Case Report

A Case of a Shiga Toxin Producing Escherichia Coli

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We encountered a patient with hemolytic uremic syndrome (HUS) with persistent isolation of shiga toxin-producing Escherichia coli (STEC) for 3 weeks despite of having no clinical symptoms. STEC has been recognized as an important food-borne pathogen that causes severe diseases such as HUS. We characterized this STEC strain via a polymerase chain reaction, reverse-passive latex agglutination and the slide agglutination method. In this STEC strain, stx2 (shiga toxin), eaeA, tir, iha (adherence genes), espADB (type III secretion genes), and hlyA, ehxA, clyA (hemolysin genes) were present. The O antigen of the strain was non-typable.

Key Words: Shiga toxin-producing Escherichia coli, hemolytic uremic syndrome, non-typable serotype STEC strain

INTRODUCTION

Shiga toxin-producing Escherichia coli (STEC) O157:H7 has been recognized as an important food-borne pathogen that causes severe diseases such as a hemolytic uremic syndrome (HUS).¹ The majority of cases of this disease are caused by strains of the serotype O157:H7, but infections by enterohemorrhagic Escherichia coli (EHEC) strains belonging to serogroups other than O157, such as O26, O103, O111 and O145, have been reported with increasing frequency.¹,² Shiga toxins (stxs) are the virulence factor of EHEC. The two groups consist of stx1 and stx2.³ Apart from stxs, there are various virulence factors of EHEC, eae (Intimin), tir (translocated intimin receptor), hlyA (EHEC hemolysin), and espADB (type III secretion proteins).¹,⁴ The locus for enterocyte effacement (LEE) is associated with intimate adherence to epithelial cells.⁵ Several protein were proposed to be adhesion factors in LEE-negative strains, including iha (an adherence-conferring protein),⁶ efa1 (an EHEC factor for adherence),⁷ Saa (an autoagglutinating adhesin)⁸ and toxB (a protein from 93-kbpplasmid pO157), which are required for O157:H7 strain Sakai expression of adherence.⁹ E. coli hemolysin (hlyA), enterohemorrhagic E. coli toxin (ehx) and cytolyisin A (clyA) are well known as repeats in the toxin (RTX) family.¹⁰⁻¹²

In Korea, we encountered a patient who had been hospitalized for a long-time, due
to long-term isolation of STEC. We conducted molecular and serotype analyses on this patient.

**CASE REPORT**

A three-year-old child was admitted to Uijeongbu St. Mary’s Hospital with abdominal pain and watery/bloody diarrhea 3-4 days after eating boiled fish paste. Physical examination upon admission was unremarkable. The abdomen computerized tomography image showed prominent submucosal edema of the total colon. Hematological tests revealed: hemoglobin 12.6 g/dL, leukocyte count 43.5×10⁹/L with neutrophilia (90%), platelet count 33×10⁹/L. The results of a blood chemistry study of the serum samples were blood urea nitrogen, 39.1 mg/dL, albumin 1.7 g/dL and CRP 5.03 mg/dL. The serum creatinine level was increased from 0.44 mg/dL to 2.33 mg/dL. Urinalysis showed four positive tests for hematuria and proteinuria. A diagnosis of HUS was made and the patient was transferred to another hospital, where she underwent hemodialysis.

She was admitted to our hospital a second time due to persistent STEC isolation, although her clinical symptoms were improved. We isolated the STEC strain from the patient’s stool. However, no other pathogenic bacteria such as *Salmonella* spp., *Shigella* spp., and *Vibrio* spp. were detected in the stool. The isolate was biochemically characterized using the API20E system (Biomerieux, Marcy l’Etoile, France). The isolate was directly inoculated into 3 mL of Luria-Bertani (LB) broth, and this was incubated overnight at 37°C. After incubation, the enriched broth culture was centrifuged at 13,000 rpm (Sorvall Biofuge Pico, Germany) for 1 min and the pellet was heated at 100°C for 10 min. Following centrifugation, 5 µL of the supernatant was used in the PCR assays. The PCR assays were performed using the primers shown in Table 1. The PCR assays were carried out in a 50 µL volume with 2 U DNA *Taq* polymerase (Takara Ex *Taq*™, Otsu, Japan) in a thermal cycler (PTC-100; MJ Research, Watertown, MA, USA). Positive DNA and distilled water were used as positive and negative controls.

Production of *stx1* and *stx2* was determined using a reversed passive latex agglutination kit (VTEC-RPLA; Denka Seiken Co., Ltd., Tokyo, Japan). One mL of the overnight culture was centrifuged and the titer of the supernatant was determined using the VTEC-RPLA test, which was performed at dilutions up to 1 : 256. The PCR and RPLA test showed that the strain produced only *stx2*. The adherence genes, *eaeA* and *tir* genes and non-LEE adhesion genes such as the *iha*, *saa*, *efa1* and *txxB* genes, were analyzed in the STEC strain. The *eaeA*, *tir*, and *iha* genes were present in the strain (Table 1). All the genes tested for type III secretion proteins, i.e., *espADB* were found in the strain in the PCR analysis. PCR analysis showed that the *hlyA*, *ehxA*, and *clyA* genes for hemolysin production were detected in the strain (Table 1).

The presence of O antigens was determined by slide agglutination with the available O (O1-O181) antisera (Universidad de Santiago de Compostela, Lugo, Spain). We performed serotyping of the strain with the antisera. However, the serotype of the strain was non-typable with the tested antisera.

