Case Report

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A Case of a Shiga Toxin Producing *Escherichia Coli*

Seung-Hak Cho,[1*] Jung-Beom Kim,[2*] Yong-Bae Park,[2] Mi-Sun Park,[1]
Hiun Suk Chae,[3] and Hae Kyung Lee[4]

[1]Division of Enteric Bacterial Infections, Center for Infectious Diseases, Korea National Institute of Health, Osong;
[2]Division of Health Research and Planning, Gyeonggi-do Research Institute of Health and Environment, Suwon;
Departments of [3]Internal Medicine and [4]Laboratory Medicine, Uijeongbu St. Mary's Hospital,
The Catholic University of Korea College of Medicine, Uijeongbu, Korea.

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Corresponding author: Dr. Hae Kyung Lee,
Department of Laboratory Medicine,
The Catholic University of Korea
College of Medicine,
65-1 Geumo-dong, Uijeongbu 480-717, Korea.
Tel: 82-31-820-3159, Fax: 82-31-847-6266
E-mail: hkl@catholic.ac.kr

*Seung-Hak Cho and Jung-Beom Kim
contributed equally to this work.

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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), *eae*A, *tir, iha* (adherence genes), *esp*ADB (type III secretion genes), and *hly*A, *ehx*A, *cly*A (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, O111and 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), *hly*A (EHEC hemolysin), and *esp*ADB (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] *efa*1 (an EHEC factor for adherence),[7] *Saa* (an autoagglutinating adhesin)[8] and *tox*B (a protein from 93-kbplasmid pO157), which are required for O157:H7 strain Sakai expression of adherence.[9] *E. coli* hemolysin (*hly*A), enterohemorrhagic *E. coli* toxin (*ehx*) and cytolysin A (*cly*A) 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 43.5×10^9/L with neutrophilia (90%), platelet count 33×10^9/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 37ºC. 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 100ºC 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 *stx*1 and *stx*2 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 *stx*2. The adherence genes, *eae*A and *tir* genes and non-LEE adhesion genes such as the *iha*, *saa*, *efa*1 and *tox*B genes, were analyzed in the STEC strain. The *eae*A, *tir*, and *iha* genes were present in the strain (Table 1). All the genes tested for type III secretion proteins, i.e., *esp*ADB were found in the strain in the PCR analysis. PCR analysis showed that the *hly*A, *ehx*A, and *cly*A 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).[13] We performed serotyping of the stain 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/TM), cephalothin (CF), imipenem (IPM), gentamicin (GM), amikacin (AN), piperacillin/tazobactam (TZP), netilamicin (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 Cephems, 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 *stx*2 but not *stx*1 had significantly developed systemic sequelae, including HUS, than patients infected with STEC 0157 harboring *stx*1 alone or both *stx*1

and *stx*2.[14] The strain in this study possessed only *stx*2 gene among *stx* genes (Table 1).

The *stx*2 toxin has been described as being 1,000 times more cytotoxic than the *stx*1 toxin, and was related with high virulence in STEC strains from HUS patients.[15] The adherence of bacteria is important for the infection. Enteropathogenic *E. coli* (EPEC) and EHEC produce an adherence factor, intimin encoded by the *eae* gene.[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 *iha* gene.[17] The LEE gene encodes the type III secretion system.[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 tubularcells.[12] The HlyA is frequently produced by *E. coli* strains that cause urinary tract infections, while *ehx*A is found in the EHEC strains of serogroupO157.[10] ClyA is the prototype of pore-forming cytotoxins.[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.[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.[1] The O antigen of the strain was non-typable. From this result, we suggest that a non-typable serotype might play an important role in causing a long-term carrier state.

