Clinical Significance of Escherichia albertii

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Discriminating Escherichia albertii from other Enterobacteriaceae is difficult. Systematic analyses showed that E. albertii represents a substantial portion of strains currently identified as eae-positive Escherichia coli and includes Shiga toxin 2f–producing strains. Because E. albertii possesses the eae gene, many strains might have been misidentified as enterohemorrhagic or enteropathogenic E. coli.

Attaching and effacing pathogens possess a locus of enteroctye effacement (LEE)–encoded type III secretion system. They form attaching and effacing lesions on intestinal epithelial cell surfaces by the combined actions of intimin, an eae–encoded outer membrane protein, and type III secretion system effectors. Attaching and effacing pathogens include enterohemorrhagic and enteropathogenic Escherichia coli (EHEC and EPEC, respectively) and Citrobacter rodentium (1, 2). Escherichia albertii have recently been added to this group (3–5). However, the clinical significance of E. albertii has yet to be fully elucidated, partly because it is difficult to discriminate E. albertii from other Enterobacteriaceae spp. by using routine bacterial identification systems based on biochemical properties (6–9). A large number of E. albertii strains might have been misidentified as EPEC or EHEC because they possess the eae gene.

The Study

We collected 278 eae-positive strains that were originally identified by routine diagnostic protocols as EPEC or EHEC. They were isolated from humans, animals, and the environment in Japan, Belgium, Brazil, and Germany during 1993–2009 (Table 1; online Technical Appendix, wwwnc.cdc.gov/pdfs/11-1401-Techapp.pdf). To characterize the strains, we first determined their intimin subtypes by sequencing the eae gene as described (online Technical Appendix). Of the 275 strains examined, 267 possessed 1 of the 26 known intimin subtypes (4 subtypes—η, ν, τ, and a subtype unique to C. rodentium—were not found). In the remaining 8 strains, we identified 5 new subtypes; each showed <95% nt sequence identity to any known subtype, and they were tentatively named subtypes N1–N5. For subtype N1, 3 variants were identified (N1.1, N1.2, and N1.3, with >95% sequence identity among the 3 variants) (Figure 1, panel A).

To determine the phylogenetic relationships of the strains, we performed multilocus sequencing analysis of 179 strains that were selected from our collection on the basis of intimin subtype and serotype (see online Technical Appendix for selection criteria and analysis protocol). Among the 179 strains, 26 belonged to the E. albertii lineage (Figure 2). The 26 E. albertii strains were from 14 humans (13 from symptomatic patients), 11 birds, and 1 cat. All of the 5 new intimin subtypes were found in the E. albertii strains. Intimin subtypes found in other E. albertii strains were also rare subtypes found in E. coli (10). This finding suggests that more previously unknown intimin subtypes may exist in the E. albertii population.

We next analyzed the pheV, selC, and pheU loci of the 26 E. albertii strains for the presence of LEE elements as described (online Technical Appendix). These 3 genomic

| Origin       | No. strains |
|--------------|-------------|
| Human, n = 193 |            |
| Symptomatic  | 154         |
| Asymptomatic | 7           |
| No information | 32         |
| Animal, n = 76 |            |
| Bird         | 38          |
| Pig          | 31          |
| Cat          | 1           |
| Deer         | 1           |
| Bovid        | 1           |
| Sheep        | 1           |
| No information | 3          |
| Environment, n = 6 | 6     |

*EPEC, enteropathogenic Escherichia coli; EHEC, enterohemorrhagic E. coli.
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Loci are the known LEE integration sites in *E. coli*. By this analysis, all *E. albertii* strains except 1 (EC05–44) contained the LEE in the *pheU* locus (the integration site in EC05–44 was not identified). This finding indicates that despite the remarkable diversity of intimin subtypes, the LEE elements are preferentially integrated into the *pheU* tRNA gene in *E. albertii*.

Because all *E. albertii* strains isolated so far contained the *cdtB* gene encoding the cytolethal distending toxin B subunit (8,9), we examined the presence and subtype of the *cdtB* gene as described (online Technical Appendix). This analysis revealed that all *E. albertii* strains except 1 (CB10113) possessed the *cdtB* gene belonging to the II/III/V subtype group (Figure 1, panel B); this finding is consistent with published findings (9). In addition, 2 strains (E2675 and HIPH08472) each of which was subtype I, possessed a second *cdtB* gene, (Figure 1, panel B).

We used PCR to further investigate the presence of Shiga toxin genes (*stx*) and their variants (online Technical Appendix) and found that 2 *E. albertii* strains possessed the *stx2f* gene (Figure 2, panel B). Stx2 production by these strains was confirmed by using a reverse-passive latex agglutination
kit (online Technical Appendix). The 2 stx2f-positive strains were those containing the subtype 1 cdtB gene in addition to the II/III/V subtype group gene: 1 (HIPH08472) was isolated from a patient with diarrhea and the other (E2675) was from a healthy Corvus sp. bird (Figure 2).

Last, we examined the phenotypic and biochemical properties of the 26 E. albertii strains and compared the results with those obtained in a previous study (9) and with those of E. albertii type strain LMG20976 (Table 2). To identify features that could discriminate E. albertii from E. coli, the results were further compared with those of E. coli (11). Consistent with findings in previous reports (5–7,9), the lack of motility and the inability to ferment xylose and lactose and to produce β-D-glucuronidase were common biochemical properties of E. albertii that could be used to discriminate E. albertii from E. coli, although 1 E. albertii strain was positive for lactose fermentation. The inability of E. albertii to ferment sucrose has been described as a common feature (9); however, a positive reaction to this test was found for 5 (19.2%) E. albertii strains. Moreover, approximately half of E. coli strains are positive for sucrose fermentation. Thus, the inability to ferment sucrose is not informative. Rather, the inability to ferment dulcitol (all E. albertii strains were negative, 60% of E. coli strains are positive) may be a useful biochemical property for differentiation.

**Conclusions**

In the current clinical laboratory setting, a substantial number of E. albertii strains are misidentified as EPEC or
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EHEC. Because 13 of the isolates were from patients with signs and symptoms of gastrointestinal infection, E. albertii is probably a major enteric human pathogen. In addition, E. albertii should be regarded as a potential Stx2f-producing bacterial species, although the clinical significance of Stx2f-producing strains is unknown.

Notable genetic, phenotypic, and biochemical properties of E. albertii, which were identified by analyzing the confirmed E. albertii strains, are 1) possession of intimin subtypes rarely or previously undescribed in E. coli, 2) possession of the II/III/V subtype group cdIB gene, 3) LEE integration into the pheU (RNA gene, 4) nonmotility, and 5) inability to ferment xylose, lactose, and dulcitol (but not sucrose) and to produce β-D-glucuronidase. These properties could be useful for facilitating identification of E. albertii strains in clinical laboratories, which would in turn improve understanding of the clinical significance and the natural host and niche of this newly recognized pathogen. In this regard, however, current knowledge of the genetic and biological properties of E. albertii might be biased toward a certain group of E. albertii strains because, even with this study, only a limited number of strains have been analyzed. To more precisely understand the properties of E. albertii as a species, further analysis of more strains from various sources is necessary.

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Table 2. Comparison of biochemical properties of Escherichia spp. strains

| Agent or test               | 26 E. albertii strains (this study)† | E. albertii LMG20976 (type strain) | E. albertii strains (9) | E. coli (11)† |
|-----------------------------|-------------------------------------|-----------------------------------|------------------------|---------------|
| Indole                      | 96.2                                | –                                 | 100                    | 98            |
| Motility                    | 0                                   | –                                 | 0                      | 95            |
| Urea                        | 0                                   | –                                 | 0                      | 1             |
| ONPG                        | 88.5                                | +                                 | ND                     | ND            |
| MUG                         | 0                                   | –                                 | ND                     | (†)†          |
| Citrate                     | 0                                   | –                                 | 0                      | 1             |
| Acetate                     | 92.3                                | +                                 | ND                     | 90            |
| Malonate                    | 0                                   | –                                 | ND                     | 0             |
| H2S on triple sugar ion     | 0                                   | –                                 | ND                     | 1             |
| Voges-Proskauer             | 0                                   | –                                 | ND                     | 0             |
| Lysine decarboxylase        | 100                                 | +                                 | 100                    | 90            |
| Ornithine decarboxylase     | 100                                 | +                                 | 100                    | 65            |
| Arginine dihydrolase        | 0                                   | –                                 | 0                      | 17            |
| Glucose, acid               | 100                                 | +                                 | 100                    | 100           |
| Glucose, gas                | 100                                 | +                                 | 100                    | 95            |
| Acid from                   |                                     |                                   |                        |               |
| Adonitol                    | 0                                   | –                                 | ND                     | 0             |
| L-arabinose                 | 100                                 | +                                 | 100                    | 99            |
| Cellulose                   | 0                                   | –                                 | ND                     | 2             |
| Dulcitol                    | 0                                   | –                                 | ND                     | 60            |
| Myo-inositol                | 0                                   | –                                 | ND                     | 1             |
| Lactose                     | 3.9                                 | –                                 | 0                      | 95            |
| Maltose                     | 88.5                                | +                                 | ND                     | 95            |
| Mannitol                    | 100                                 | +                                 | 100                    | 100           |
| L-rhamnose                  | 0                                   | –                                 | ND                     | 0             |
| Salicin                     | 26.9                                | –                                 | ND                     | 40            |
| D-sorbitol                  | 57.7                                | –                                 | V                      | 94            |
| Sucrose                     | 19.2                                | –                                 | 0                      | 50            |
| Trehalose                   | 96.2                                | +                                 | ND                     | 98            |
| D-xylose                    | 0                                   | –                                 | 0                      | 95            |

*ONPG, ortho-nitrophenyl-β-D-galactoside; MUG, methylumbelliferyl-β-D-glucuronide; –, negative; +, positive; ND, not determined.
†Average (%) of positive strains.
††Most E. coli strains produce β-D-glucuronidase.

