Human Gastroenteritis Outbreak Associated with *Escherichia albertii*, Japan

Tadasuke Ooka, Eisuke Tokuoka, Masato Furukawa, Tetsuya Nagamura, Yoshitoshi Ogura, Kokichi Arisawa, Seiya Harada, and Tetsuya Hayashi

Although *Escherichia albertii* is an emerging intestinal pathogen, it has been associated only with sporadic human infections. In this study, we determined that a human gastroenteritis outbreak at a restaurant in Japan had *E. albertii* as the major causative agent.

Escherichia albertii is an emerging human and bird pathogen that belongs to the attaching and effacing group of pathogens. This group of pathogens forms lesions on intestinal epithelial cell surfaces by the combined action of intimin, an *eae* gene–encoded outer membrane protein, and type III secretion system effectors (*1–4*).

Recently, we found that *E. albertii* represents a substantial proportion of the strains that had previously been identified as *eae*-positive *Escherichia coli*, enteropathogenic *E. coli* or enterohemorrhagic *E. coli*; 26 of the 179 *eae*-positive strains analyzed were found to be *E. albertii* (*5*). Furthermore, *E. albertii* is also a potential Shiga toxin 2f (Stx2f)–producing bacterial species (*5*). However, no *E. albertii*–associated gastroenteritis outbreak has been reported, which generates doubts regarding the clinical role of this microorganism. In this study, we revisited an outbreak of gastroenteritis that was presumed to have been caused by *eae*-positive atypical *E. coli* OUT:HNM (*6*) to determine if it was actually caused by *E. albertii*.

The Study

An outbreak of gastroenteritis occurred at the end of May 2011 in Kumamoto, Japan, among persons who attended 1 of 2 parties held in a Japanese restaurant on May 29. We reviewed case records for the 94 persons who attended the parties. A total of 48 persons became ill; 43 of them attended the first party (a total of 86 attended), and 5 attended the second party (a total of 8 attended). The ill participants had not eaten any food in common except for the meals served at the restaurant. The main symptoms of the patients were diarrhea (83%), abdominal pain (69%), fever (44%; mean temperature 37.2°C), and nausea (29%). The mean incubation period was 19 h.

A routine protocol to identify bacteria and viruses (online Technical Appendix, wwwnc.cdc.gov/EID/pdfs/12-0646-Techapp.pdf) was used by our laboratory to examine 54 fecal specimens from 44 party participants and 10 members of the restaurant kitchen staff (7 party participants and all of the kitchen staff were asymptomatic). Atypical *E. coli* (lactose negative; OUT:HNM) strains harboring the *eae* gene and *E. coli* OUT:H18 strains harboring the *stx2d* and *astA* (but not *eae*) genes were isolated from 24 and 3 specimen, respectively; 7 specimens yielded both strains (Table 1). The *stx2*-positive/*eae*-negative *E. coli* strains were found to be serotype O183 (a recently described O serotype) by agglutination testing with O183-specific antiserum (S. Iyoda, M. Ohnishi, unpub. data).

All atypical *E. coli* strains showed identical or nearly identical *Xba*I-digested DNA banding patterns by pulsed-field gel electrophoresis, and the 10 *E. coli* O183:H18 strains also exhibited identical patterns (Figure). The source of the infection was most likely the meals served in the restaurant, but a bacteriological examination of the meal or of the ingredients used to prepare the meal was not possible because none of the food was preserved for analysis.

The lactose-negative/*eae*-positive features of the OUT:HNM strains suggested that these strains might be *E. albertii*. We examined additional biochemical properties of these strains and found that they exhibited the *E. albertii*–specific features described (*4,5*). These features include nonmotility, inability to ferment xylose and lactose, and inability to produce β-D-glucuronidase. The *E. coli* O183:H18 strains demonstrated common phenotypic and biochemical properties of *E. coli* (*7*).

To determine whether the *E. albertii*–like OUT:HNM strains were *E. albertii*, we randomly selected 6 strains and determined their phylogeny by multilocus sequence analysis as described (*5*) (online Technical Appendix Table). Results indicated that although the *E. coli* O183:H18 strain analyzed in parallel belongs to *E. coli sensu stricto*, the *E. albertii*–like OUT:HNM strains belong to the *E. albertii* lineage; all 6 strains showed identical sequences (online Technical Appendix Figure).