There have been several reports of Stx producing *E. coli*

Table 1. Detection of Virulence Genes in the Shiga Toxin Producing *Escherichia Coli* (STEC) Strain Isolated from a Long-Term Hospitalized Patient

Description	Target genes	Primer sequence (5' to 3')	Size of PCR product (bp)	Presence of target gene
Shiga toxin genes	*stx*1	CGTACGGGGATGCAGATAAATCGC CAGTCATTACATAAGAACGCCCAC	210	No
	*stx*2	GTTCTGCGTTTTGTCACTGTCAC GTCGCCAGTTATCTGACATTCTGG	326	Yes
LEE adhesion genes	*eae*A	ATGCTGGCATTTGGTCAGGTCGG TGACTCATGCCAGCCGCTCATGCG	233	Yes
	tir	GCTTGCAGTCCATTGATCCT GGGCTTCCGTGATATCTGA	107	Yes
Non-LEE adhesion genes	*iha*	CAGTTCAGTTTCGCATTCACC GTATGGCTCTGATGCGATG	1,305	Yes
	saa	CGTGATGAACAGGCTATTGC ATGGACATGCCTGTGGCAAC	119	No
	*tox*B	ATACCTACCTGCTCTGGATTGA TTCTTACCTGATCTGATGCAGC	602	No
	*efa*1	GAGACTGCCAGAGAAAG GGTATTGTTGCATGTTCAG	479	No
Type III secretion genes	*esp*A	GTTTTTCAGGCTGCGATTCT AGTTTGGCTTTCGCATTCTT	187	Yes
	*esp*D	AAAAAGCAGCTCGAAGAACA CCAATGGCAACAACAGCCCA	145	Yes
	*esp*B	GCCGTTTTTGAGAGCCAGAA AAAGAACCTAAGATCCCCA	106	Yes
Genes for hemolysin	*hly*A	GCATCATCAAGCGTACGTTCC AATGAGCCAAGCTGGTTAAGCT	519	Yes
	*ehx*A	GGTGCAGCAGAAAAAGTTGTAG TCTCGCCTGATAGTGTTTGGTA	1,551	Yes
	*cly*A	GAGGCGAATGATTATGACTG ACTTCAGGTACCTCAAAGAG	920	Yes

PCR, polymerase chain reaction; bp, base pair; LEE, locus for enterocyte effacement.

Table 2. Antibiotic Resistance Patterns of the Shiga Toxin Producing *Escherichia Coli* (STEC) Isolate

Antimicrobial agents	Antibiotic resistance
ß-lactams	
Ampicillin (AM)	R
ß-lactam/ß-lactamase inhibitor combinations	
Ampicillin-sulbactam (SAM)	S
Piperacillin/tazobactam (TZP)	S
Cephems	
Cephalothin (CF)	R
Cefepime (FEP)	S
Cefotetan (CTT)	S
Cefotaxime (CTX)	S
Cefoxitin (FOX)	S
Carbapenems	
Imipenem (IPM)	S
Aminoglycosides	
Amikacin (AN)	S
Gentamicin (GM)	R
Tobramycin (NN)	R
Netilamicin (NET)	S
Tetracyclines	
Tetracycline (TE)	R
Monobactams	
Aztreonam (ATM)	S
Folate pathway inhibitors	
Trimethoprim-sulfamethoxazole (SXT)	R

R, resistant; S, sensible.

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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REFERENCES