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The International Conference on Emerging Infectious Diseases was first convened in 1998; ICEID 2012 marks its eighth occurrence. The conference brings together public health professionals to encourage the exchange of scientific and public health information on global emerging infectious disease issues. The program will include plenary and panel sessions with invited speakers as well as oral and poster presentations on emerging infections. Major topics to be included are current work on surveillance, epidemiology, research, communication and training, bioterrorism, and prevention and control of emerging infectious diseases, both in the United States and abroad.

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*Whom are they affecting?*

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Technical Appendix

Bacterial strains, growth conditions, and DNA extraction

The 275 bacterial strains used were isolated in the laboratories participating in this study or from strain stocks from each laboratory. The sources of their isolation and other strain information are summarized in Technical Appendix Table 1. In brief, the 275 strains were isolated in Japan, Brazil, Germany, and Belgium. All of the strains had been originally identified as EPEC or EHEC. Among the 193 human isolates, 154 were isolated from patients with the clinical symptoms of a gastrointestinal infection, such as diarrhea (bloody or non-bloody), abdominal pain, vomiting, and fever. As for the remaining 39 human isolates, we confirmed that 7 were from asymptomatic carriers, while clinical records on the others were not available. The 76 animal isolates were mainly from wild birds, which were found dead due to unknown reasons and thus subjected to laboratory examinations, and healthy pigs; these isolates included several strains from other domestic and wild animals. The environmental strains were isolated from sand pit courts at elementary schools, parks, and shrines.) The *E. albertii* type strain LMG20976 was provided by RIKEN BioResource Center (Ibaraki, Japan). Bacterial cells were grown aerobically at 37°C in Luria-Bertani (LB) medium or on LB agar. Bacterial DNA used as template DNA for PCR was prepared by the alkaline-boiling method as described previously (1).

Sequence-based intimin subtyping

DNA sequences of the entire *eae* genes were determined as described by Lacher *et al.* (2). Briefly, the 5′ half of the gene and its upstream region were amplified by PCR using the cesT-F9/eae-F1 primer pair and KAPATaq (NIPPON Genetics, Tokyo, Japan), and the 3′ half and the downstream region were amplified using the eae-R3/escD-R1 primer pair. Amplicons were sequenced with the primers used for PCR amplification on the ABI 3710 autosequencer (Life Technologies Corporation, CA). To fully sequence the 3′ half, an additional sequence primer
(1669-1688) was used. Primer sequences and amplification conditions are listed in Technical Appendix Table 2.

Predicted amino acid sequences were aligned with those of the reference intimin subtypes listed in Technical Appendix Table 3 by the ClustalW program in MEGA4 (3). A phylogenetic tree was constructed with the neighbor-joining algorithm using MEGA4. Poisson correction was used to calculate protein distances. Bootstrap analysis with 1000 replicates was performed to evaluate the significance of internal branches. To define new intimin subtypes, we employed the cutoff value of 95% nucleotide sequence identity (4).

**Multi-locus sequence (MLS) analysis**

To determine the phylogenetic relationships of the eae-positive strains, we performed MLS analysis. For this analysis, we selected one or two representative strains for each intimin subtype. When different serotypes were found within an intimin subtype, we selected one or two strains for each serotype; thus, 179 strains were analyzed in total.

MLS analysis was performed using the nucleotide sequences of 7 housekeeping genes (adk, fumC, gyrB, icd, mdh, purA, and recA). Target genes were amplified and sequenced according to the protocol provided from the UCC Web site (http://mlst.ucc.ie/). Using the concatenated nucleotide sequences of the 7 genes and the maximum composite likelihood model, a neighbor-joining tree was constructed using MEGA4 software. EcoR collection strains (5) and genome-sequenced E. coli, E. fergusonii, E. albertii, Shigella sp., and Salmonella enterica serovar Typhi strains were included in a phylogenetic representation.

**PCR detection and sequencing of the stx and cdtB genes**

PCR screening was performed for the genes for Shiga toxins 1 and 2 (stx1, stx2 and stx2-variants) and the B subunit of cytolethal-distending toxin (cdtB). All primers and PCR conditions used for this screening are shown in Technical Appendix Table 2. PCR amplification was performed using KAPATaq Extra DNA polymerase (KAPA Biosystems, Inc., MA). Subtypes and phylogenetic relationships of the cdtB genes were determined by direct sequencing of the amplicons on the ABI 3710 autosequencer using the primers used for PCR amplification.
Detection of Stx production with or without mitomycin C (MMC) induction

The production of Stx2f by stx2f-positive E. albertii strains was determined by using a reverse-passive latex agglutination kit (VTEC-RPLA; Denka Seiken Co., Ltd., Tokyo, Japan). Bacterial cell were pre-cultured in 1 mL of Casamino Acids-yeast extract (CAYE) broth (Denka Seiken, Tokyo, Japan) overnight with shaking, and then inoculated to adjust OD₆₀₀ = 0.1 into 2mL of fresh CAYE broth and followed by 16 hrs incubation (MMC-). For mitomycin C (MMC; Kyowa Hakko Kirin Co., Ltd., Tokyo, Japan) induction, 0.5 µl of 2 mg/mL MMC solution was add to the 2 mL culture at an hour incubation (final concentration of 50 µg/mL) and followed by 15 hrs incubation. Of the cell suspension after 16 hrs incubation, 1 mL culture was treated with 1 mL of polymyxin B (Sigma-Aldrich Japan, Tokyo, Japan; final concentration of 5,000 U/mL) for 1 hr at 37°C. The solution were centrifuged for 10 min at 9,000 rpm at 4°C and used for VTEC-RPLA assay according to the manufactures instruction. E. albertii strain LMG20976 (type strain; stx-negative) and strain CB9786 (stx-negative) were used as negative controls. EHEC O128:HNM strain EC1463 (stx2f-positive) and EHEC O157:H7 strain Sakai (stx1- and stx2-positive) were used as positive control (stx2f and stx genes, respectively). The result of this analysis was shown in Technical Appendix Table 4.

Determination of the LEE integration sites

Three integration sites that have so far been identified for the LEE elements in various E. coli strains are the pheV, selC, and pheU tRNA gene loci. It is also known that although the gene organization of LEE core regions is highly conserved between strains, accessory regions of highly variable sizes and genetic structures often exist just downstream of the core region (6–8). In contrast, no or only small accessory regions have been identified upstream of the core region; thus, the genetic structures of the left (upstream) chromosome/LEE junctions are relatively well conserved. Therefore, by employing long-range PCR targeted to the escR gene in the LEE core region and chromosomal regions outside of the left chromosome/LEE junctions, we performed a systematic survey of the pheV, selC, and pheU loci of the eae-positive strains for the presence of LEE elements.

Long-range PCR screening was performed by using TaKaRa LA Taq polymerase (Takara Bio Inc. Ohtsu, Japan). Each locus was examined by PCR using an inside primer (escR-R) in
combination with outside primers targeted to the genomic regions adjacent to each tRNA gene locus. The outside primers were designed based on the genome sequences of the K-12 strain MG1655 (9) and 5 EHEC and EPEC strains (6,10,11). Primer sequences and amplification conditions are listed in Technical Appendix Table 5.

**Phenotype and biochemical characterization of E. albertii strains**

The phenotypic and biochemical properties of the strains identified as E. albertii in this study and the E. albertii type strain (LMG20976) were examined by conventional methods (12). Carbohydrate-fermenting abilities were determined after 7 days of incubation at 37°C in Andrade peptone water (Oxoid, Cambridge, UK) containing one of the following 15 carbohydrates (Wako Pure Chemicals, Osaka, Japan): adonitol, arabinose, cellobiose, dulcitol, glucose, inositol, lactose, maltose, mannitol, rhamnose, salicin, sorbitol, sucrose, trehalose, and xylose. The β-glucuronidase activity was examined using CLIG medium (Kyokuto Pharmaceutical, Tokyo, Japan).

**Nucleotide sequence accession numbers**

All nucleotide sequences obtained in this study have been deposited into the DDBJ/EMBL/GenBank database. The accession numbers are AB647359-AB647618 (for the eae genes), AB647619-AB647655 (for the cdtB genes), and AB647656-648908 (for the 7 housekeeping genes [adk, fumC, gyrB, icd, mdh, purA, and recA] used for MLS analysis).

