Emergence of a Novel Shiga Toxin-Producing *Escherichia coli* O Serogroup Cross-Reacting with *Shigella boydii* Type 10[†]

Atsushi Iguchi,^{1*} Sunao Iyoda,² Kazuko Seto,³ and Makoto Ohnishi²
on behalf of the EHEC Study Group‡

Interdisciplinary Research Organization, University of Miyazaki, Miyazaki, Japan¹; Department of Bacteriology I, National Institute of Infectious Diseases, Tokyo, Japan²; and Division of Bacteriology, Osaka Prefectural Institute of Public Health, Osaka, Japan³

Received 16 June 2011/Returned for modification 2 August 2011/Accepted 17 August 2011

This is the first report of the isolation of Shiga toxin-producing *Escherichia coli* (STEC) strains whose O antigens were genetically and serologically identical to those of *Shigella boydii* type 10, from human feces. The novel STEC O serogroup may be widespread in Japan and associated with diarrhea and hemorrhagic colitis.

Shiga toxin-producing *Escherichia coli* (STEC) represents one of the most important groups of food-borne pathogens worldwide, as it can cause gastroenteritis that may be complicated by hemorrhagic colitis or hemolytic-uremic syndrome (HUS) (9). More than 170 O serogroups of *E. coli* have been identified (13), and the serotyping of *E. coli* strains with O somatic antigens and H flagellar antigens is an effective method for identifying strains with pathogenic potential and classifying them into clonal groups. Most STEC outbreaks and sporadic cases have been attributed to O157 strains (5, 11), but infections caused by other STEC O serogroups (including O26, O91, O103, O104, O111, and O145) have also been associated with severe illness (1, 6, 12).

STEC O serogroup-untypeable (OUT) strains have been isolated from patients with diarrhea, hemorrhagic colitis, and HUS (8, 15), and these strains are also a threat to human health. Unlike for other STEC strains, the clonal relatedness of STEC OUT strains could not be predicted without further

genetic examination, because O serogrouping as our primary classification of STEC was unavailable for them. Additionally, their characteristics, such as prevalence and pathogenicity, are poorly understood. In the search for the O antigen of STEC OUT strains, we noticed that the O antigen of a STEC strain was genetically and serologically identical to that of *Shigella boydii* type 10. Here, we investigated the prevalence of the STEC strain expressing the type 10 O antigen of *S. boydii* in Japan.

A Shiga toxin 1 (Stx1)-producing *E. coli* strain (EHOUT32) was isolated from a patient with hemorrhagic colitis in Miyazaki prefecture, Japan, in 2008. EHOUT32 had a typical *E. coli* biochemical profile as determined by API-20E (bio-Mérieux, Marcy l'Etoile, France) or by standard agar reagents (all from Nissui Pharmaceutical Co., Ltd., Tokyo, Japan). For example, lactose, sucrose, sorbitol, and fucose were fermented, lysine was decarboxylated, and gas production and motility were confirmed. PCR-based screens for *E. coli* and *Shigella* species virulence-related genes (*stx*₁, *stx*₂, *eae*, *ehxA*, *bfpA*, *elt*, *est*, *astA*, *aggR*, or *ipaH*) (see Table S1 in the supplemental material) showed that EHOUT32 carried only *stx*₁. Serotyping was performed by conventional slide agglutination tests using antisera against all recognized *E. coli* O antigens (O1 to O181 except O31, O47, O67, O72, O93, O94, and O122 [purchased from Statens Serum Institut {SSI}, Copenhagen, Denmark]). The O serogroup of EHOUT32 was untypeable, but the strain's H antigen was determined as H18 (H-antigen antisera from SSI).