We further examined the intimin subtype by sequencing the *eae* gene, the chromosome integration site of the locus of enterocyte effacement encoding the *eae* gene and a

Author affiliations: University of Miyazaki, Miyazaki, Japan (T. Ooka, Y. Ogura, T. Hayashi); Kumamoto Prefectural Institute of Public Health and Environmental Science, Kumamoto, Japan (E. Tokuoka, M. Furukawa, T. Nagamura, S. Harada); and University of Tokushima, Tokushima, Japan (K. Arisawa)

DOI: http://dx.doi.org/10.3201/eid1901.120646

Table 1. Isolates from fecal specimens of party participants during outbreak of gastroenteritis associated with *Escherichia albertii*, Japan*

	Origin of isolates				
	Participants, n = 44			Kitchen staff, n = 10	
Isolate	Symptomatic	Asymptomatic	No information	Symptomatic	Asymptomatic
E. albertii†	21	1	0	0	2
E. albertii† and *E. coli* O183:H18‡	7	0	0	0	0
E. coli O183:H18‡	3	0	0	0	0
None	6	5	1	0	8

*None, negative for both pathogens.
†Initially identified as atypical (lactose negative) *E. coli* OUT:HNM harboring the intimin (*eae*) gene.
‡Initially identified as *eae* negative *E. coli* OUT:H18 harboring the Shiga toxin 2d and enteroaggregative *E.coli* heat-stable toxin genes.

set of type III secretion system genes, and the presence and subtype of the *cdtB* gene as described (*5*). Results showed that the *E. albertii* strains had intimin σ, which is rarely identified in enteropathogenic *E. coli* or enterohemorrhagic *E. coli*; the locus of enterocyte effacement was integrated into the *pheU* tRNA gene; and the *cdtB* gene of the II/III/V subtype group was present. These features are consistent with recently described genetic features of *E. albertii* (*5*).

We divided the party participants into 4 groups according to strain isolation patterns and statistically assessed the association of strain isolation patterns with incidence of clinical symptoms (Table 2). The results indicated that persons infected with only *E. albertii* or persons infected with *E. albertii* and *E. coli* O183:H18 had diarrhea and abdominal pain more frequently than did uninfected persons ($p<0.05$) and that the incidence of asymptomatic carriers was lower among persons infected only with *E. albertii*.

Nucleotide sequences obtained in this study have been deposited in the DNA Data Bank of Japan/European Molecular Biology Laboratory/GenBank database. Accession numbers and other information on sequence analyses are shown in the online Technical Appendix.

Conclusions

In this gastroenteritis outbreak, *E. albertii* or *stx2*-positive *E. coli* O183:H18 was isolated from 24 ill patients; both strains were isolated from 7 patients. Thus, although the responsible meal or food was not identified, it was most likely contaminated with these 2 microorganisms. The contribution or involvement of *E. coli* O183:H18 in this outbreak is unknown because there were 3 patients from whom only *E. coli* O183:H18 was isolated and because there were no differences in clinical symptoms between persons infected with *E. coli* O183:H18 and persons not infected (Tables 1, 2). In contrast, *E. albertii* was isolated