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Technical Appendix Table 1. Detailed information of the strains used in this study

| Serotype† | strain name | year of isolate | origin | countries | symptoms or notes | intimin subtypes | LEE integration sites (tRNA gene) | presence or absence | MLST analysis | References |
|-----------|-------------|-----------------|--------|-----------|------------------|-----------------|----------------------------------|-------------------|--------------|------------|
| O51:H40   | EC06-71     | 2006            | human  | Japan     | symptomatic      | theta           | NT                               | -                 | Y            | this study  |
| O40/33:H34| EC06-80     | 2006            | human  | Japan     | symptomatic (diarrhea, abdominal pain, fever) | (lambda)        | selC                             | -                 | -            | Y          |
| O88:H6    | EC06-90     | 2006            | human  | Japan     | symptomatic      | iota1           | selC                             | -                 | Y            | this study  |
| O51:H40   | EC06-118    | 2006            | human  | Japan     | symptomatic (diarrhea, abdominal pain) | theta           | NT                               | -                 | Y            | this study  |
| OUT:H34   | EC06-119    | 2006            | human  | Japan     | symptomatic (diarrhea, abdominal pain) | iota1           | selC                             | -                 | Y            | this study  |
| O175:NM   | EC06-170    | 2006            | human  | Japan     | symptomatic      | xi               | pheU                             | -                 | Y            | this study  |
| O55:H6    | 18H89       | 2006            | human  | Japan     | symptomatic      | iota1           | selC                             | -                 | Y            | this study  |
| O145:H34  | 19H198      | 2007            | human  | Japan     | symptomatic (diarrhea, abdominal pain, fever) | iota1           | selC                             | -                 | Y            | this study  |
| O23:H8    | 19H226      | 2007            | human  | Japan     | symptomatic (diarrhea, abdominal pain) | theta           | NT                               | -                 | Y            | this study  |
| O65:NM    | 20H38       | 2008            | human  | Japan     | symptomatic (diarrhea) | sigma           | pheU                             | -                 | Y            | this study  |
| O152/115:N M | 20H183   | 2008            | human  | Japan     | symptomatic (diarrhea) | N1.3            | pheU                             | -                 | Y            | this study  |
| O113:H19  | 20H186      | 2008            | human  | Japan     | symptomatic (diarrhea) | epsilon2        | selC                             | -                 | Y            | this study  |
| O114:H19  | 20H215      | 2008            | human  | Japan     | symptomatic (diarrhea) | epsilon2        | selC                             | -                 | Y            | this study  |
| O101:NM   | 20H250      | 2009            | human  | Japan     | symptomatic (diarrhea, abdominal pain, fever) | (iota2)         | pheU                             | -                 | Y            | this study  |
| O101:NM   | 21H147      | 2009            | human  | Japan     | symptomatic (diarrhea) | iota2           | pheU                             | -                 | Y            | this study  |
| O21:H8    | EC01-376    | 2001            | environmen t | Japan | sand pit court | theta           | selC                             | -                 | Y            | this study  |
| O66:H21   | EC01-380    | 2001            | environmen t | Japan | sand pit court | theta           | NT                               | -                 | Y            | this study  |
| O142:H34  | EC01-383    | 2001            | environmen t | Japan | sand pit court | alpha1          | selC                             | -                 | Y            | this study  |
| OUT:H21   | EC01-386    | 2001            | environmen t | Japan | sand pit court | theta           | NT                               | -                 | Y            | this study  |
| O51:H49   | EC01-403    | 2001            | environmen t | Japan | sand pit court | alpha1          | selC                             | -                 | Y            | this study  |
| OUT:H34   | EC01-406    | 2001            | environmen t | Japan | sand pit court | alpha2          | selC                             | -                 | Y            | this study  |
| O128:NM   | EC01-460    | 2001            | human  | Japan     | symptomatic (diarrhea, abdominal pain, fever) | beta1           | NT                               | -                 | Y            | this study  |
| O5:NM     | EC03-71     | 2003            | human  | Japan     | symptomatic (diarrhea, bloody stool, fever, abdominal pain) | beta1           | NT                               | stx1&stx2         | -            | Y          |
| OUT:H34   | EC03-82     | 2003            | human  | Japan     | symptomatic (diarrhea, bloody stool, fever, abdominal pain) | iota1           | selC                             | -                 | Y            | this study  |
| O51:H40   | EC03-93     | 2003            | human  | Japan     | symptomatic (diarrhea, abdominal pain) | epsilon1        | NT                               | -                 | Y            | this study  |
| OUT:H6    | EC03-126    | 2003            | human  | Japan     | symptomatic (diarrhea) | beta2           | selC                             | -                 | Y            | this study  |
| Serotype† | strain name | year of isolate | origin | countries | symptoms or notes | intimin subtypes | LEE integration sites (RNA gene) | presence or absence | MLST analysis | References |
|-----------|-------------|----------------|--------|-----------|------------------|----------------|-------------------------------|-------------------|--------------|------------|
| O181:NM   | EC03-127    | 2003           | human  | Japan     | symptomatic (diarrhea) | epsilon03 | pheU                          | -     | +           | Y‡         | this study  |
| O180:NM   | EC03-144    | 2003           | human  | Japan     | asymptomatic carrier | rho      | pheU                          | -     | -           | Y          | this study  |
| O153:H21  | EC03-152    | 2003           | human  | Japan     | symptomatic (diarrhea, fever) | theta | NT                         | -     | -           | Y          | this study  |
| OUT:NM    | EC03-195    | 2003           | human  | Japan     | symptomatic        | N6       | pheU                          | -     | +           | Y‡         | this study  |
| OUT:H2    | EC03-207    | 2003           | animal | Japan     | asymptomatic      | theta    | seIC                          | -     | -           | Y          | this study  |
| OUT:H2    | EC03-211    | 2003           | animal | Japan     | asymptomatic      | theta    | NT                          | -     | -           | Y          | this study  |
| OUT:H6    | EC03-224    | 2003           | animal | Japan     | asymptomatic      | beta2    | seIC                          | -     | -           | Y          | this study  |
| OUT:H34   | EC04-81     | 2004           | human  | Japan     | symptomatic (abdominal pain, vomiting) | iota1   | seIC                          | -     | -           | Y          | this study  |
| O88:H25   | EC04-258    | 2004           | human  | Japan     | symptomatic (diarrhea, vomiting) | epsilon2 | seIC                          | -     | -           | Y          | this study  |
| O21:H6    | EC04-268    | 2004           | human  | Japan     | symptomatic (diarrhea) | alpha2   | seIC                          | -     | -           | Y          | this study  |
| O117:H21  | EC04-311    | 2004           | human  | Japan     | symptomatic (diarrhea, vomiting) | theta    | NT                          | -     | -           | Y          | this study  |
| O152:H38  | EC04-437    | 2004           | human  | Japan     | symptomatic (abdominal pain) | epsilon1 | NT                          | -     | -           | Y          | this study  |
| OUT:H2    | EC04-500    | 2004           | human  | Japan     | symptomatic (diarrhea) | beta1    | NT                          | -     | -           | Y          | this study  |
| OUT:H34   | EC04-569    | 2004           | human  | Japan     | symptomatic (diarrhea, vomiting) | iota1    | seIC                          | -     | -           | Y          | this study  |
| OUT:H2    | EC04-572    | 2004           | human  | Japan     | symptomatic (diarrhea, abdominal pain) | beta1    | NT                          | -     | -           | Y          | this study  |
| O49:H10   | EC04-588    | 2004           | human  | Japan     | symptomatic (kappa) | seIC    | -                            | -     | -           | Y          | this study  |
| OUT:NM    | EC05-44     | 2005           | human  | Japan     | symptomatic      | N4       | NT                         | -     | +           | Y‡         | this study  |
| O129/13:H11 | EC05-63    | 2005           | human  | Japan     | symptomatic (diarrhea) | epsilon1 | NT                          | -     | -           | Y          | this study  |
| O108:H40  | EC05-66     | 2005           | human  | Japan     | symptomatic (diarrhea, abdominal pain) | epsilon1 | NT                          | -     | -           | Y          | this study  |
| OUT:NM    | EC05-81     | 2005           | human  | Japan     | symptomatic      | N3       | pheU                          | -     | +           | Y‡         | this study  |
| O70:H11   | EC05-86     | 2005           | human  | Japan     | asymptomatic carrier | epsilon1 | NT                          | -     | -           | Y          | this study  |
| O128:NM   | EC05-93     | 2005           | human  | Japan     | symptomatic      | beta1    | NT                          | -     | -           | N          | this study  |
| OUT:H34   | EC05-94     | 2005           | human  | Japan     | symptomatic      | alpha2   | seIC                          | -     | -           | N          | this study  |
| O71:H49   | EC05-95     | 2005           | human  | Japan     | asymptomatic carrier | kappa    | seIC                          | -     | -           | Y          | this study  |
| O10:NM    | EC05-134    | 2005           | human  | Japan     | symptomatic (diarrhea, abdominal pain) | iota1    | seIC                          | -     | -           | Y          | this study  |
| OUT:NM    | EC05-160    | 2005           | human  | Japan     | symptomatic      | sigma    | pheU                          | -     | +           | Y‡         | this study  |
| OUT:H4    | EC05-165    | 2005           | human  | Japan     | symptomatic      | alpha1   | seIC                          | -     | -           | Y          | this study  |
| O171:H19  | 12H133      | 2000           | human  | Japan     | symptomatic      | epsilon2 | seIC                          | -     | -           | Y          | this study  |
| O119:H2   | 12H377      | 2000           | human  | Japan     | symptomatic      | beta1    | NT                          | -     | -           | Y          | this study  |
| O2:H49    | 17H285      | 2005           | human  | Japan     | symptomatic      | iota1    | seIC                          | -     | -           | Y          | this study  |
| OUT:HND   | 93010       | 1993.6.18      | human  | Japan     | symptomatic (diarrhea) | mu      | seIC                          | -     | -           | Y          | this study  |
| OUT:H40   | 94037       | 1994.6.29      | human  | Japan     | symptomatic (diarrhea, fever) | (eta2)  | seIC                          | -     | -           | Y          | this study  |
| OUT:HND   | 94046-2     | 1994.7.25      | human  | Japan     | symptomatic (bloody diarrhea) | epsilon2 | seIC                          | -     | -           | Y          | this study  |
| Serotype† | strain name | year of isolate | origin | countries | symptoms or notes | intimin subtypes | LEE integration sites (rRNA gene) | presence or absence | MLST analysis | References |
|-----------|-------------|----------------|--------|-----------|------------------|----------------|---------------------------------|------------------|--------------|------------|
| OUT:H7    | 94064       | 1994.9.19      | human  | Japan     | symptomatic (diarrhea) | theta           | NT                             | -                | -            | Y          | this study |
| O86a:HND  | 94308       | 1994.6.25      | human  | Japan     | NI               | iota1           | selC                            | -                | -            | Y          | this study |
| O55:H7    | 94327       | 1994.7.12      | human  | Japan     | NI               | gamma1          | selC                            | -                | -            | Y          | this study |