To gain information about the genetic characteristics of the O antigen in EHOUT32, we amplified the chromosomal region containing the O-antigen biosynthesis gene cluster using a primer pair, O55re-1F and O55re-1R (3). The PCR product (ca. 18 kb) was sequenced by the shotgun method. The gene organization of the O-antigen gene cluster was identical to that of *S. boydii* type 10 (14), and sequence comparison showed that their deduced amino acid sequences were highly conserved between the strains (Fig. 1). Consistent with the sequence analysis, EHOUT32 serologically reacted with anti-*S. boydii* type 10 O-antigen serum. We provisionally name its O serogroup OSB10 (*S. boydii* type 10) in *E. coli*. A specific primer pair against the *wzx*_{OSB10} gene on the OSB10-antigen gene

* Corresponding author. Mailing address: Interdisciplinary Research Organization, University of Miyazaki, 5200 Kihara, Miyazaki 889-1692, Japan. Phone and fax: 81-985-85-9765. E-mail: iguchi@med.miyazaki-u.ac.jp.

‡ Members of the EHEC Study Group include Reiko Arai (Niigata Prefectural Institute of Public Health and Environmental Sciences), Nanami Asoshima (Fukuoka City Institute for Hygiene and the Environment), Midori Hiroi (Shizuoka Institute of Environment and Hygiene), Kazumi Horikawa, Yoshiki Etoh, and Shachiko Ichihara (Fukuoka Institute of Health and Environmental Sciences), Akiko Kaneko (Yamagata Prefectural Institute of Public Health), Kimiko Kawano and Shuji Yoshino (Miyazaki Prefectural Institute for Public Health and Environment), Tomomi Kouno (Shiga Prefectural Institute of Public Health and Environmental Science), Kazutoshi Matsumoto, Eisuke Tokuoka, and Masato Furukawa (Kumamoto Prefectural Institute of Public Health and Environmental Science), Yuko Matsumoto (Yokohama City Institute of Public Health), Kikuyo Ogata (Oita Prefectural Institute of Health and Environment), Ikuyo Shimono (Tokushima Prefectural Centre for Public Health and Environmental Sciences), Yutaka Shiraki (Gifu Prefectural Research Institute for Health and Environmental Sciences), Jun-ichi Tanabe (Niigata City Institute of Public Health and Environment), Kiyoshi Tominaga (Yamaguchi Prefectural Institute of Public Health and Environment), and Eiji Yokoyama (Chiba Prefectural Institute of Public Health).

† Supplemental material for this article may be found at <http://jcm.asm.org/>.

[‡] Published ahead of print on 24 August 2011.

FIG. 1. Comparison of the O-antigen biosynthesis gene clusters of EHOUT32 (*E. coli* OSB10 serogroup) and *S. boydii* type 10 (accession no. AY693427; 13,403 bp).

cluster (wzxOSB10-F, 5'-CGCAGTTTATTCCGTTTGGT-3'; wzxOSB10-R, 5'-CCATTGATATGTTTCGCTCCA-3') was designed (product size, 631 bp) and shown to be specific to OSB10 among the entire *E. coli* O-serogroup collection (O1 to O181) by PCR using the following conditions: denaturation at 94°C for 20 s, annealing at 58°C for 20 s, and elongation at 72°C for 30 s (25 cycles; 10 ng of genomic template DNA per 15 µl reaction mixture).

To evaluate the prevalence of the OSB10 serogroup, 20 STEC OUT strains isolated from human feces during 2007 to 2010 in Japan were screened serologically by using anti-*S. boydii* type 10 O-antigen serum and genetically by using the OSB10-specific PCR designed in this study. The experiments confirmed that 11 of 20 STEC OUT strains were of the OSB10 serogroup. Biochemical characteristics and virulence-related genes of all OSB10 strains were identical to those of EHOUT32. Including isolate EHOUT32, a total of 12 STEC OSB10 strains were obtained (Table 1). Although nine strains were isolated from asymptomatic patients, three were from patients with diarrhea (including a case of bloody diarrhea). All OSB10 strains had confirmed production of Stx1 by a reversed passive latex agglutination assay (VTEC-RPLA; Denka Seiken, Tokyo, Japan) and showed the H18 serogroup. They did not carry the *ipaH* gene, a useful marker for detection of all *Shigella* spp. as well as enteroinvasive *E. coli*. One strain was isolated in 2007, two strains were isolated in 2008, and the remaining nine strains were isolated in 2010. Sources of strains were the Miyazaki ($n = 4$), Fukuoka ($n = 4$), Yamagata ($n =$