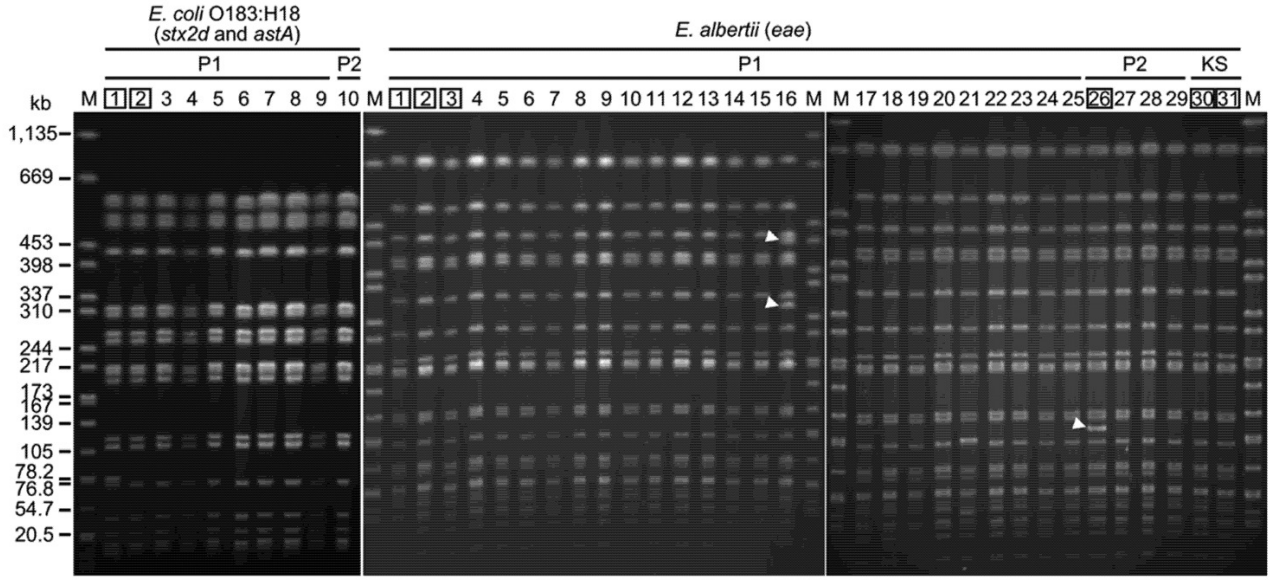


Figure. *Xba*I-digested pulsed-field gel electrophoresis profiles of isolates from fecal specimens collected from patients during an outbreak of human gastroenteritis associated with *Escherichia albertii*, Japan. Extra bands observed in 2 *E. albertii* isolates are indicated by arrowheads (only 1 or 2 band differences). The 2 *E. coli* O183:H18 and 6 *E. albertii* isolates indicated by numbers in boxes were subjected to multilocus sequence analysis (see text and online Technical Appendix, wwwnc.cdc.gov/EID/pdfs/12-0646-Techapp.pdf). *stx2d*, Shiga toxin 2d gene; *astA*, enteroaggregative *E. coli* heat-stable toxin gene; *eae*, intimin gene. Lane M, *Salmonella enterica* serovar Braenderup strain H9812 (used as a DNA size standard); lanes P1, Party 1; lanes P2, Party 2; lanes KS, kitchen staff.

Table 2. Clinical symptoms of party participants during outbreak of gastroenteritis associated with *Escherichia albertii*, by pathogen identified, Japan*

Symptom	*E. albertii*, n = 21†	*E. albertii* and *E. coli* O183:H18, n = 7	*E. coli* O183:H18, n = 3	None,‡ n =11§
Diarrhea	17 (81)¶	7 (100)§	1 (33)	4 (36)
Abdominal pain	16 (76)¶	6 (86)§	2 (67)	3 (27)
Nausea	5 (24)	5 (71)§	0	1 (9)
Fever	8 (38)	4 (57)	2 (67)	4 (36)
None	1 (5)¶	0	0	5 (45)

*Values are no. (%). None, negative for both pathogens.
†One symptomatic person was excluded because no clinical record was available.
‡Negative for both pathogens.
§One person was excluded because no clinical record was available.
¶A 2-tailed Fisher exact test ($p<0.05$) showed significant differences between the groups from which *E. albertii* or *E. coli* O183:H18 was isolated and the groups from which they were not isolated.

from a larger number of patients, and many fecal specimens yielded only *E. albertii* (Table 1).

The proportion of persons who had clinical symptoms was also higher for *E. albertii*–positive party participants than for uninfected persons (Table 2). Therefore, it is plausible that *E. albertii* was the major causative pathogen of this outbreak. This information indicates that *E. albertii* can cause gastroenteritis outbreaks among humans (*5*).

More attention should be given to sporadic cases and outbreak cases caused by this emerging pathogen. It may also be informative to revisit past outbreak cases caused by *eae*-positive atypical *E. coli* if pathogens were recorded as being nonmotile, unable to ferment lactose and xylose, and unable to produce β-D-glucuronidase.

Acknowledgments

We thank Sunao Iyoda and Makoto Ohnishi for sharing their unpublished results of *E. coli* serotyping and Keigo Ekinaga, Haruki Tokunaga, and Ryuusei Higashi for providing materials and epidemiologic information.

This study was supported by a grant-in-aid for scientific research from MEXT Japan (Wakate-B, 23790480) to T.O. and a grant from the Yakult Foundation.

Dr Ooka is an assistant professor in the Department of Infectious Diseases, Faculty of Medicine, University of Miyazaki, Miyazaki, Japan. His research interests include bacterial genomics and pathogenicity.