| O26:H21   | 94358       | 1994.8.6       | human  | Japan     | NI               | theta           | NT                             | -                | N            | Y          | this study |
| OUT:HND   | 94368       | 1994.8.16      | human  | Japan     | NI               | theta           | NT                             | -                | Y            | Y          | this study |
| OUT:HNM   | 94389       | 1994.9.8       | human  | Japan     | NI               | sigma           | phiU                           | +                | Y            | Y          | this study |
| OUT:HNM   | 94414       | 1994.10.12     | human  | Japan     | NI               | theta           | NT                             | -                | Y            | Y          | this study |
| O55:H7    | 95012       | 1995.5.11      | human  | Japan     | symptomatic (diarrhea) | gamma1          | selC                            | -                | N            | Y          | this study |
| O119:H2   | 95028       | 1995.6.12      | human  | Japan     | symptomatic (diarrhea) | beta1           | NT                             | -                | Y            | Y          | this study |
| OUT:HND   | 95032       | 1995.6.16      | human  | Japan     | symptomatic (diarrhea) | iota1           | selC                            | -                | Y            | Y          | this study |
| O26:HNM   | 95036-2     | 1995.6.18      | human  | Japan     | symptomatic (diarrhea) | beta1           | NT                             | -                | Y            | Y          | this study |
| OUT:HND   | 95037       | 1995.6.19      | human  | Japan     | symptomatic (diarrhea, fever) | epsilon2        | selC                            | -                | Y            | Y          | this study |
| O15:HND   | 95301       | 1995.5.9       | human  | Japan     | symptomatic (bloody diarrhea, fever, abdominal pain) | iota1           | selC                            | -                | Y            | Y          | this study |
| O153:H7   | 95473       | 1995.10.27     | human  | Japan     | NI               | beta1           | NT                             | -                | Y            | Y          | this study |
| O26:HNM   | 960064      | 1996.7.2       | human  | Japan     | symptomatic (bloody diarrhea) | beta1           | selC                            | -                | Y            | Y          | this study |
| O126:HND  | 960134      | 1996.8.2       | human  | Japan     | symptomatic (diarrhea, fever) | beta1           | NT                             | -                | Y            | Y          | this study |
| OUT:HND   | 960135      | 1996.8.2       | human  | Japan     | symptomatic (diarrhea, fever) | gamma1          | selC                            | -                | Y            | Y          | this study |
| O20:HND   | 960175      | 1996.8.23      | human  | Japan     | symptomatic (diarrhea, fever) | beta2           | selC                            | -                | N            | Y          | this study |
| OUT:HND   | 960185      | 1996.8.29      | human  | Japan     | symptomatic (bloody diarrhea) | beta2           | selC                            | -                | Y            | Y          | this study |
| OUT:HND   | 960192      | 1996.8.31      | human  | Japan     | symptomatic (diarrhea, fever) | gamma1          | selC                            | -                | Y            | Y          | this study |
| O115:HND  | 960719      | 1996.9.30      | human  | Japan     | symptomatic (diarrhea) | theta           | NT                             | -                | N            | Y          | this study |
| O20:H6    | 960241      | 1996.3.18      | human  | Japan     | NI               | beta2           | selC                            | -                | Y            | Y          | this study |
| O115:HND  | 960242      | 1996.3.19      | human  | Japan     | symptomatic (bloody stool, abdominal pain, vomiting) | beta2           | selC                            | -                | Y            | Y          | this study |
| O15:H2    | 960261      | 1996.5.13      | human  | Japan     | symptomatic (bloody stool, fever, abdominal pain) | beta1           | NT                             | -                | Y            | Y          | this study |
| OUT:HND   | 960296      | 1996.6.25      | human  | Japan     | NI               | iota1           | selC                            | -                | Y            | Y          | this study |
| O119:HND  | 960337      | 1996.7.15      | human  | Japan     | NI               | zeta3           | selC                            | -                | Y            | Y          | this study |
| NI        | 960349      | 1996.7.19      | human  | Japan     | NI               | zeta3           | selC                            | -                | Y            | Y          | this study |
| OUT:HND   | 960446      | 1996.8.8       | human  | Japan     | NI               | beta2           | selC                            | -                | Y            | Y          | this study |
| O26:HND   | 960462      | 1996.8.29      | human  | Japan     | NI               | kappa           | selC                            | -                | Y            | Y          | this study |
| OUT:HND   | 960468      | 1996.9.6       | human  | Japan     | NI               | eta2            | selC                            | -                | Y            | Y          | this study |
| O26:HNM   | 960496      | 1996.10.14     | human  | Japan     | NI               | beta1           | selC                            | -                | N            | Y          | this study |
| O119:HNM  | 97054-1     | 1997.8.13      | human  | Japan     | symptomatic (diarrhea) | theta           | NT                             | -                | Y            | Y          | this study |
| Serotype† | strain name | year of isolate | origin | countries | symptoms or notes | intimin subtypes | LEE integration sites (rRNA gene) | presence or absence | MLST analysis | References |
|-----------|-------------|-----------------|--------|-----------|-------------------|------------------|-------------------------------|------------------|--------------|------------|
| OUT:HND   | 97105       | 1997.8.8        | human  | Japan     | symptomatic (diarrhea, vomiting) | beta1            | pheU                          | - -            | Y            | this study |
| NI        | 97144       | 1997.9.11       | human  | Japan     | symptomatic (diarrhea)          | theta            | NT                            | - -            | Y            | this study |
| O26:HNM   | 97207       | 1997.11.14      | human  | Japan     | symptomatic (diarrhea)          | beta1            | NT                            | - -            | N            | this study |
| O55:H7    | 97214       | 1997.11         | human  | Japan     | symptomatic                  | gamma1           | selC                          | - -            | N            | this study |
| O55:HND   | 97253-2     | 1997.12.20      | human  | Japan     | symptomatic (bloody diarrhea)  | gamma1           | selC                          | - -            | N            | this study |
| O157:HND  | 97255       | 1997.12.17      | human  | Japan     | symptomatic (diarrhea)          | alpha1           | selC                          | - -            | Y            | this study |
| O167:HND  | 97603       | 1997.6.9        | human  | NI        | symptomatic (diarrhea)          | beta1            | NT                            | - -            | Y            | this study |
| O168:HND  | 97604       | 1997.6.10       | human  | Japan     | symptomatic (diarrhea)          | gamma1           | selC                          | - -            | Y            | this study |
| O128:HND  | 97651       | 1997.6.21       | human  | Japan     | symptomatic (diarrhea, abdominal pain, vomiting, fever) | beta1            | NT                            | - -            | Y            | this study |
| OUT:HND   | 97674-2     | 1997.6.30       | human  | Japan     | symptomatic (bloody diarrhea, abdominal pain) | epsilon2         | selC                          | - -            | Y            | this study |
| O128:H2   | 97756       | 1997.7.19       | human  | Japan     | symptomatic (diarrhea)          | beta1            | NT                            | - -            | Y            | this study |
| OUT:HND   | 97845       | 1997.8.8        | human  | Japan     | symptomatic (diarrhea)          | iota1            | selC                          | - -            | Y            | this study |
| O126:H4   | 97846       | 1997.8.8        | human  | Japan     | symptomatic (diarrhea, fever)   | alpha2           | selC                          | - -            | Y            | this study |
| O146:H7   | 97938       | 1997.9.1        | human  | Japan     | symptomatic (diarrhea, abdominal pain) | epsilon1         | NT                            | - -            | Y            | this study |
| OUT:HND   | 971107      | 1997.12.20      | human  | Japan     | symptomatic (diarrhea)          | epsilon1         | NT                            | - -            | Y            | this study |
| O55:H7    | 98078       | 1998.5.26       | human  | Japan     | symptomatic (diarrhea)          | beta1            | pheU                          | - -            | Y            | this study |
| O55:H7    | 98117       | 1998.6.24       | human  | Japan     | symptomatic (diarrhea)          | gamma1           | selC                          | - -            | N            | this study |
| OUT:HND   | 98257       | 1998.10.28      | human  | Japan     | symptomatic (bloody diarrhea)   | theta            | NT                            | - -            | Y            | this study |
| O153:H7   | 98275       | 1998.11.6       | human  | NI        | symptomatic (diarrhea)          | beta1            | NT                            | - -            | Y            | this study |
| O20:HNM   | 98288       | 1998.11.30      | human  | Japan     | symptomatic (diarrhea, vomiting, fever) | beta2            | selC                          | - -            | Y            | this study |
| O55:H7    | 99600       | 1999.7.8        | human  | Japan     | symptomatic (diarrhea, abdominal pain) | gamma1           | selC                          | - -            | N            | this study |
| OUT:H2    | 99622       | 1999.8.11       | human  | Japan     | symptomatic (diarrhea, abdominal pain) | beta1            | selC                          | - -            | Y            | this study |
| O119:HNM  | 99638       | 1999.8.23       | human  | Japan     | symptomatic (diarrhea)          | beta1            | selC                          | - -            | Y            | this study |
| OUT:HND   | 99066       | 1999.8.3        | human  | Japan     | symptomatic (diarrhea, abdominal pain) | iota1            | selC                          | - -            | Y            | this study |
| O127a:H40 | 99067       | 1999.8.2        | human  | Japan     | symptomatic (diarrhea, abdominal pain) | theta            | NT                            | - -            | Y            | this study |
| O153:H7   | 99674       | 1999.9.30       | human  | Japan     | symptomatic (diarrhea)          | beta1            | selC                          | - -            | Y            | this study |
| O119:HNM  | 99697       | 1999.10.17      | human  | Japan     | symptomatic (diarrhea, fever)   | beta1            | pheU                          | - -            | N            | this study |
| O26:HNM   | 540         | 2000.4          | human  | Japan     | symptomatic (diarrhea)          | beta1            | NT                            | - -            | N            | this study |
| Serotype† | strain name | year of isolate | origin | countries | symptoms or notes | intimin subtypes | LEE integration sites (tRNA gene) | presence or absence | MLST analysis | References |
|-----------|-------------|-----------------|--------|-----------|-------------------|----------------|-----------------------------------|-------------------|--------------|------------|
| O55:H7    | 544         | 2000.4.21       | human  | Japan     | symptomatic (bloody stool) | gamma1          | seIC                              | -                 | -            | N          | this study |
| OUT:HND   | 24          | 2000.4.14       | human  | Japan     | symptomatic (diarrhea, abdominal pain) | epsilon3        | pheU                              | -                 | +            | Y‡         | this study |
| O55:H7    | 594         | 2000.7          | human  | Japan     | symptomatic (diarrhea, abdominal pain, vomiting) | gamma1          | seIC                              | -                 | -            | N          | this study |
| O128:H2   | 595         | 2000.7.22       | human  | Japan     | symptomatic (diarrhea, abdominal pain) | beta1           | NT                                | -                 | -            | N          | this study |