3), and Oita ($n = 1$) prefectures (Miyazaki, Fukuoka, and Oita are located on Kyushu island, and Yamagata is located in the northeast of the main island). EHOUT52, EHOUT53, and EHOUT54 strains originated from three members of a single household, and EHOUT62 and EHOUT63 were also from two members of a single household. The others were isolated from sporadic cases. STEC OSB10 infections were geographically and temporally dispersed, suggesting that this pathogen is widespread throughout Japan. Based on the concatenated nucleotide sequences (3,423 bp) of seven housekeeping genes (*adhA*, *fumC*, *gyrB*, *icd*, *mdh*, *purA*, and *recA*), we performed phylogenetic analysis as previously described (4) and compared STEC OSB10 strains with the well-known STEC serotype collection. These sequences from STEC OSB10 strains were identical, and they formed a cluster distinct from nine other STEC serotype strains (Fig. 2). The pulsed-field gel electrophoresis (PFGE) pattern analysis revealed a diverse population of STEC OSB10 isolates, except for relationships within isolates from each domestic case (Fig. 3).

The analysis of housekeeping gene sequences indicated that *E. coli* and *Shigella* were very closely related, as reported elsewhere (10). Indeed, some O-antigenic cross-reactions between *E. coli* and *Shigella* strains are known to occur, and the O-antigen gene clusters in these strains were also identical or closely

TABLE 1. Characterization of STEC OSB10:H18-serotype strains and the patients' symptoms

Strain	Yr of isolation	Source (prefecture in Japan)	Patient clinical status or symptom(s) ^a
EHOUT32	2008	Miyazaki	AP, Fe, and BD
EHOUT16	2007	Fukuoka	AC
EHOUT29	2008	Oita	AC
EHOUT51	2010	Fukuoka	AC
EHOUT52	2010	Yamagata	AC
EHOUT53	2010	Yamagata	AC
EHOUT54	2010	Yamagata	AC
EHOUT55	2010	Fukuoka	AC
EHOUT60	2010	Fukuoka	AP, Fe, and Di
EHOUT61	2010	Miyazaki	AC
EHOUT62	2010	Miyazaki	AP and Di
EHOUT63	2010	Miyazaki	AC

^a AC, asymptomatic carrier; Di, diarrhea; AP, abdominal pain; Fe, fever; BD, bloody diarrhea.

FIG. 2. Phylogenetic relationships of OSB10:H18-serotype strains among nine well-characterized STEC serotype strains. The phylogenetic tree was constructed based on the concatenated sequences of 7 housekeeping genes by using the neighbor-joining algorithm. Bootstrap analysis was performed with 1,000 replicates. The tree was rooted by *Escherichia albertii* 9194. The numerical values in the tree represent bootstrap results.

FIG. 3. Dendrogram generated from the Dice coefficients of XbaI PFGE profiles of 12 STEC OSB10 strains. M, lambda PFGE ladder marker (New England BioLabs, Beverly, MA).

related (7). The O serogroup of *S. boydii* type 10, however, was unique to *Shigella* and was not related to all recognized *E. coli* O serogroups. Therefore, this is the first report of the presence of an *E. coli* isolate cross-reacting with *Shigella boydii* type 10.

Although many disease-related STEC serogroups possess the locus of enterocyte effacement (LEE) chromosomal pathogenicity island that is involved in the formation of attaching and effacing lesions, LEE-negative STEC strains are also associated with sporadic and outbreak cases of severe disease (2). Despite the Stx1 production, potential pathogenic factors of STEC OSB10 were not identified in this study. Because some STEC OSB10 isolates were obtained from patients with

diarrhea and hemorrhagic colitis and it is likely that the isolated strains caused these conditions, STEC OSB10 strains could be a threat to human health. To gain more information about trends in STEC OSB10 epidemiology, further studies of global OSB10 isolates are needed. The serological and PCR-based methodologies described in this study may help the surveillance and monitoring of the OSB10 serogroup.

