Isolation of an *Escherichia coli* O157:H7 strain producing Shiga toxin 1 but not Shiga toxin 2 from a patient with hemolytic uremic syndrome in Korea

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**Abstract**

*Escherichia coli* strains isolated from patients with diarrhea or hemolytic uremic syndrome (HUS) at Pusan University Hospital, South Korea, between 1990 and 1996 were examined for traits of the O157:H7 serogroup. One strain isolated from a patient with HUS belonged to the O157:H7 serotype, possessed a 60-MDa plasmid, the *eae* gene, and ability to produce Shiga toxin 1 but not Shiga toxin 2. Arbitrarily primed PCR analysis suggested that this strain is genetically very close to a O157:H7 strain isolated in Japan. © 1998 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

**Keywords:** Enterohemorrhagic *Escherichia coli*; Shiga toxin; Hemolytic uremic syndrome

1. Introduction

*Escherichia coli* O157:H7 is an important cause of bloody diarrhea and hemolytic uremic syndrome (HUS) [1]. This organism can infect humans through consumption of contaminated food or water or by person-to-person transmission [1]. Ability to produce one or both of two Shiga toxins (Stx: Stx1 and Stx2) and possession of a 60-MDa plasmid and the *eae* gene are considered important virulence-associated traits of this organism [1,2]. Infection by this organism is a serious problem in developed countries and reports on the isolation of this organism in the developing countries are rare [1,3]. Isolation of *E. coli* strains belonging to the O157 serogroup from patients has been reported only from Japan, India, and China within Asia [1,4–6]. Of these strains, only those that were isolated in Japan have been well characterized [5,6]. The report on the isolation of *E. coli* belonging to serogroup O157 in other...
Asian countries is limited so far; we recently reported isolation of *E. coli* O157:H7 strains possessing all the virulence-associated traits from beef marketed in Malaysia [7].

In this study, we examined *E. coli* strains isolated from patients with diarrhea or HUS in a university hospital in South Korea and found an O157:H7 strain. Relatedness of this strain with O157:H7 strains isolated in Japan and Malaysia was investigated by a genetic method.

2. Materials and methods

2.1. Bacterial strains

Korean *E. coli* strains examined in this study were isolated from the patients at Pusan University Hospital, Pusan, South Korea, between 1990 and 1996. The stool specimens of the patients with acute diarrhea or HUS were inoculated onto DHL agar medium (Nissui Pharmaceutical Co., Tokyo, Japan). Representative red and transparent colonies were subjected to standard biochemical tests for identification of *E. coli* [8]. The strains identified as *E. coli* were screened for production of the cytotoxin against cultured Vero cells. The test strains were grown in modified syncase broth and the Vero cytotoxicity assay was carried out as described previously [9] except that the culture supernatant was filtered through a membrane with a pore size of 0.45 μm and only this preparation was used as the test sample. The strains producing the cytotoxin were maintained in a nutrient broth-based semi-solid medium at room temperature. The strains were passed through this medium three to five times and were further characterized as described below during the period between November 1996 and April 1997. *E. coli* O157:H7 strains isolated in Malaysia, Japan, and the United States were reported previously [6,7,10,11].

2.2. Characterization of the strains

The O:H serotypes of the test strains were determined using a commercially available serotyping kit (*Escherichia coli* antisera Seiken; Denka Seiken Co., Tokyo, Japan) according to the manufacturer’s specification. Presence or absence of the *eae* gene and a 60-MDa plasmid in the test strains was determined by the DNA probe method and plasmid profile analysis, respectively, as described previously [7]. Ability of the test strains to produce Stx was determined by three methods: the toxins produced into the culture supernatant were detected by a reversed passive latex agglutination test as reported previously [7]; presence or absence of the *stx* genes was examined by the PCR method as described previously [7] and by the DNA probe method. The DNA probes used to detect the *stx1* and *stx2* genes were, respectively, the VT1- and VT2-specific probes described previously [12]. The probes were used in the DNA colony hybridization test under high stringency conditions as described previously [12].

<table>
<thead>
<tr>
<th>Strain</th>
<th>Year and month of isolation</th>
<th>Patient’s symptom</th>
<th>Serotype</th>
<th>Ability to produce Stx</th>
<th>Presence of 60-MDa plasmid</th>
<th>Presence of <em>eae</em> gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>K-H-1</td>
<td>1995, April</td>
<td>Diarrhea</td>
<td>O143:H–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>K-H-2</td>
<td>1995, April</td>
<td>Diarrhea</td>
<td>OUT:H–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>K-H-3</td>
<td>1995, August</td>
<td>Diarrhea</td>
<td>OUT:H–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>K-H-4</td>
<td>1995, August</td>
<td>Diarrhea</td>
<td>O128:H2</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>K-H-5</td>
<td>1996, June</td>
<td>HUS</td>
<td>O26:H–</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>K-H-6</td>
<td>1996, June</td>
<td>HUS</td>
<td>O111:H21</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>K157</td>
<td>1996, April</td>
<td>HUS</td>
<td>O157:H7</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

*a Strains K-H-5 and K-H-6 were isolated from the same HUS patient.