| O26:H11   | 608         | 2000.8.4        | human  | Japan     | symptomatic (diarrhea, fever) | beta1           | NT                                | -                 | -            | N          | this study |
| O128:H2   | 618         | 2000.8.10       | human  | Japan     | symptomatic (diarrhea, abdominal pain) | beta1           | pheV                              | -                 | -            | N          | this study |
| O26:HUT   | 626         | 2000.8          | human  | Japan     | symptomatic (diarrhea, vomiting) | theta           | NT                                | -                 | -            | Y          | this study |
| O119:HNM  | 629         | 2000.8.25       | human  | Japan     | NI                  | beta1           | pheU                              | -                 | -            | N          | this study |
| O159:HNM  | 664         | 2000.10.16      | human  | Japan     | symptomatic (diarrhea) | theta           | NT                                | -                 | -            | Y          | this study |
| O128:H2   | 674         | 2000.10.27      | human  | Japan     | NI                  | beta1           | NT                                | -                 | -            | N          | this study |
| OUT:HND   | 80          | 2000.10.6       | human  | Japan     | symptomatic (bloody stool) | theta           | NT                                | -                 | -            | Y          | this study |
| OUT:H19   | 1558        | 2001.6.16       | human  | Japan     | symptomatic (diarrhea, abdominal pain, vomiting) | iota1           | seIC                              | -                 | -            | Y          | this study |
| O124:H16  | 01601-2     | 2001.7.28       | human  | Japan     | NI                  | rho              | pheU                              | -                 | -            | Y          | this study |
| OUT:HNM   | 1065        | 2001.7          | human  | Japan     | symptomatic (diarrhea) | theta           | NT                                | -                 | -            | Y          | this study |
| O128:H2   | 1614        | 2001.8.16       | human  | Japan     | NI                  | beta1           | NT                                | -                 | -            | N          | this study |
| O20:H6    | 1086        | 2001.8.17       | human  | Japan     | symptomatic (diarrhea, vomiting) | beta2           | seIC                              | -                 | -            | N          | this study |
| O114:H19  | 1631        | 2001.9.4        | human  | Japan     | NI                  | epsilon2        | seIC                              | -                 | -            | Y          | this study |
| OUT:H21   | 1121        | 2001.10.10      | human  | Japan     | symptomatic (diarrhea) | theta           | seIC                              | -                 | -            | Y          | this study |
| OUT:H6    | 1128        | 2001.10.18      | human  | Japan     | symptomatic (bloody stool, abdominal pain, fever) | beta2           | seIC                              | -                 | -            | Y          | this study |
| O55:H7    | 1687        | 2001.11         | human  | Japan     | symptomatic (bloody stool, fever) | gamma1          | seIC                              | -                 | -            | N          | this study |
| O55:H7    | 01689-1     | 2001.11.27      | human  | Japan     | symptomatic (diarrhea, fever) | gamma1          | seIC                              | -                 | -            | N          | this study |
| O119:HNM  | 1691        | 2001.11.29      | human  | Japan     | symptomatic (diarrhea, abdominal pain) | beta1           | pheU                              | -                 | -            | N          | this study |
| O119:HNM  | 2528        | 2002.4.8        | human  | Japan     | symptomatic (bloody stool) | beta1           | pheU                              | -                 | -            | N          | this study |
| OUT:HND   | 2059        | 2002.6.5        | human  | Japan     | symptomatic (diarrhea, vomiting, fever) | alpha2           | seIC                              | -                 | -            | Y          | this study |
| O55:H7    | 2075        | 2002.6.27       | human  | Japan     | symptomatic (diarrhea) | beta1           | pheV                              | -                 | -            | Y          | this study |
| O128:H2   | 2584        | 2002.7.13       | human  | Japan     | symptomatic (diarrhea, fever) | beta1           | pheV                              | -                 | -            | N          | this study |
| Serotype† | strain name | year of isolate | origin | countries | symptoms or notes | intimin subtypes | LEE integration sites (IRNA gene) | presence or absence | MLST analysis | References |
|-----------|-------------|-----------------|--------|-----------|-------------------|-----------------|-----------------------------------|-------------------|---------------|------------|
| O128:HND  | 2595        | 2002.7.22       | human  | Japan     | symptomatic (diarrhea, fever) | beta1           | pheV                               | -                 | -             | N          | this study |
| O55:H7    | 2604        | 2002.8.3        | human  | Japan     | NI                | gamma1          | selC                               | -                 | -             | N          | this study |
| O55:H7    | 2612        | 2002.8.12       | human  | Japan     | NI                | gamma1          | selC                               | -                 | -             | N          | this study |
| O55:H7    | 2626        | 2002.8.26       | human  | Japan     | symptomatic (diarrhea, fever) | gamma1          | selC                               | -                 | -             | N          | this study |
| OUT:HND   | 2184        | 2002.11.25      | human  | Japan     | symptomatic (diarrhea, vomiting) | beta2           | selC                               | -                 | -             | Y          | this study |
| O55:H7    | 3114        | 2003.7.30       | human  | Japan     | symptomatic (diarrhea, fever) | gamma1          | selC                               | -                 | -             | N          | this study |
| O26:H     | 3641        | 2003.7.7        | human  | Japan     | symptomatic (diarrhea)       | beta1           | NT                                | -                 | -             | N          | this study |
| OUT:HND   | 3124        | 2003.8.5        | human  | Japan     | symptomatic (bloody stool, abdominal pain) | epsilon2         | selC                               | -                 | -             | Y          | this study |
| O55:H7    | 3649        | 2003.8.1        | human  | Japan     | symptomatic (diarrhea)       | gamma1          | selC                               | -                 | -             | N          | this study |
| O128:H2   | 3705        | 2003.8.29       | human  | Japan     | symptomatic (diarrhea)       | beta1           | NT                                | -                 | -             | N          | this study |
| O26:HN   | 03706-2    | 2003.9.3        | human  | Japan     | NI                 | beta1           | NT                                | -                 | -             | N          | this study |
| O55:H7    | 4676        | 2004.9.17       | human  | Japan     | symptomatic (diarrhea)       | iota1           | selC                               | -                 | -             | N          | this study |
| O153:HND  | 4679        | 2004.9.24       | human  | Japan     | symptomatic (diarrhea)       | beta1           | NT                                | -                 | -             | N          | this study |
| O142:HUT  | 6592        | 2006.6.2        | human  | Japan     | symptomatic (diarrhea, abdominal pain) | alpha1          | selC                               | -                 | -             | Y          | this study |
| O55:H7    | 7575        | 2007.5.16       | human  | Japan     | symptomatic (diarrhea)       | gamma1          | selC                               | -                 | -             | N          | this study |
| O55:H7    | 7675        | 2007.7.23       | human  | Japan     | symptomatic (diarrhea)       | gamma1          | selC                               | -                 | -             | N          | this study |
| O55:H7    | 7693        | 2007.8.1        | human  | Japan     | symptomatic (diarrhea)       | gamma1          | selC                               | -                 | -             | N          | this study |
| O55:H7    | 7707        | 2007.8.8        | human  | Japan     | symptomatic (diarrhea)       | gamma1          | selC                               | -                 | -             | N          | this study |
| O119:3-H  | 7753        | 2007.8.30       | human  | Japan     | symptomatic (diarrhea)       | beta1           | pheU                               | -                 | -             | N          | this study |
| O124:HUT  | 7852        | 2007.10.23      | human  | Japan     | symptomatic (diarrhea)       | theta            | NT                                | -                 | -             | Y          | this study |
| O26:H     | 7857        | 2007.10.29      | human  | Japan     | NI                 | beta1           | NT                                | -                 | -             | N          | this study |
| O74:HND   | 7871        | 2007.11.9       | human  | Japan     | symptomatic (diarrhea)       | iota1           | selC                               | -                 | -             | Y          | this study |
| O103:3-H  | 7929        | 2007.12.4       | human  | Japan     | symptomatic (bloody stool)  | beta1           | NT                                | -                 | -             | Y          | this study |
| O103      | NIAH_Por_1  | 2007           | pig    | Japan     | rectal swab, healthy       | beta1           | NT                                | -                 | -             | Y          | this study |
| OUT       | NIAH_Por_2  | 2007           | pig    | Japan     | rectal swab, healthy       | gamma1          | selC                               | -                 | -             | N          | this study |
| OUT       | NIAH_Por_4  | 2007           | pig    | Japan     | rectal swab, healthy       | gamma1          | selC                               | -                 | -             | N          | this study |
| O49       | NIAH_Por_5  | 2007           | pig    | Japan     | rectal swab, healthy       | kappa            | selC                               | -                 | -             | Y          | this study |
| O171      | NIAH_Por_8  | 2007           | pig    | Japan     | rectal swab, healthy       | theta            | pheV                               | -                 | -             | N          | this study |
| OUT       | NIAH_Por_9  | 2007           | pig    | Japan     | rectal swab, healthy       | gamma1          | selC                               | -                 | -             | N          | this study |
| O88       | NIAH_Por_10 | 2007           | pig    | Japan     | rectal swab, healthy       | beta1           | pheU                               | -                 | -             | Y          | this study |
| O76       | NIAH_Por_11 | 2007           | pig    | Japan     | rectal swab, healthy       | theta            | NT                                | -                 | -             | N          | this study |
| O145      | NIAH_Por_12 | 2007           | pig    | Japan     | rectal swab, healthy       | gamma1          | selC                               | -                 | -             | Y          | this study |
| OUT       | NIAH_Por_13 | 2007           | pig    | Japan     | rectal swab, healthy       | xi                | NT                                | -                 | -             | N          | this study |
| O26       | NIAH_Por_14 | 2007           | pig    | Japan     | rectal swab, healthy       | xi                | NT                                | -                 | -             | Y          | this study |
| O2        | NIAH_Por_15 | 2007           | pig    | Japan     | rectal swab, healthy       | iota1           | selC                               | -                 | -             | Y          | this study |
| O145      | NIAH_Por_16 | 2007           | pig    | Japan     | rectal swab, healthy       | gamma1          | selC                               | -                 | -             | N          | this study |
| O53       | NIAH_Por_17 | 2007           | pig    | Japan     | rectal swab, healthy       | gamma1          | selC                               | -                 | -             | Y          | this study |

