as follows: initial denaturation at 95 °C for 12 min, followed by 30 cycles of denaturation at 94 °C for 30 seconds, annealing at 60 °C for 40 seconds, extension at 72 °C for 60 seconds, followed by a final 10 min extension at 72 °C for multiplex PCR sets of 2 and 3. For set 1, the conditions were the same except the annealing conditions were 55 °C for 40 seconds. After amplification, the PCR products were separated by electrophoresis in a 2% agarose gel, stained in ethidium bromide solution, and visualized with a GelDoc 2000 gel documentation system (BioRad, Hercules, USA) [15,17,18].

**Antibiotic Susceptibility Testing**

The disk diffusion method was used to determine antibiotic susceptibility of the clinical isolates on Muller Hinton agar (Difco, Franklin Lakes, USA). Each isolate was tested for antibiotic susceptibility using a panel of the following antibiotics: ampicillin (10 μg), gentamicin (10 μg), cefoxitin (30 μg), cefuroxime (30 μg), ceftazidime (30 μg), cephalothin (30 μg), trimethoprim/sulfamethoxazole (1.25/23.75 μg), ciprofloxacin (5 μg), imipenem (10 μg), and nitrofurantoin (300 μg) (BioLab, Budapest, Hungary). The plates were incubated at 37 °C for 24 h, and inhibition zones were measured. The results were interpreted according to the criteria recommended by Clinical Laboratory Standard Institute (CLSI 2017) [19]. Multiple drug resistance (MDR) was identified as the resistance to at least three different antimicrobial groups. *E. coli* ATCC 25922 strain was used as a control for the drug susceptibility.
**Statistical analysis**

Statistical analysis was performed by using Fisher’s exact test, and the Chi-square test. The level of significance was set at $p$ value of $\leq 0.05$.

**Results**

*E. coli* isolated from the stool samples were further characterized into EAEC, and DPAE pathotypes by using HEp-2 adherence pattern. Out of 341 isolates, 97 (28.4%) had AA adhesion pattern, and 25 (7.3%) had DA adhesion pattern (Figure 1). EAEC strains were classified as typical if they carried a gene encoding for the transcriptional activator aggR, atypical EAEC strains do not encode this gene [20]. Out of 97 isolates that yielded the AA pattern, 17 were only aggR positive, and 2 were aggR+/app+ as detected by PCR. One *E. coli* isolate harbored aggR, and eae genes, which were considered LEE-positive EAEC. 20 (20.6%) strains belonged to the typical EAEC (tEAEC) category, whereas 77 (79.4%) strains were identified as atypical EAEC (aEAEC). Among the 25 DA pattern isolates, only 2 (7.7%) possessed the daaE+ gene.

A total of 341 isolates of *E. coli* were obtained and screened for the presence of eae, bfp, stx 1, stx 2, lt, stII, and ipaH genes by multiplex PCR (Table 2). In the present study, neither *E. coli* isolate harbored bfpA, and eae genes, which were considered LEE-positive EAEC. 20 (20.6%) strains belonged to the typical EAEC category whereas 77 (79.4%) strains were identified as atypical EAEC (aEAEC). Out of 97 DA pattern isolates, 2 (2.1%) possessed the daaE+ gene.

<table>
<thead>
<tr>
<th>Polymerase chain reaction</th>
<th>AggR+/eae+</th>
<th>AggR+</th>
<th>AggR-</th>
<th>Eae+</th>
<th>Eae-/bfp+</th>
<th>stx2+/eae+</th>
<th>Lt+/eae+</th>
<th>Lt+</th>
<th>ipaH+</th>
</tr>
</thead>
<tbody>
<tr>
<td>tEAEC</td>
<td>20</td>
<td>77</td>
<td>2</td>
<td>23</td>
<td>50</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>aEAEC</td>
<td>25</td>
<td>50</td>
<td>6</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Table 2. Detection of diarrheagenic *E. coli* pathotypes by HEp-2 adherence assay and polymerase chain reaction.*

ETEC: enterotoxigenic *E. coli*; aEAEC: atypical enterotoxigenic *E. coli*; DAEC: diffusely adherent *E. coli*; aEPEC: atypical enteropathogenic *E. coli*; EHEC: enterohaemorrhagic *E. coli*; EIEC: enteroinvasive *E. coli*.