1. Nataro JP, Kaper JB. Diarrheagenic Escherichia coli. Clin Microbiol Rev 1998;11:142-201.
2. Tozzi AE, Caprioli A, Minelli F, Gianviti A, De Petris L, Edefonti A, et al. Shiga toxin-producing Escherichia coli infections associated with hemolytic uremic syndrome, Italy, 1988-2000. Emerg Infect Dis 2003;9:106-8.
3. Melton-Celsa AR, O'Brien AD. Animal models for STEC-mediated disease. Methods Mol Med 2003;73:291-305.
4. Makino S, Tobe T, Asakura H, Watarai M, Ikeda T, Takeshi K, et al. Distribution of the secondary type III secretion system locus found in enterohemorrhagic Escherichia coli O157:H7 isolates among Shiga toxin-producing E. coli strains. J Clin Microbiol 2003;41:2341-7.
5. Clarke SC, Haigh RD, Freestone PP, Williams PH. Virulence of enteropathogenic Escherichia coli, a global pathogen. Clin Microbiol Rev 2003;16:365-78.
6. Tarr PI, Bilge SS, Vary JC Jr, Jelacic S, Habeeb RL, Ward TR, et al. Iha: a novel Escherichia coli O157:H7 adherence-conferring molecule encoded on a recently acquired chromosomal island of conserved structure. Infect Immun 2000;68:1400-7.
7. Nicholls L, Grant TH, Robins-Browne RM. Identification of a novel genetic locus that is required for in vitro adhesion of a clinical isolate of enterohaemorrhagic Escherichia coli to epithelial cells. Mol Microbiol 2000;35:275-88.
8. Paton AW, Srimanote P, Woodrow MC, Paton JC. Characterization of Saa, a novel autoagglutinating adhesin produced by locus of enterocyte effacement-negative Shiga-toxigenic Escherichia coli strains that are virulent for humans. Infect Immun 2001;69:6999-7009.
9. Tatsuno I, Horie M, Abe H, Miki T, Makino K, Shinagawa H, et al. toxB gene on pO157 of enterohemorrhagic Escherichia coli O157:H7 is required for full epithelial cell adherence phenotype. Infect Immun 2001;69:6660-9.
10. Welch RA, Forestier C, Lobo A, Pellett S, Thomas W Jr, Rowe G. The synthesis and function of the Escherichia coli hemolysin and related RTX exotoxins. FEMS Microbiol Immunol 1992;5:29-36.
11. Bauer ME, Welch RA. Characterization of an RTX toxin from enterohemorrhagic Escherichia coli O157:H7. Infect Immun 1996;64:167-75.
12. König B, Ludwig A, Goebel W, König W. Pore formation by the Escherichia coli alpha-hemolysin: role for mediator release from human inflammatory cells. Infect Immun 1994;62:4611-7.
13. Guinée PA, Agterberg CM, Jansen WH. Escherichia coli O antigen typing by means of a mechanized microtechnique. Appl Microbiol 1972;24:127-31.
14. Ostroff SM, Tarr PI, Neill MA, Lewis JH, Hargrett-Bean N, Kobayashi JM. Toxin genotypes and plasmid profiles as determinants of systemic sequelae in Escherichia coli O157:H7 infections. J Infect Dis 1989;160:994-8.
15. Tarr PI, Gordon CA, Chandler WL. Shiga-toxin-producing Escherichia coli and haemolytic uraemic syndrome. Lancet 2005;365:1073-86.
16. Jerse AE, Gicquelais KG, Kaper JB. Plasmid and chromosomal elements involved in the pathogenesis of attaching and effacing Escherichia coli. Infect Immun 1991;59:3869-75.
17. Toma C, Martínez Espinosa E, Song T, Miliwebsky E, Chinen I, Iyoda S, et al. Distribution of putative adhesins in different seropathotypes of Shiga toxin-producing Escherichia coli. J Clin Microbiol 2004;42:4937-46.
18. Oscarsson J, Westermark M, Löfdahl S, Olsen B, Palmgren H, Mizunoe Y, et al. Characterization of a pore-forming cytotoxin ex-

pressed by Salmonella enterica serovars typhi and paratyphi A. Infect Immun 2002;70:5759-69.
19. Coombes BK, Wickham ME, Mascarenhas M, Gruenheid S, Finlay BB, Karmali MA. Molecular analysis as an aid to assess the public health risk of non-O157 Shiga toxin-producing Escherichia coli strains. Appl Environ Microbiol 2008;74:2153-60.
20. Mukherjee J, Chios K, Fishwild D, Hudson D, O'Donnell S, Rich SM, et al. Human Stx2-specific monoclonal antibodies prevent systemic complications of Escherichia coli O157:H7 infection. Infect Immun 2002;70:612-9.
21. Stephan R, Untermann F. Virulence factors and phenotypical traits of verotoxin-producing Escherichia coli strains isolated from asymptomatic human carriers. J Clin Microbiol 1999;37:1570-2.