Antimicrobial susceptibility of the isolate to the following 16 antibiotics was determined by agar disk diffusion (Kirby-Bauer method) using Mueller-Hinton agar (Difco) : ampicillin/sulbactam (SAM), ampicillin (AM), tetracycline (TE), aztreonam (ATM), cefotetan (CTT), cefepime (FEP), cefoxitin (FOX), cefotaxime (CTX), tobramycin (NN), trimethoprim/sulfamethoxazole (SXT/TEM), cephalothin (CF), imipenem (IPM), gentamicin (GM), amikacin (AN), piperacillin/tazobactam (TZP), netilamycin (NET). *E. coli* ATCC 25922 and *E. coli* ATCC 35218 were used as quality controls. As shown in Table 2, the isolate showed resistance to Ampicillin, Tetracycline and Trimethoprim-sulfamethoxazole, while it was sensible to two antibiotics of β-lactam/β-lactamase inhibitor combinations and Imipenem. Among the Cephalosporins, the isolate showed resistance only to Cephalothin. On hospital day (HD) 17, cefuroxime was started due to persistent STEC isolation. On HD38, cefepime was started for four days. On HD 49, she was discharged after having negative results for stool STEC isolation for a week.

**DISCUSSION**

In the present study, we characterized the virulence genes of the STEC strain. Our results showed that the strain in this study possessed many virulent factors for infection, for example, toxins, adhesins and hemolysins.

The Shiga-toxin genotype of the infecting strain may influence the risk of developing microangiopathic sequelae and the outcome of infection. Patients infected with STEC 0157 possessing *stx2* but not *stx1* had significantly developed systemic sequelae, including HUS, than patients infected with STEC 0157 harboring *stx1* alone or both *stx1* and *stx2*.
and stx2.\textsuperscript{14} The strain in this study possessed only stx2 gene among stx genes (Table 1).

The stx2 toxin has been described as being 1,000 times more cytotoxic than the stx1 toxin, and was related with high virulence in STEC strains from HUS patients.\textsuperscript{15} The adherence of bacteria is important for the infection. Enteropathogenic \textit{E. coli} (EPEC) and EHEC produce an adherence factor, intimin encoded by the \textit{eae} gene.\textsuperscript{16} This gene is detected in the locus of enterocyte effacement (LEE), which is required for attachment-effacement lesions. Another study showed that the most widely distributed STEC adhesin gene was the \textit{iha} gene.\textsuperscript{17} The LEE gene encodes the type III secretion system.\textsuperscript{4} The strain possessed all the tested genes related with the hemolysin. HlyA lyses cells via the creation of pores in the cell membrane and this affects erythrocytes, leukocytes, and renal tubular cells.\textsuperscript{12} The HlyA is frequently produced by \textit{E. coli} strains that cause urinary tract infections, while \textit{ehxA} is found in the EHEC strains of serogroup O157.\textsuperscript{16} ClyA is the prototype of pore-forming cytotoxins.\textsuperscript{18}

Although STEC O157:H7 is known to be the predominant serotype associated with most STEC-related outbreaks worldwide, previous epidemiological studies have implied that non-O157 STEC infection is becoming problematic in public health.\textsuperscript{19} Among those non-O157 STEC associated with human disease are the serotypes O8:H-, O26:H11, O91:H21, O103:H2, O111:H-, O113:H21, and O128:H2.\textsuperscript{1} The O antigen of the strain was nontypable. From this result, we suggest that a nontypable serotype might play an important role in causing a long-term carrier state.

There have been several reports of Stx producing \textit{E. coli}

| Description                  | Target genes | Primer sequence (5' to 3')          | Size of PCR product (bp) | Presence of target gene |
|------------------------------|--------------|-------------------------------------|--------------------------|-------------------------|
| Shiga toxin genes            | stx1         | CGTACCGGGATGCGAGATAATCGGC          | 210                      | No                      |
|                              | stx2         | GTCCTGCGTTTTGTCAGTGCAC             | 326                      | Yes                     |
| LEE adhesion genes           | eaeA         | ATGCCTGCAATGTTGTCAGGTCG            | 233                      | Yes                     |
|                              | tir          | GCTTGCAGTCCATTTATCC               | 107                      | Yes                     |
| Non-LEE adhesion genes       | iha          | CAGTTTCAGTTTTCGATCCACC             | 1,305                    | Yes                     |
|                              | saa          | CGTGATGGACTGCTGCTCGG              | 119                      | No                      |
|                              | toxB         | ATACCTACCTGCTCTGATGTCG            | 602                      | No                      |
| Type III secretion genes     | espA         | GTTTTTTACGGTCTGGATTCT             | 187                      | Yes                     |
|                              | espD         | AAAAACGACGCTCGAAGAAACA            | 145                      | Yes                     |
|                              | espB         | GCGGTTTTTGGAGGCCAGAAA             | 106                      | Yes                     |
| Genes for hemolysin          | hlyA         | GCAATCATCAACGGCTGCTGCC            | 519                      | Yes                     |
|                              | ehxA         | GGTCGCCAGAGAAAAAGTTGAGT           | 1,551                    | Yes                     |
|                              | clyA         | GAGGCGAATGATTGACTG                | 920                      | Yes                     |

PCR, polymerase chain reaction; bp, base pair; LEE, locus for enterocyte effacement.
from asymptomatic human carriers.20,21 The patient may have produced antibodies against the STEC strain during the long-term infection. However, further study of the mechanisms of long-term isolation in the patient is needed.

In conclusion, we suggest that this result provides epidemiological information for a prototype of a molecular mechanism showing how the STEC strain can remain in a patient for a long time without causing symptoms.

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