Phylogenetic of Shiga Toxin-Producing *Escherichia coli* and a typical Enteropathogenic *Escherichia coli* Strains Isolated From Human and Cattle in Kerman, Iran

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**Background:** Shiga toxin-producing *Escherichia coli* (STEC) have emerged as the important zoonotic food-borne pathogens and confirming the risk to public health. Enteropathogenic *Escherichia coli* (EPEC) is a major cause of children diarrhoea in developing countries. *E. coli* strains can be assigned to four main phylogenetic groups, A, B1, B2 and D.

**Objectives:** The aim of the current study was to analyze the distribution of phylogenetic groups and presence of STEC and atypical EPEC pathotypes in *E. coli* isolated from human diarrhoea and fecal samples of healthy cattle in Kerman, Iran by PCR.

**Materials and Methods:** A total of 188 *E. coli* isolates were isolated from human diarrhoeic (94 isolates) and fecal healthy cattle (94 isolates) samples. The isolates were identified by standard bacteriological tests. The confirmed isolates were examined to detect the phylogenetic groups and a selection of virulence genes including *stx*1, *stx*2 and *eae* by PCR.

**Results:** Phylotyping of isolates from diarrhoeic human showed that 38.29% belonged to A, 20.21% to B1, 14.89% to B2 and 26.59% to D phylo groups. The isolates of healthy cattle distributed in A (34.04%), B1 (47.88%), B2 (7.44 %) and D (10.64%) phylo-groups. Prevalence of *eae* gene in human diarrhoeic isolates was 5.32% (5 isolates), whereas none of the human diarrhoeic isolates were positive for *stx*1 and *stx*2 genes. Among cattle isolates 7.44% (7 isolates) were positive for *stx*1 gene and 5.32% (5 isolates) possessed *eae* gene. Of the all isolates examined, none were positive for the *stx*2 gene. The *eae* gene were positive for isolates of human diarrhoea distributed in A and B2 phylo-groups and isolates possessed *stx*1 and *eae* genes from healthy cattle fell into A (4 isolates), B1 (7) and B2 (one isolate).

**Conclusions:** The isolates of human diarrhoea samples and fecal healthy cattle were distributed into different phylogenetic groups, which mostly distributed in A and B phylo-groups. In addition, results of this study revealed the lower prevalence of SETC and aEPEC in isolates.

**Keywords:** *Escherichia coli*; Diarrhea; Shiga-Toxigenic Escherichia coli

1. **Background**

Shiga toxin-producing *Escherichia coli* (STEC) and enteropathogenic *E. coli* (EPEC) produce the characteristic attaching and effacing (A/E) lesions in the gut mucosa of humans and animals (1). Based on the molecular studies, currently EPEC is responsible, on average, for 5-10% of pediatric diarrheal episodes in the developing world. Diarrheagenic *E. coli* have been classified into six categories based on epidemiologic, clinical, and molecular criteria: enteropathogenic *E. coli* (EPEC); entero toxinogenic *E. coli* (ETEC); Shiga toxin–producing *E. coli* (STEC), also known as enterohemorrhagic *E. coli* (EHEC) or verotoxin-producing *E. coli* (VTEC); entero invasive *E. coli* (EIEC); enteroga gregative *E. coli* (EAEC or EAggEC); and diffusely adherent *E. coli* (DAEC) (2). Domestic ruminants, especially cattle and sheep harboring STEC may constitute an important reservoir for STEC infection of humans (3). STEC can cause severe diseases in humans, such as hemorrhagic colitis (HC), hemolytic uremic syndrome (HUS), and thrombocytopenic purpura (TP), which may prove fatal in immunodeficiency patients (4). Typical EPEC strains express *eae* gene, which encodes intimin, and the bundle-forming pili (BFP) responsible for enterocyte attaching and effacing lesions, whereas strains with the A/E genotype which do not possess bfpA2 gene are classified as atypical EPEC (aEPEC) (5). STEC with and without the *eaeA* genotype, may expresses one or two shiga-like toxin encoding genes, *stx*1 and *stx*2 (6). *E. coli* strains can be classified to one of the main phylogenetic groups: A, B1, B2 or D. The

Implication for health policy/practice/research/medical education:

This study carried out in order to determine the prevalence of shiga toxin-producing *Escherichia coli* and atypical enteropathogenic *Escherichia coli* pathotypes and also determined phylogenetic groups of isolates in cattle and human. Transmission of shiga toxin-producing *Escherichia coli* strains is important from food specially cattle to human.