References

1. Albert MJ, Alam K, Islam M, Montanaro J, Rahman AS, Haider K, et al. *Hafnia alvei*, a probable cause of diarrhea in humans. Infect Immun. 1991;59:1507–13.
2. Albert MJ, Faruque SM, Ansaruzzaman M, Islam MM, Haider K, Alam K, et al. Sharing of virulence-associated properties at the phenotypic and genetic levels between enteropathogenic *Escherichia coli* and *Hafnia alvei*. J Med Microbiol. 1992;37:310–4. http://dx.doi.org/10.1099/00222615-37-5-310
3. Huys G, Cnockaert M, Janda JM, Swings J. *Escherichia albertii* sp. nov., a diarrhoeagenic species isolated from stool specimens of Bangladeshi children. Int J Syst Evol Microbiol. 2003;53:807–10. http://dx.doi.org/10.1099/ijs.0.02475-0
4. Oaks JL, Besser TE, Walk ST, Gordon DM, Beckmen KB, Burek KA, et al. *Escherichia albertii* in wild and domestic birds. Emerg Infect Dis. 2010;16:638–46. http://dx.doi.org/10.3201/eid1604.090695
5. Ooka T, Seto K, Kawano K, Kobayashi H, Etoh Y, Ichihara S, et al. Clinical significance of *Escherichia albertii*. Emerg Infect Dis. 2012;18:488–92. http://dx.doi.org/10.3201/eid1803.111401
6. Tokuoka E, Furukawa M, Nagamura T, Harada S, Ekinaga K, Tokunaga H, et al. Food poisoning outbreak due to atypical EPEC OUT:HNM, May 2011—Kumamoto. Infectious Agents Surveillance Report. 2012;33:8–9.
7. Nataro JP, Bopp CA, Fields PI, Kaper JB, Strockbine NA. *Escherichia, Shigella*, and *Salmonella*. In: Murray PR, Baron EJ, Jorgensen JH, Landry ML, Pfaller MA, editors. Manual of clinical microbiology, 9th ed. Washington (DC): American Society for Microbiology Press; 2007. p. 670–87.

Address for correspondence: Tetsuya Hayashi, Division of Bioenvironmental Science, Frontier Science Research Center, University of Miyazaki, 5200 Kiyotake, Miyazaki 889-1692, Japan; email: thayash@med.miyazaki-u.ac.jp

Article DOI: http://dx.doi.org/10.3201/eid1901.120646

Human Gastroenteritis Outbreak Associated with *Escherichia albertii*, Japan

Technical Appendix

Protocol used to identify bacteria and viruses in fecal specimens obtained during a human gastroenteritis outbreak associated with *Escherichia albertii*, Japan

Detection and Isolation of Causative Agents

We determined the causative agents for the outbreak by our routine laboratory protocol. To isolate bacterial pathogens, fecal specimens from 44 party participants and 10 members of the restaurant kitchen staff were directly placed and cultivated on the following media: deoxycholate-hydrogen sulfide-lactose (DHL) agar (Nissui Pharamceutical Co. Ltd., Tokyo, Japan) and MacConkey agar (Difco, Detroit, MI, USA) for enteric bacteria; CHROMagar Vibrio (CHROMagar, Paris, France) for the genus *Vibrio*; mannitol salt agar with egg yolk (Nissui Pharamceutical Co. Ltd.) for *Staphylococcus aureus*; NaCl glycine Kim and Goepfert agar (Nissui Pharamceutical Co. Ltd.) with egg yolk for *Bacillus cereus*; modified charcoal-cefoperazone-deoxycholate agar (Oxoid, Basingstoke, UK) for the genus *Campylobacter*; and *Clostridium welchii* egg yolk agar (Nissui Pharamceutical Co. Ltd.) with kanamycin for *Clostridium perfringens*. Bacterial colonies were grown on DHL and MacConkey agar plates (33 specimens).

Five colonies (including white and red colonies when both were present) were picked from each of the DHL agar plates and subjected to PCR for detection of pathogenic *Escherichia coli* marker genes. Species were identified by using the Api20E System (bioMérieux, Lyon, France).

For virus investigations, 5 fecal specimens were randomly selected from 37 patients (symptomatic persons) and subjected to reverse transcription PCR to detect norovirus, sapovirus, rotavirus, adenovirus, astrovirus, and kobuvirus, according to described protocols (*1*). Results were negative for all 6 viruses whose presence was assessed.