*b OUT, untypable for O serogroup. H–, nonmotile and thus H serotype could not be determined.

+c, capable; –, incapable. The results of the examination by three different methods (described in the text) were identical.

+d, present; –, absent.
2.3. AP-PCR

Analysis of the test strains by the arbitrarily primed PCR (AP-PCR) method was carried out using purified total DNA of the test strains and commercially available primer sets followed by agarose gel electrophoresis as described previously [7].

The AE 19 and AE 20 primers to amplify a 1087-bp sequence within the eae gene and MSF1F and MSF1R primers to amplify a 166-bp sequence within the 60-MDa plasmid [13] were employed for PCR to ensure the quality of the above DNA preparations used for the AP-PCR. The amplification conditions were as follows: an initial denaturation at 96°C for 5 min followed by 35 cycles, with 1 cycle consisting of 1 min at 94°C, 1 min at 55°C, and 1 min at 72°C, with a final chain elongation at 72°C for 7 min.

3. Results

3.1. Characteristics of Korean strains

One hundred forty-seven strains of E. coli were isolated from patients with diarrhea and three E. coli strains were isolated from HUS patients during the survey period. Of these strains, seven strains gave a positive result in the Vero cytotoxicity assay and were examined for the O:H serotype, presence of the eae gene and a 60-MDa plasmid, and ability to produce Shiga toxins. The results are summarized in Table 1. One strain, designated K157, isolated from a HUS patient was found to be an O157:H7 strain carrying all the virulence-associated traits of O157:H7. Of the other strains, K-H-1, K-H-4, K-H-5, and K-H-6 belonged to the serogroups of enteroinvasive, enteropathogenic, enterohemorrhagic, and enteroaggregative E. coli categories, respectively [2]. K-H-4 and K-H-5 carried the eae gene, one of the important virulence genes of their respective pathogenic E. coli groups [2]. In addition, K-H-5 carried a 60-MDa plasmid. However, none of the strains other than K157 had the ability to produce Stx. The results of the PCR assay for the stx genes are shown in Fig. 1.

3.2. Comparison of Korean, Japanese, and Malaysian O157:H7 strains

The Korean O157:H7 strain, K157, was compared with the O157:H7 strains isolated in other Asian countries by the AP-PCR method (Fig. 2). The strains were selected from available Japanese and Malaysian strains; the Japanese strains represented five major genetic groups as analyzed by a pulsed field gel electrophoresis method [6] and the Malaysian strains represented five different genetic types as determined by the AP-PCR method [7]. EDL933, a standard O157:H7 strain isolated in the USA [10,11],
was also included. Four different primers were used and the profiles of the amplicons were compared (Fig. 2A–D). The quality of the DNA template preparations used for the AP-PCR was shown to be acceptable by examining the same preparations for PCR amplification of the chromosomal eae gene (Fig. 2E) and the 60-MDa plasmid sequence (Fig. 2F). Comparative analysis of the results obtained with four primers (Fig. 2A–D) confirmed that the Japanese strains (lanes 5–9) and Malaysian strains (lanes 10–14) were genetically diversified within each group except that two Malaysian strains (lanes 10 and 11) could not be distinguished in this analysis. K157 (lane 4) was judged to be almost indistinguishable from a Japanese strain, 2 (lane 5) and closely related to EDL933 (lane 3) and a Malaysian strain MA17 (lane 12).

4. Discussion

In this communication, we report for the first time the isolation from a HUS patient of an E. coli O157:H7 strain in Korea. This strain, K157, had all important virulence traits of O157:H7 serogroup. However, of the traits, ability to produce Stx was unusual in that this strain can produce only Stx1. Strains producing only Stx1 are rare among E. coli O157:H7 strains isolated from HUS patients [14–16] and Stx2 is considered more important than Stx1 for progression of E. coli O157:H7 infection to HUS [1,2]. Almost all O157:H7 strains isolated in Japan and all O157:H7 strains isolated in Malaysia produced Stx2 with or without Stx1 [6,7]. We wished to examine whether K157 is significantly different from these strains by the AP-PCR analysis. The results suggest that K157 is genetically very close to one of the O157:H7 strains isolated in Japan. Therefore, K157 does not seem to be a unique O157:H7 strain. Of the seven Korean strains that were cytotoxic to Vero cells (Table 1), only K157 possessed the stx gene. Ability of the test strains to produce Stx was examined after subcultivation. The stx genes could possibly be lost from E. coli upon subcultivation [17]. Therefore, the possibility that K157 originally had the stx2 gene but lost this gene upon subcultivation cannot be ruled out.

Acknowledgments

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References