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diarrheagenic *E. coli* strains belong to groups A, B1 and D, the commensal strains to groups A and B1, whilst the extra-intestinal pathogenic strains usually belong to groups B2 and D (7, 8).

2. Objectives

Healthy asymptomatic bovine are the best-recognized animal reservoir for STEC strains. Sources of human infection include primarily foods of cattle origin; mainly uncooked beef products, unpasteurized milk, and direct contact with bovine and person to person transmission. The aim of this study was to analyze the distribution of phylogenetic groups (A, B1, B2 and D) and occurrence of STEC and atypical EPEC pathotypes encoding genes *stx1*, *stx2*, and *eaeA* in *E. coli* isolated from patients with diarrhea and fecal samples of healthy cattle in Kerman, Iran by PCR.

2. Materials and Methods

2.1. Source of the *E. coli* Isolates

A total of 94 *E. coli* isolates were obtained from diarrheic samples of human and 94 isolates were taken from rectal swabs of the healthy cattle. The human samples were related to both male (n=51) and female (n=43). The isolates were collected during 2010 to 2011 in Kerman province, Iran. In the laboratory, samples were cultured on Mac Conkey agar and EMB (Biolife Laboratories, Milano, Italy). *E. coli* isolates were isolated and identified by standard biochemical and bacteriological methods. Isolates were stored in Luria-Bertani broth (Invitrogen, Paisley, Scotland) with 30% sterile glycerol at -20°C.

2.2. Reference Strains

In this study two *E. coli* strains were used as positive controls: *E. coli* Sakai for EHEC and atypical EPEC (*stx1+, stx2+ and eaeA+*) and *E. coli* ECOR62 for (*chuA+, yjaA+* and TspE4.C2+). *E. coli* strain MG1655 was used as a negative control for virulence genes and as a positive control for phylogenetic ECOR group A (*chuA*, *yjaA* and TspE4.C2). All the reference strains were from the bacterial culture collection of Microbiology Department of Ecole Nationale Veterinaire Toulouse, France.

2.3. Pathotype and Phylotype Determination by PCR Assay

DNA of *E. coli* isolates and reference strains was extracted by lysis method (9). The phylogenetic analyses of the isolates were carried out by combinations of three genetic markers *chuA*, *yjaA* and DNA fragment TspE4.C2 by a triplex PCR method (10). All isolates were tested by multiplex PCR assay for the presence of the genes encoding intimin, *stx1* and *stx2* described by China et al. (11). The primers used for amplification of the virulence genes to determine STEC and atypical EPEC pathotypes and phylogenetic groups are presented in Table 1.

4. Results

PCR assays for phylotyping of isolates indicated that 188 *E. coli* isolates from diarrheic samples of human and fecal samples of healthy cattle fall into four phylogenetic groups, whereas 36.17% (68 isolates) fell into A, 34.04% (64) B1, 11.17% (21) B2 and 18.61% (35) D phylogenetic groups. The combinations of three genetic markers *chuA* (279 bp), *yjaA* (211 bp) and TspE4.C2 (152 bp) used in determination of phylogenetic groups. Among 94 *E. coli* isolates from diarr

<table>
<thead>
<tr>
<th>Table 1. Oligonucleotide Primers Used in This Study</th>
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<tbody>
<tr>
<td>EPEC &amp; STEC a</td>
</tr>
<tr>
<td><strong>Gene</strong></td>
</tr>
<tr>
<td>eaeA</td>
</tr>
<tr>
<td>stx1</td>
</tr>
<tr>
<td>stx2</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Phylo-group</td>
</tr>
<tr>
<td><strong>Gene or probe name</strong></td>
</tr>
<tr>
<td>yjaA</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>TspE4.C2</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>chuA</td>
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a Abbreviations: EPEC, enteropathogenic *E. coli*; STEC, shiga toxin-producing *Escherichia coli*
rhic human 36 isolates (38.29%) belonged to A, 19 (20.21%) to B1, 14 (14.89%) to B2 and 25 (26.59%) to D phylo groups. PCR results showed that 94 isolates from healthy cattle distributed into four phylo groups including 32 isolates (34.04%) in A, 45 (47.88%) in B1, 7 (7.44%) in B2 and 10 (10.64%) in D group (Figure 1). Overall seventeen (9.04%) of the 188 E. coli isolates analyzed carried the STEC and aEPEC encoding genes, while stx1 gene (388 bp) were detected in 3.72% (7 isolates) and eae gene (570 bp) in 5.32% (10 isolates) of isolates. Of the all isolates investigated, none were positive for the stx2 gene. Prevalence of eae gene in human diarrheic isolates was 5.32% (5 isolates), whereas none of the human diarrheic isolates were positive for stx1 and stx2 genes. Among 94 E. coli isolates of cattle 7.44% (7 isolates) were positive for stx1 gene and 5.32% (5 isolates) possessed eae gene (Figure 2). Among isolates examined 9.04% isolates were positive for STEC and aEPEC pathotypes, which distributed in A (5 isolates), B1 (7 isolates) and B2 (5 isolates) phylogenetic groups. Among E. coli isolates from human diarrheic samples 5 isolates were positive for eae gene, which distributed in two phylogenetic groups A (one isolate) and B2 (4 isolates) (Table 2). Seven stx positive isolates from healthy cattle fell into A (3 isolates), B1 (3 isolates) and B2 (one isolate) and five isolates were positive for eae gene belonged to A (one isolate) and B1 (4 isolates) phylo-groups (Table 2).