One strain included in the present study. According to the HEp-2 adherence assay and multiplex PCR results, EAEC was the most common DEC identified in 97 samples (28.4%), followed by atypical EPEC identified in 50 samples (14.7%), DAEC identified in 25 samples (7.3%), EHEC identified in 6 samples (1.8%), ETEC identified in 4 samples (1.2%), and EIEC identified in 1 sample (0.3%). Thus, the DEC pathogens were detected in 53.7% of 341 *E. coli* isolates.

The results of antimicrobial resistance and multiple drug resistance (MDR) studies of isolates of each DEC pathotype are summarized in Table 3. For all DEC isolates detected, the frequencies of resistance to commonly tested antibiotics were as follows: cephalothin, 155 isolates (84.7%); ampicillin, 134 isolates (73.2%); trimethoprim-sulfamethoxazole, 128 isolates (69.9%); gentamicin and ciprofloxacin, 67 isolates (36.6%); ceftazidime 50 isolates (27.3%); cefuroxime, 26 isolates (14.2%); cefoxitin 16 isolates (8.8%); and nitrofurantoin 7 isolates (3.8%). All DEC strains were susceptible to imipenem. Among the 183 DEC strains, 27 (14.8%) were extended spectrum beta-lactamase (ESBL) producing isolates, and 125 (68.3%) isolates were considered as multiple drug resistant (MDR). The resistance to trimethoprim sulfamethoxazole and ampicillin was statistically associated with the presence of the aggreg gene ($p < 0.05$). DEC strains had $\geq 50\%$ resistance to cephatholin, ampicillin, and trimethoprim/sulfamethoxazole. One
isolate of DAEC was resistant to 9 antibiotics out of the 10 tested. 5 isolates were resistant to 8 antibiotics out of 10, of which 3 were EAEC, 1 was EPEC, and 1 was EIEC.

**Discussion**

DEC is a significant causative agent for development of diarrheal disease throughout the world [23]. DEC strains are usually characterized by phenotypic assays in most laboratories, but it is not possible to identify all six pathotypes of DEC by these methods. DEC can be identified by molecular methods based on the presence of different chromosomal and/or plasmid encoded virulence genes that are absent in commensal *E. coli*. So far, the identification of DEC strains is still required to differentiate it from nonpathogenic members of the normal flora [12]. Thus, to identify DEC strains, factors that determine their virulence should be extensively tested [24]. Further studies evaluating the epidemiological characteristics of HEp-2 cell adherent *E. coli* in diarrheal disease are required, particularly in developing countries [25]. HEp-2 cell adherent *E. coli* strains that show localized adherence (LA), aggregative adherence (AA), diffuse adherence (DA), and localized adherence-like (LAL) patterns have been implicated as diarrheal pathogens [12,15].

In this study, we examined a total of 341 *E. coli* strains isolated from diarrheal stool samples by multiplex PCR and HEp-2 assay, and showed that 28.4%, 14.7%, 7.3%, 1.8%, 1.2%, and 0.3% of them were EAEC, EPEC, DAEC, EHEC, ETEC, and EIEC, respectively. Our results were in agreement with the data reported by Habib *et al.* [26] which shows EAEC (36%), EPEC (26%), ETEC (52.5%), and EHEC (4.3%) as the most commonly recovered pathotypes. Among Nicaraguan children ward aged 0-60 months, an outbreak of DEC was detected (53.8%) in the diarrhea group. Detecting of EAEC, ETEC, EPEC, and EIEC among children with diarrhea were 27.8%, 20.5%, 16.0%, and 0.8%, respectively [27]. In Egypt, a total of 729 children with acute diarrhea were isolated. The most frequently identified DEC were EAEC (30.2%), followed by DAEC (15%), and EPEC (5.2%) [28].