Nucleotide sequence accession number. The sequence of the OSB10-antigen gene cluster was deposited in the GenBank/EMBL/DDBJ database under accession number AB627352.

We thank Tetsuya Hayashi for extensive discussions and Atsuko Akiyoshi and Hitomi Satou for technical assistance. This work was supported by grants-in-aid for the Young Scientists from Japan Society for the Promotion of Science (to A.I. and S.I.) and for the Model Program for Young Principal Investigators from the University of Miyazaki and also by the Program to Disseminate Tenure Tracking System from the Japanese Ministry of Education, Culture, Sports, Science and Technology.

REFERENCES

1. Askar, M., et al. 2011. Update on the ongoing outbreak of haemolytic uraemic syndrome due to Shiga toxin-producing *Escherichia coli* (STEC) serotype O104, Germany, May 2011. *Euro Surveill.* **16**:pii:19883.
2. Beutin, L., G. Krause, S. Zimmermann, S. Kaulfuss, and K. Gleier. 2004. Characterization of Shiga toxin-producing *Escherichia coli* strains isolated from human patients in Germany over a 3-year period. *J. Clin. Microbiol.* **42**:1099–1108.
3. Iguchi, A., et al. 2008. Genomic comparison of the O-antigen biosynthesis gene clusters of *Escherichia coli* O55 strains belonging to three distinct lineages. *Microbiology* **154**:559–570.
4. Iguchi, A., et al. 2009. Complete genome sequence and comparative genome analysis of enteropathogenic *Escherichia coli* O127:H6 strain E2348/69. *J. Bacteriol.* **191**:347–354.
5. Jenke, C., et al. 2010. Phylogenetic analysis of enterohemorrhagic *Escherichia coli* O157, Germany, 1987–2008. *Emerg. Infect. Dis.* **16**:610–616.
6. Lathrop, S., K. Edge, and J. Bareta. 2009. Shiga toxin-producing *Escherichia coli*, New Mexico, U. S. A., 2004–2007. *Emerg. Infect. Dis.* **15**:1289–1291.
7. Liu, B., et al. 2008. Structure and genetics of *Shigella* O antigens. *FEMS Microbiol. Rev.* **32**:627–653.
8. Mellmann, A., et al. 2008. Analysis of collection of hemolytic uraemic syndrome-associated enterohemorrhagic *Escherichia coli*. *Emerg. Infect. Dis.* **14**:1287–1290.
9. Nataro, J. P., and J. B. Kaper. 1998. Diarrheagenic *Escherichia coli*. *Clin. Microbiol. Rev.* **11**:142–201.
10. Pupo, G. M., R. Lan, and P. R. Reeves. 2000. Multiple independent origins of *Shigella* clones of *Escherichia coli* and convergent evolution of many of their characteristics. *Proc. Natl. Acad. Sci. U. S. A.* **97**:10567–10572.
11. Rangel, J. M., P. H. Sparling, C. Crowe, P. M. Griffin, and D. L. Seward. 2005. Epidemiology of *Escherichia coli* O157:H7 outbreaks, United States, 1982–2002. *Emerg. Infect. Dis.* **11**:603–609.
12. Rivas, M., et al. 2008. Risk factors for sporadic Shiga toxin-producing *Escherichia coli* infections in children, Argentina. *Emerg. Infect. Dis.* **14**:763–771.
13. Scheut, F., T. Cheasty, D. Woodward, and H. R. Smith. 2004. Designation of O174 and O175 to temporary O groups OX3 and OX7, and six new *E. coli* O groups that include verocytotoxin-producing *E. coli* (VTEC): O176, O177, O178, O179, O180 and O181. *APMIS* **112**:569–584.
14. Senchenkova, S. N., et al. 2005. Structural and genetic characterization of the *Shigella boydii* type 10 and type 6 O antigens. *J. Bacteriol.* **187**:2551–2554.
15. Seto, K., M. Taguchi, K. Kobayashi, and S. Kozaki. 2007. Biochemical and molecular characterization of minor serogroups of Shiga toxin-producing *Escherichia coli* isolated from humans in Osaka prefecture. *J. Vet. Med. Sci.* **69**:1215–1222.