### Table 2. Distribution of STEC and Atypical EPEC Pathotypes in Phylogenetic Groups

<table>
<thead>
<tr>
<th>Gene</th>
<th>Human Diarrhea</th>
<th>Fecal Healthy Cattle</th>
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<tbody>
<tr>
<td></td>
<td>A</td>
<td>B1</td>
</tr>
<tr>
<td>stx1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>stx2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>eae</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>1</td>
<td>-</td>
</tr>
</tbody>
</table>

**Figure 1.** Positive Multiplex PCR Results for the Detection of E. coli Phylogenetic Groups Among the Patients with Diarrhea and Fecal Samples of Healthy Cattle.

**Figure 2.** The Multiplex PCR Results for stx1 and eaeA Genes.
5. Discussion

STEC strains, the cause of human infections, occur after consumption of contaminated food or contact with an infected animal. Cattle are thought to be the main reservoir of STEC and often carry this pathotype in their intestinal flora and serve as source of food contamination (12).

Prevalence of EPEC varies in human related to differences in study population, age group, diagnostic criteria and methods used for diagnosis. Recent studies suggest that atypical EPEC are more prevalent than typical EPEC in both developed and developing countries (2). In the present study, STEC and atypical pathotypes occurred at lower frequencies in human with diarrhea and fecal of healthy cattle in the studied region of Kerman (Iran).

In studies on the capital of Iran (Tehran) 808 isolates which obtained from patients with acute diarrhea 7.92% isolates were positive for STEC and 1.48% for aEPEC, which stx1 and stx2 genes were detected in 34.3% and 43.7% of SETC strains, respectively (13). In the study of Akta et al. (14) on faeces of healthy cattle, sheep and pigs entering abattoirs 8.06% isolates were eae gene positive that presumptively identified A/E. All isolates were positive for eae gene, five of these isolates possessed stx1 gene and none of them were positive for stx2 gene. The results from the present study are in accordance with the mentioned study by Akta et al. (14), which showed none of the faeces of healthy cattle isolates were positive for stx2 gene. In a study on Iran, 29 STEC strains were isolated in northern (Mazandaran province) and southwest (Ilam province), which 28 of them revealed the presence of stx1 and one strain possessed stx2 gene. None of the strains carried the eae gene (15). Reports revealed discrepancies in prevalence of STEC in different countries because this pathogen has not been isolated from diarrhea specimen in human (13, 16). Food-borne outbreaks caused by STEC can affect large numbers of people. Since there is currently no specific treatment for infections of this pathotype, an understanding of the epidemiology of STEC infections is urgently needed. In the current study frequency of aEPEC in human was 5.32%, as was found in study conducted in Thailand (5.5%) (17); however, in Brazil (34%) and Korea (56%) EPEC were detected with high frequency (18). Alikhani et al. (6) reported that aEPEC strains possess the eaeA gene are a common cause of diarrhea in three Iranian provinces, Tehran, Ilam and Mazandaran of Iran. In Spain, distribution of the types of the eae gene among a collection of AECE strains isolated from healthy cattle and healthy sheep was investigated, which healthy sheep isolates were high percentage types of eae gene than healthy cattle isolates (18).

In the current study indicated the distribution of the main phylogenetic groups among E. coli strains isolated from human diarrhea and healthy cattle. Carlos et al. (19) concluded that geographic variation of the E. coli population structure related to different phylogenetic groups.

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Authors’ Contribution

All the authors participated in the manuscript preparation and experiment procedures equally.

Financial Disclosure

There is no conflict of interest.

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References