EPEC in the present study accounted for 14.7% of the DEC, of which the incidence was higher compared with the finding of Canizalez-Roman *et al.* (5.1%) in Mexico [20] and Nguyen *et al.* (6.6%) in Vietnam [29]. However, in our findings, presence of EPEC was lower than those reported from China (22.2%) [10] and Iran (47.5%) [30]. These differences may be related to difference in methodology, sampling size, and socioeconomic conditions. In this study, all EPEC strains (50/341) positive for only *eae* gene were aEPEC. aEPEC was more common than tEPEC, in concordance with current data suggesting that aEPEC is more prevalent than tEPEC in both developed and developing countries [31].

EHEC has the ability to produce a hemolytic uremic syndrome which was a life-threatening disease in 15% of patients with 5% mortality rate [32]. In our study, 1.8% were EHEC. This frequency was higher than that in a previously conducted study in Mongolia [15]. In a study in Iran, it was reported that the prevalence of EHEC was 2.8% [32]. In another study conducted in Norway, the rate of EHEC was 0.67% in patients with gastrointestinal symptoms [33]. Bolukaoto *et al.* showed that the frequency of EHEC was 2.3% in patients with diarrhea in South Africa [34].

Shiga toxin-producing *E. coli* (STEC) strains harboring *eae* are suggested to be more pathogenic with a higher risk of developing haemolytic uremic

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**Table 3. Antimicrobial resistance among diarrheagenic *E. coli* strains isolated from diarrhea cases.**

<table>
<thead>
<tr>
<th>Diarrheagenic <em>E. coli</em> pathotypes</th>
<th>tEAEC</th>
<th>aEAEC</th>
<th>aEPEC</th>
<th>DAEC</th>
<th>EHEC</th>
<th>ETEC</th>
<th>EIEC</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>n = 20</strong></td>
<td><strong>n = 77</strong></td>
<td><strong>n = 50</strong></td>
<td><strong>n = 25</strong></td>
<td><strong>n = 6</strong></td>
<td><strong>n = 4</strong></td>
<td><strong>n = 1</strong></td>
<td><strong>n = 183</strong></td>
</tr>
<tr>
<td><strong>Antibiotic resistance, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AM</td>
<td>19 (95)</td>
<td>55 (71.4)</td>
<td>19 (76)</td>
<td>33 (66)</td>
<td>3 (50)</td>
<td>4 (100)</td>
<td>1 (100)</td>
<td>134 (73.2)</td>
</tr>
<tr>
<td>CN</td>
<td>9 (45)</td>
<td>29 (37.7)</td>
<td>7 (28)</td>
<td>18 (36)</td>
<td>1 (16.7)</td>
<td>2 (50)</td>
<td>1 (100)</td>
<td>67 (36.6)</td>
</tr>
<tr>
<td>FOX</td>
<td>2 (10)</td>
<td>9 (11.8)</td>
<td>1 (4)</td>
<td>3 (6)</td>
<td>-</td>
<td>-</td>
<td>1 (100)</td>
<td>16 (8.8)</td>
</tr>
<tr>
<td>CXM</td>
<td>3 (15)</td>
<td>12 (15.6)</td>
<td>3 (12)</td>
<td>7 (14)</td>
<td>-</td>
<td>-</td>
<td>1 (100)</td>
<td>26 (14.2)</td>
</tr>
<tr>
<td>CAZ</td>
<td>5 (25)</td>
<td>22 (28.6)</td>
<td>6 (24)</td>
<td>13 (26)</td>
<td>1 (16.7)</td>
<td>2 (50)</td>
<td>1 (100)</td>
<td>50 (27.3)</td>
</tr>
<tr>
<td>NF</td>
<td>16 (80)</td>
<td>65 (84.4)</td>
<td>22 (80)</td>
<td>42 (84)</td>
<td>5 (83.3)</td>
<td>4 (100)</td>
<td>1 (100)</td>
<td>155 (84.7)</td>
</tr>
<tr>
<td>SXT</td>
<td>19 (95)</td>
<td>53 (68.8)</td>
<td>17 (68)</td>
<td>31 (62)</td>
<td>3 (50)</td>
<td>4 (100)</td>
<td>1 (100)</td>
<td>128 (69.9)</td>
</tr>
<tr>
<td>CIP</td>
<td>3 (15)</td>
<td>30 (39)</td>
<td>7 (28)</td>
<td>23 (46)</td>
<td>1 (16.7)</td>
<td>2 (50)</td>
<td>1 (100)</td>
<td>67 (36.6)</td>
</tr>
<tr>
<td>IPM</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MDR</td>
<td>18 (90)</td>
<td>49 (63.6)</td>
<td>18 (72)</td>
<td>32 (64)</td>
<td>3 (50)</td>
<td>4 (100)</td>
<td>1 (100)</td>
<td>125 (68.3)</td>
</tr>
</tbody>
</table>