References
- this study
| Serotype† | strain name         | year of isolate | origin | countries | symptoms or notes               | intimin subtypes | LEE integration sites (rRNA gene) | presence or absence | MLST analysis | References       |
|-----------|--------------------|-----------------|--------|-----------|---------------------------------|-----------------|-----------------------------------|---------------------|---------------|-----------------|
| O117      | NIAH_Por_18        | 2007            | pig    | Japan     | rectal swab, healthy            | theta           | pheV                              | -                   | N             | this study      |
| O172      | NIAH_Por_19        | 2007            | pig    | Japan     | rectal swab, healthy            | gamma1          | seiC                              | -                   | Y             | this study      |
| O172      | NIAH_Por_20        | 2007            | pig    | Japan     | rectal swab, healthy            | gamma1          | seiC                              | -                   | N             | this study      |
| O117      | NIAH_Por_21        | 2007            | pig    | Japan     | rectal swab, healthy            | theta           | pheV                              | -                   | N             | this study      |
| O117      | NIAH_Por_22        | 2007            | pig    | Japan     | rectal swab, healthy            | theta           | pheV                              | -                   | N             | this study      |
| O117      | NIAH_Por_23        | 2007            | pig    | Japan     | rectal swab, healthy            | theta           | pheV                              | -                   | N             | this study      |
| O117      | NIAH_Por_24        | 2007            | pig    | Japan     | rectal swab, healthy            | gamma1          | seiC                              | -                   | N             | this study      |
| O156      | NIAH_Por_25        | 2007            | pig    | Japan     | rectal swab, healthy            | theta           | NT                                | -                   | N             | this study      |
| O98       | NIAH_Por_26        | 2007            | pig    | Japan     | rectal swab, healthy            | theta           | pheU                              | -                   | N             | this study      |
| OUT       | NIAH_Por_27        | 2007            | pig    | Japan     | rectal swab, healthy            | xi               | pheU                              | -                   | N             | this study      |
| O49       | NIAH_Por_33        | 2007            | pig    | Japan     | rectal swab, healthy            | kappa           | seiC                              | -                   | N             | this study      |
| OUT       | NIAH_Por_34        | 2007            | pig    | Japan     | rectal swab, healthy            | pheV            | -                                 | -                   | N             | this study      |
| O172      | NIAH_Por_35        | 2007            | pig    | Japan     | rectal swab, healthy            | gamma1          | seiC                              | -                   | N             | this study      |
| O8        | NIAH_Por_36        | 2007            | pig    | Japan     | rectal swab, healthy            | gamma1          | seiC                              | -                   | Y             | this study      |
| O145      | NIAH_Por_37        | 2007            | pig    | Japan     | rectal swab, healthy            | gamma1          | seiC                              | -                   | N             | this study      |
| O145      | NIAH_Por_38        | 2007            | pig    | Japan     | rectal swab, healthy            | gamma1          | seiC                              | -                   | N             | this study      |
| O53       | NIAH_Por_40        | 2007            | pig    | Japan     | rectal swab, healthy            | gamma1          | seiC                              | -                   | N             | this study      |
| O71       | NIAH_Bird_1        | 2003            | Bird   | Japan     | feces, Treron sieboldi          | kappa           | seiC                              | -                   | Y             | this study      |
| OUT       | NIAH_Bird_2        | 2002            | Bird   | Japan     | feces, Sturnus cinereus         | sigma           | pheU                              | +                   | Y‡            | this study      |
| O115      | NIAH_Bird_3        | 2004            | Bird   | Japan     | feces, Puffinus tenuirostris    | N1.1            | pheU                              | -                   | +             | Y‡             |
| O137      | NIAH_Bird_4        | 2004            | Bird   | Japan     | feces, Passer montanus          | beta2           | seiC                              | -                   | -             | Y              |
| O128      | NIAH_Bird_5        | 2004            | Bird   | Japan     | feces, Puffinus tenuirostris    | beta3           | pheU                              | -                   | +             | Y‡             |
| O117      | NIAH_Bird_6        | 2004            | Bird   | Japan     | feces, Hirundo rustica          | mu               | seiC                              | -                   | -             | Y              |
| O117      | NIAH_Bird_7        | 2004            | Bird   | Japan     | feces, Passer montanus          | mu               | seiC                              | -                   | -             | N              |
| O64       | NIAH_Bird_8        | 2004            | Bird   | Japan     | feces, Egretta garzetta         | epsilon4        | pheU                              | -                   | +             | Y‡             |
| O21       | NIAH_Bird_9        | 2004            | Bird   | Japan     | feces, Hirundo rustica          | beta1           | NT                                | -                   | -             | Y              |
| O81       | NIAH_Bird_10       | 2004            | Bird   | Japan     | feces, Anas poecilorhyncha      | beta2           | seiC                              | -                   | +             | Y              |
| O55       | NIAH_Bird_11       | 2005            | Bird   | Japan     | feces, Emberiza certhya         | theta           | NT                                | -                   | -             | N              |
| O2        | NIAH_Bird_12       | 2005            | Bird   | Japan     | feces, Sturnus cinereus         | beta1           | NT                                | -                   | -             | Y              |
| OUT       | NIAH_Bird_13       | 2005            | Bird   | Japan     | feces, Hypsipetes amaurots      | xi               | pheU                              | -                   | +             | Y‡             |
| O55       | NIAH_Bird_15       | 2005            | Bird   | Japan     | feces, Cyanopica cyanus         | theta           | NT                                | -                   | -             | N              |
| O103      | NIAH_Bird_16       | 2005            | Bird   | Japan     | feces, Passer montanus          | NT.1            | pheU                              | -                   | +             | Y‡             |
| O55       | NIAH_Bird_17       | 2005            | Bird   | Japan     | feces, Streptopelia orientalis  | theta           | NT                                | -                   | -             | N              |
| O120      | NIAH_Bird_18       | 2005            | Bird   | Japan     | feces, Anas strepera            | pi               | seiC                              | -                   | -             | N              |
| O132      | NIAH_Bird_19       | 2005            | Bird   | Japan     | feces, Columba livia            | alpha2          | seiC                              | -                   | -             | N              |
| O132      | NIAH_Bird_20       | 2005            | Bird   | Japan     | feces, Columba livia            | alpha2          | seiC                              | -                   | -             | Y              |
| O50       | NIAH_Bird_21       | 2005            | Bird   | Japan     | feces, Streptopelia orientalis  | alpha1          | seiC                              | -                   | Y             | this study      |
| Serotype† | strain name | year of isolate | origin | countries | symptoms or notes | intimin subtypes LEE integration sites (tRNA gene) | presence or absence | MLST analysis | References |
|-----------|-------------|----------------|--------|-----------|-------------------|------------------------------------------------|-------------------|--------------|------------|
| O171      | NIAH_Bird_22 | 2005           | Bird   | Japan     | feces, *Phalacrocorax carbo* | epsilon2 selC | -     | Y ‡    | this study |
| O58       | NIAH_Bird_23 | 2006           | Bird   | Japan     | feces, *Phalacrocorax carbo* | epsilon1 pheU | -     | +     | Y ‡    | this study |
| O147      | NIAH_Bird_24 | 2006           | Bird   | Japan     | feces, *Cyanopica cyanus* | sigma pheU | -     | +     | Y ‡    | this study |
| O8        | NIAH_Bird_25 | 2006           | Bird   | Japan     | feces, *Passer montanus* | sigma pheU | -     | +     | Y ‡    | this study |
| O128      | NIAH_Bird_26 | 2006           | Bird   | Japan     | feces, *Hypsipetes amaurotis* | beta3 pheU | -     | +     | Y ‡    | this study |
| O8        | NIAH_Bird_27 | 2006           | Bird   | Japan     | feces, *Phalacrocorax carbo* | beta1 NT | -     | -     | N   | this study |
| O137      | NIAH_Bird_28 | 2006           | Bird   | Japan     | feces, *Zosterops japonica* | beta2 selC | -     | -     | N   | this study |
| O56       | NIAH_Bird_29 | 2005           | Bird   | Japan     | foot, *Hypsipetes amaurotis* | beta2 selC | -     | -     | Y   | this study |
| O55       | NIAH_Bird_30 | 2005           | Bird   | Japan     | foot, *Phalacrocorax carbo* | theta NT | -     | -     | N   | this study |
| O132      | NIAH_Bird_31 | 2005           | Bird   | Japan     | foot, *Columba livia* | alpha2 selC | -     | -     | N   | this study |
| O120      | NIAH_Bird_32 | 2005           | Bird   | Japan     | foot, *Columba japonica* | pi selC | -     | -     | Y   | this study |
| O110      | NIAH_Bird_33 | 2006           | Bird   | Japan     | foot, *Puffinus tenuirostris* | beta2 selC | -     | -     | Y   | this study |
| O2        | NIAH_Bird_34 | 2006           | Bird   | Japan     | foot, *Columba livia* | kappa selC | -     | -     | Y   | this study |
| O55       | NIAH_Bird_35 | 2006           | Bird   | Japan     | foot, *Hypsipetes amaurotis* | theta NT | -     | -     | N   | this study |
| O8        | NIAH_Bird_36 | 2006           | Bird   | Japan     | foot, *Phalacrocorax carbo* | beta1 NT | -     | -     | Y   | this study |
| O55       | NIAH_Bird_37 | 2006           | Bird   | Japan     | foot, *Sturnus cineraceus* | theta NT | -     | -     | N   | this study |
| O55       | NIAH_Bird_38 | 2006           | Bird   | Japan     | foot, *Columba livia* | theta NT | -     | -     | N   | this study |
| O103:H2   | 00E001      | 2000           | human  | Japan     | symptomatic (diarrhea, abdominal pain, fever) | epsilon1 NT | stx1 | -     | Y   | this study |
| O150:H11  | 00E019      | 2000           | human  | Japan     | symptomatic (diarrhea, bloody stool, abdominal pain, fever) | beta1 pheU stx1 | - | Y   | this study |
| O103:H11  | 01E015      | 2001           | human  | Japan     | symptomatic (diarrhea, abdominal pain, fever) | beta1 pheU stx1 | - | Y   | this study |
| O103:H2   | 02E028      | 2002           | human  | Japan     | symptomatic (diarrhea, abdominal pain, fever) | epsilon1 NT | stx1 | - | N   | this study |
| O165:H-   | 04E077      | 2000           | human  | Japan     | symptomatic (diarrhea, abdominal pain, fever) | epsilon1 NT | stx2 | - | N   | this study |
| O121:H14  | 06E050      | 2006           | human  | Japan     | symptomatic (diarrhea, bloody stool) | epsilon1 NT | stx2 | - | Y   | this study |
| O103:H2   | 07E030      | 2007           | human  | Japan     | symptomatic (diarrhea, bloody stool, abdominal pain) | epsilon1 NT | stx1 | - | N   | this study |
| O63:H6    | 07E033      | 2000           | human  | Japan     | symptomatic (diarrhea, abdominal pain) | (alpha2) selC stx2f | + | Y   | this study |
| O165:H-   | 07E051      | 2007           | human  | Japan     | symptomatic (diarrhea, bloody stool, abdominal | epsilon1 NT | stx1&2 | - | Y   | this study |