PCR Detection of Pathogenic *E. coli* Marker Genes

PCR screening was performed for 9 pathogenic *E. coli* marker genes: *stx1*, *stx2*, *invE*, *eae*, *bfp*, *aggR*, *astA*, the heat-labile enterotoxin gene, and the heat-stable enterotoxin gene. All primers used for screening have been described (*2–4*). KAPATaq EXtra DNA polymerase (KAPA Biosystems, Inc., Woburn, MA, USA) was used for PCR amplification.

DNA Sequencing and Subtype Determination of the *stx2* Gene

The *stx2* gene of *E. coli* O183:H18 was amplified by using primers 5′-GATGGCGGTCCATTATC-3′ (*5*) and 5′-CGCCATAAACATCTTCTTCA-3′, which were designed on the basis of the nucleotide sequence of a highly conserved region in the gene encoding Stx2 subunit B, and KAPATaq EXtra DNA polymerase. The nucleotide sequence of the PCR product was determined by direct sequencing of the amplicon by using an ABI 3710 Autosequencer (Life Technologies, Carlsbad, CA, USA) with primers used for PCR amplification. The subtype of the *stx2* gene was determined by using a blastx homology search against known *stx2* sequences (www.ncbi.nlm.nih.gov/BLAST/).

Sequencing of Other Genes and Nucleotide Sequence Accession Numbers

In addition to the *stx2* gene of *E. coli* O183:H18, we determined the sequences of the *eae* and *cdtB* genes and 7 housekeeping genes (*adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA*, and *recA*) of 6 *E. albertii* strains and the same 7 housekeeping genes of 2 *E. coli* O183:H18 strains as described (*6*). These 8 strains were randomly selected and are indicated by boxed numbers in the Figure in the main text.

Because nucleotide sequences of these genes were identical among the 6 *E. albertii* strains and between the 2 *E. coli* O183:H18 strains, sequences of the *E. albertii* strain KU20110014 and *E. coli* O183:H18 strain KU2011009 have been deposited in the DNA Data Bank of Japan/European Molecular Biology Laboratory/GenBank database. Both strains were isolated from the same patient, who had diarrhea and abdominal pain. Accession numbers of the deposited sequences are AB714729 (*eae* of KU20110014), AB714730 (*cdtB* of KU20110014), AB741082 (*stx2d* of KU2011009), and AB714731-AB714744 (*adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA*, and *recA* of KU20110014 and KU2011009).

References

1. Harada S, Okada M, Yahiro S, Nishimura K, Matsuo S, Miyasaka J, et al. Surveillance of pathogens in outpatients with gastroenteritis and characterization of sapovirus strains between 2002 and 2007 in Kumamoto Prefecture, Japan. J Med Virol. 2009;81:1117–27. PubMed http://dx.doi.org/10.1002/jmv.21454

2. Sueyoshi M, Fukui H, Tanaka S, Nakazawa M, Ito K. A new adherent form of an attaching and effacing *Escherichia coli* (*eaeA*+, *bfp*-) to the intestinal epithelial cells of chicks. J Vet Med Sci. 1996;58:1145–7. PubMed http://dx.doi.org/10.1292/jvms.58.11_1145

3. Kobayashi K, Seto K, Yatsuyanagi J, Saito S, Terao M, Kaneko M, et al. Presence of the genes regarding adherence factors of *Escherichia coli* isolates and a consideration of the procedure for detection of a diarrheagenic strain. Kansenshogaku Zasshi. 2002;76:911–20. PubMed