**tEAEC:** typical enteroaggregative *E. coli*; **aEAEC:** atypical enteroaggregative *E. coli*; **aEPEC:** atypical enteropathogenic *E. coli*; **DAEC:** diffusely adherent *E. coli*; **EHEC:** enterohemorrhagic *E. coli*; **ETEC:** enterotoxigenic *E. coli*; **EIEC:** enteroinvasive *E. coli*. AM: ampicillin; CN: gentamicin; FOX: cefoxitin; CXM: cefuroxime; CAZ: ceftazidime; KF: cephalothin; SXT: trimethoprim sulfamethoxazole; CIP: ciprofloxacin; IPM: imipenem; F: nitrofurantoin; MDR: multiple drug resistant; (-): not detected.
syndrome (HUS)[35]. We found that 33.3% (2/6) of clinical STEC strains were eae positive. Therefore, it is necessary to focus on improving the detection and diagnosis of EHEC.

In this study, EAEC was the most prevalent pathogen among the DEC categories. EAEC is an emerging enteric pathogen associated with acute and persistent diarrhea and may cause malnutrition and growth defects in children. It has been identified as travelers’ diarrhea in both developing and developed countries, and has been isolated in immunocompromised patients [12,36]. In 2003, Sarantuya et al. reported that the incidence of EAEC was 15.1%, and that EHEC was not found in Mongolian children [15]. Prevalence of EAEC has increased from 15.1% to 28.4%, and EHEC has increased to 1.8% since 2003 in Mongolia. Hegde et al. (31.9%), and Khairy et al. (47%) reported a higher prevalence of EAEC in diarrheal patients, which is similar to our results [24,37]. 20 (20.6%) strains belonged to the typical EAEC (tEAEC) category, whereas 77 (79.4%) strains were identified as atypical EAEC (aEAEC) in our study. Interestingly, AA plasmid-positive EAEC was dominant among children and AA plasmid-negative EAEC was dominant among adults. This result may be because we included people of all ages in our study.

A high incidence of MDR strains was also detected amongst the present isolates. 68.3% of DEC strains demonstrated MDR phenotype and showed resistance to three or more of the tested antibiotics. The most common resistance pattern was cephalothin/ampicillin/co-trimoxasole. Similar results were obtained from other studies [36,38,39]. The rate of MDR in DEC was 39.5% in Qatar [40]. In another study performed in Southwest China, the rate of MDR isolates was 81.1% [41]. MDR causes major consequences such as the empirical therapy of Escherichia coli categorization of diarrheagenic Escherichia coli and a possible co-selection of the antimicrobial resistance which is mediated by MDR plasmids [42]. Selective antibiotic pressure associated with the inappropriate use of antibiotics may be responsible for antimicrobial resistance.

The causative agents of diarrhea should be determined to prevent outbreaks and reduce sporadic cases by taking preventative measures. This study revealed a high prevalence of MDR rate and DEC among clinical isolates of E. coli. The clinical laboratories in Mongolia still use traditional methods for some EPEC and ETEC serotypes [15], hence, DEC strains are probably under-detected. Overall, our findings highlight the importance of the role of DEC isolates in the etiology of diarrhea in Mongolia.

Conclusions
In this study, we have detected all DEC pathotypes (EAEC, DAEC, EPEC, EHEC, ETEC, EIEC) and a high prevalence of antimicrobial resistance. Among them, EAEC was the most frequent pathotype and this is the first report of EHEC in Ulaanbaatar, Mongolia.

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**Conflict of interests:** No conflict of interests is declared.