References: this study
| Serotype† | strain name | year of isolate | origin | countries | symptoms or notes (pain) | intimin subtypes | LEE integration sites (RNA gene) | presence or absence of stx1 & 2f and cdtB | MLST analysis | References |
|-----------|-------------|-----------------|--------|-----------|--------------------------|-----------------|-------------------------------|--------------------------------------------|--------------|------------|
| O118:H3   | 07E054      | 2007            | human  | Japan     | symptomatic (diarrhea, abdominal pain) | beta1           | NT                            | stx1 -                                     | Y            | this study |
| O103:H2   | 08E011      | 2008            | human  | Japan     | asymptomatic carrier        | epsilon1        | NT                            | stx1 -                                     | N            | this study |
| O103:HUT  | 08E021      | 2008            | human  | Japan     | asymptomatic carrier        | theta           | NT                            | stx1 -                                     | N            | this study |
| O121:H19  | 08E027      | 2008            | human  | Japan     | symptomatic (diarrhea, bloody stool, abdominal pain, fever) | epsilon1        | NT                            | stx2 -                                     | N            | this study |
| O76:H6    | 08E035      | 2008            | human  | Japan     | asymptomatic carrier        | gamma1          | NT                            | stx1 -                                     | Y            | this study |
| O165:HNM  | 08E132      | 2008            | human  | Japan     | symptomatic (bloody stool, abdominal pain, vomiting, fever) | epsilon1        | NT                            | stx1 & 2f -                                 | N            | this study |
| O165:SEN  | osen07-074  | 2007            | Bovid  | Japan     | food                       | epsilon1        | NT                            | stx2 -                                     | N            | this study |
| OUT       | CB10113     | 2004            | cat    | Brazil    | domestic (asymptomatic carrier) | ypsilon          | pheU                          | - -                                        | Y‡           | Morato et al., 2009f |
| O180      | CB9776      | 2003            | human  | Germany   | symptomatic (diarrhea)      | rho             | pheU                          | - -                                        | N            | this study |
| O65       | CB9786      | 2003            | human  | Germany   | symptomatic (diarrhea)      | rho             | pheU                          | - -                                        | Y            | this study |
| O168      | CB9791      | 2003            | human  | Germany   | symptomatic (diarrhea)      | rho             | pheU                          | - +                                        | Y‡           | this study |
| O160      | DG172/5     | 1990            | sheep  | Japan     | asymptomatic carrier        | rho             | pheU                          | - -                                        | N            | this study |
| NT:H19    | 0471-1      | 1989            | human  | Germany   | symptomatic (diarrhea)      | rho             | pheU                          | - -                                        | Y            | Ooka et al., 2008‡ |
| NT:HNM    | 0451-6      | 1989            | human  | Brazil    | symptomatic (diarrhea)      | omicron         | pheU                          | - +                                        | Y‡           | Ooka et al., 2008‡ |
| NI        | A09/332.1   | 2008.11         | deer   | Belgium   | Capreolus capreolus        | epsilon2        | selC                          | - -                                        | Y            | Bardiau et al., 2010¶ |
| O115:HNM  | HPH08472    | 2008.8          | human  | Japan     | symptomatic (diarrhea)      | N2              | pheU                          | stx2f -                                    | Y‡           | this study |
| OUT:H1    | E2675       | 2007            | bird   | Japan     | feces swab, Corvus spp.     | N1.2            | pheU                          | stx2f -                                    | Y‡           | this study |
| O156:H25  | RIMD0509178 | 2003            | human  | Japan     | asymptomatic carrier        | zeta            | pheV                          | stx1 -                                     | Y            | this study |
| O55:H6    | F76193      | 1989            | human  | SSI       | symptomatic (diarrhea)      | alpha2          | selC                          | - -                                        | Y            | Iida et al., 2001§ |
| O153:HNM  | HPH07217    | 2007.8          | human  | Japan     | symptomatic (diarrhea, fever) | beta1           | NT                            | stx2f +                                    | Y            | this study |
| O63:H6    | HPH07137    | 2007.8          | human  | Japan     | symptomatic (diarrhea, abdominal pain) | alpha2          | selC                          | stx2f -                                    | Y            | this study |
| O145:H34  | HPH08592    | 2008.10         | human  | Japan     | symptomatic (diarrhea, fever) | iota1           | selC                          | stx2f -                                    | Y            | this study |
| O128:HNM  | EC2175      | 2002.7.28       | human  | Japan     | symptomatic (one year old, diarrhea, bloody mucus stool, vomiting) | beta1           | NT                            | stx2f +                                    | Y            | this study |
| O63:H6    | EC2689      | 2006.9.16       | human  | Japan     | symptomatic (four years old, fever, cough, soft stool) | alpha2          | selC                          | stx2f +                                    | N            | this study |
| O145:H34  | E2473       | 2006.8          | human  | Japan     | symptomatic (diarrhea, bloody stool, abdominal pain, fever) | iota1           | selC                          | stx2f +                                    | N            | this study |

References:

Iida et al., 2001§
Morato et al., 2009f
Ooka et al., 2008‡
Bardiau et al., 2010¶
| Serotype† | strain name | year of isolate | origin | countries | symptoms or notes | intimin subtypes | LEE integration sites (rRNA gene) | presence or absence | MLST analysis | References |
|-----------|-------------|-----------------|--------|-----------|-------------------|-----------------|---------------------------------|-------------------|-------------|------------|
| O63:H6    | A32         | 2003.8          | human  | Japan     | symptomatic (diarrhea, abdominal pain) | alpha2          | selC                            | stx1&2&2f         | -           | this study |

NI: no information, NT: not typed
†: Determined by the serotyping system for E. coli.
‡: E. albertii strains (confirmed by MLS analysis).
ƒ: Morato et al. (2009) Domestic cats constitute a natural reservoir of human enteropathogenic Escherichia coli Types. Zoonoses Public Health. 56: 229-237.
‡: Ooka T et al. (2007) Characterization of tccP2 carried by atypical enteropathogenic Escherichia coli. FEMS Microbiol Lett. 271: 126-135.
¶: Bardiau M et al. (2010) Enteropathogenic (EPEC), enterohaemorrhagic (EHEC) and verotoxigenic (VTEC) Escherichia coli in wild cervids. J Appl Microbiol. 109: 2214-2222.
§: Iida K et al. (2001) Type 1 fimbriation and its phase switching in diarrheagenic Escherichia coli strains. Clin Diagn Lab Immunol. 8: 489–495.
 Technical Appendix Table 2. PCR primers for detection and sequencing of the stx and cdt genes

| target gene | primer name | sequence (5’-3’) | PCR conditions (30 cycles) | Size of amplicon (bp) | References |
|-------------|-------------|------------------|----------------------------|-----------------------|------------|
| 5’ half of eae | eae-F1 | ACTCGATTCCTCTGGTGAC | 55°C, 60 s / 72°C, 2 min | around 1.3 kb | Lacher et al., 2006f |
| 5’ half of eae | eae-R3 | TCTTGTCGCTTTGGCTT | 92°C, 60 s / 92°C, 60 s / 72°C, 2 min | 92 | Lacher et al., 2006f |
| 3’ half of eae | escD-R1 | GTATCAACATCTCCGCCA | 52°C, 60 s / 72°C, 2 min | around 1.6 kb | Lacher et al., 2006f |
| inside of eae | 1669-1688§ | CAGTTGGGGAACAGGACTT | — | — | this study |
| stx1 | stx1-F | GTCATTGCCCTCTGCAATAGGTAC | 94°C, 30 s / 64°C, 30 s / 72°C, 90 s | 151 | Ooka et al., 2009 |
| stx1 | stx1-R | GCCGTAGATTATTAACCGCCT | 94°C, 30 s / 64°C, 30 s / 72°C, 90 s | 181 | Ooka et al., 2009 |
| stx2 | stx2-F | CCATGCAACCGGACACCTT | 94°C, 30 s / 64°C, 30 s / 72°C, 90 s | 466 | Toth et al., 2003† |
| stx2 | stx2-R | CTGCTGTGACAGTGACAAAACG | 94°C, 30 s / 64°C, 30 s / 72°C, 90 s | 466 | Toth et al., 2003† |
| cdtB | CDT-s1 | GAAAGTAAATGGAATATAAATGTCCG | 94°C, 30 s / 64°C, 30 s / 72°C, 90 s | 466 | Toth et al., 2003† |
| cdtB | CDT-s2 | GAAATATAATGGAAACACACATGTCGG | 94°C, 30 s / 64°C, 30 s / 72°C, 90 s | 466 | Toth et al., 2003† |
| cdtB | CDT-as1 | AAATCACCAAGAATCATCCAGTTA | 94°C, 30 s / 64°C, 30 s / 72°C, 90 s | 466 | Toth et al., 2003† |

§: This primer is used only for sequencing of the eae gene.
ƒ: Lacher DW et al. (2006) Allelic subtyping of the intimin locus (eae) of pathogenic Escherichia coli by fluorescent RFLP. FEMS Microbiol Lett. 261:80-87.
*: Schmidt H et al. (2000) A new Shiga toxin 2 variant (Stx2f) from Escherichia coli isolated from pigeons. Appl Environ Microbiol. 66:1205-1208.
†: Toth I et al. (2003) Production of cytotoxic distending toxins by pathogenic Escherichia coli strains isolated from human and animal sources: establishment of the existence of a new cdt variant (Type IV). J Clin Microbiol. 41:4285-4291.
### Technical Appendix Table 3. Reference sequences of the eae genes

| subtype | species of origin | Serotype | Strain name | accession No. |
|---------|------------------|----------|-------------|---------------|
| α1 (alpha1) | Escherichia coli | O127:H6  | E2348/69   | AF022236      |
| α2 (alpha2) | Escherichia coli | O125:H6  | C712-65    | DQ52600       |
| α8 (alpha8) | Escherichia albertii | - | I2005002880 36 | FJ609835 |
| β1 (beta1) | Escherichia coli | O26:H- | 413/89-1 | AJ277443 |
| β2 (beta2) | Escherichia coli | O119:H6  | 0659-79    | DQ52605       |
| β3 (beta3) | Escherichia coli | O103:H2  | MT#80      | DQ529406      |
| ε2 (epsilon2) | Escherichia coli | O116:[H9] | 0659-79 | DQ523605 |
| ε3 (epsilon3) | Escherichia coli | O119:H6  | 98B3       | DQ529414      |
| ε4 (epsilon4) | Escherichia coli | O142:[H21] | 012-050982 | AJ876565 |
| γ1 (gamma1) | Escherichia coli | O157:H7  | BAB37982.1 | AJ876565 |
| γ2 (gamma2) | Escherichia coli | O111:H- | 95NR1      | AF025311      |
| ι1 (iota1) | Escherichia coli | O55:[H34/47] | 1252-59 | DQ52601 |
| ι2 (iota2) | Shigella boydii | 13 | C-425     | AY696842      |
| ι (iota) | Escherichia coli | O49:[H10] | 64B4      | DQ523611      |
| κ (kappa) | Escherichia coli | O33:[H34] | 57A1      | DQ523609      |
| μ (mu) | Escherichia coli | O55:[H51] | MA551/1 | DQ523607 |
| ν (nu) | Escherichia albertii | - | 106A5   | DQ523615 |
| ο (omicron) | Escherichia albertii | - | 19982   | AY696838 |
| π (pi) | Escherichia coli | O57 | AEEC-191.2 | AY705052 |
| ρ (rho) | Escherichia coli | 9314 | 9314  | DQ523613 |
| σ (sigma) | Escherichia coli | O86:K61:H- | EPEC-EC74699 | AJ781125 |
| τ (tau) | Shigella boydii | 7 | K-1      | AY696839 |
| θ (theta) | Escherichia coli | O111:H8 | CL-37     | AF449418      |
| ξ (xi) | Escherichia coli | O5:[H2] | 60A3      | DQ523610      |
| υ (upsilon) | Escherichia coli | ONT | CB10113 | AM116755.1 |
| ζ (zeta) | Escherichia coli | 92-1B | AF449417 |
| ζ3 (zeta3) | Escherichia coli | O85:H31 | FV10126 | FM872423 |
| C. rodentium | Citrobacter | 10 | DBS100 | AF311901 |

### Technical Appendix Table 4. Shiga toxin production by the two stx2f-positive E. albertii strains

| Strain name | Species | prevalence of stx genes | VTEC-RPLA† | References |
|-------------|---------|--------------------------|------------|------------|
| HIPH08472   | E. albertii | stx2f               | MMC+   | this study |
| E2675       | E. albertii | stx2f               | MMC+   | this study |
| LMG20976    | E. albertii | stx2f               | MMC+   | this study |
| CB9786      | E. albertii | stx2f               | MMC+   | this study |
| O128:HNM    | E. albertii | stx2f               | MMC+   | this study |
| EC1463      | E. coli  | stx2f               | MMC+   | Isobe et al., 2004 |
| O157:H7 Sakai| E. coli  | stx1, stx2           | MMC+   | Hayashi et al., 2000 |

†: The maximum dilution of culture supernatant that exhibited agglutination is shown in parentheses.

§: Hayashi T et al. (2001) Complete genome sequence of enterohemorrhagic Escherichia coli O157:H7 and genomic comparison with a laboratory strain K-12. DNA Res. 8: 11-22.
| Primer name | Sequence (5'-3') | Target locus | Location of the primers on the sequenced *E. coli* strains | Expected amplicon size (bp) | References |
|-------------|-----------------|--------------|-----------------------------------------------------------|-----------------------------|------------|
| escR-R      | ACTGGCGGATACCATCATC | escR gene on the LEE core region | - | - | this study |
| pheV-Ro1    | CAGGTATGTACCTTCACC | pheV 3'-end of K-12 strain 26,337 bp downstream of the pheV 3'-end of MG1655 | around 30 kb | this study |
| pheV-gIcB   | ACAATGAGTCAAACCATA | pheV 3' end of K-12 strain 13,367 bp downstream of the pheV 3' end of MG1655 | around 17 kb | this study |
| selC-Ro1    | CACGGCGGCAATCAGAA | selC 3'-end of O103:H2 strain 1,984 bp downstream of the selC 3'-end of 12009 | around 5 kb | this study |
| 433-f       | ACGCGGGATTGTTTGG | pheU 3'-end of O157:H7 strain 14,687 bp downstream of the pheU 3'-end of Sakai | around 18 kb | Ohnishi *et al.*, 2002§ |

†: PCR cycle; 2 min at 96°C, followed by 30 cycles of 20 s at 96°C and 16 min at 69°C.
§: Ohnishi M *et al.* (2002) Genomic diversity of enterohemorrhagic Escherichia coli O157 revealed by whole genome PCR scanning. Proc Natl Acad Sci USA, 99:17043-17048.