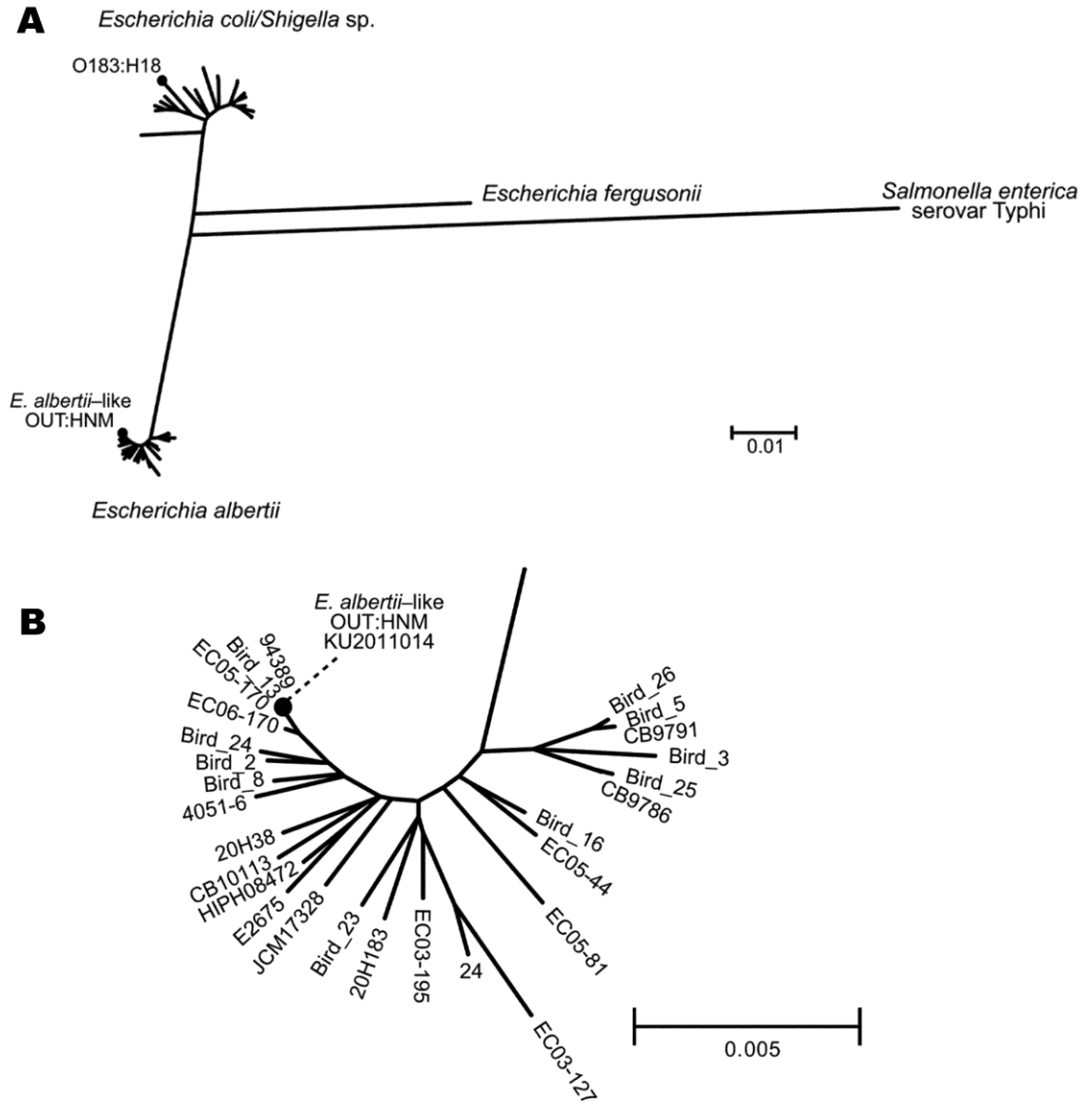
4. Taguri T, Noguchi H, Hirayama H. The simultaneous detection method of 18 species of food-borne pathogenic bacteria by multiplex PCR. Annual Report of Nagasaki Prefectural Institute of Public Health and Environmental Sciences. 2002;48:43–56.

5. Paton AW, Paton JC, Manning PA. Polymerase chain reaction amplification, cloning and sequencing of variant *Escherichia coli* Shiga-like toxin type II operons. Microb Pathog. 1993;15:77–82. PubMed http://dx.doi.org/10.1006/mpat.1993.1058

6. Ooka T, Seto K, Kawano K, Kobayashi H, Etoh Y, Ichihara S, et al. Clinical significance of *Escherichia albertii.* Emerg Infect Dis. 2012;18:488–92. PubMed http://dx.doi.org/10.3201/eid1803.111401

Technical Appendix Table. Reference strains used in multilocus sequence analysis of fecal specimens obtained from party participants during outbreak of gastroenteritis associated with *Escherichia albertii*, Japan

Bacteria, strain name (serotype)	Reference or accession no.
Escherichia albertii	
Bird_2	Ooka et al. (*6*)
Bird_3	
Bird_5	
Bird_8	
Bird_13	
Bird_16	
Bird_23	
Bird_24	
Bird_25	
Bird_26	
EC03–127	
EC03–195	
EC05–44	
EC05–81	
EC05–160	
EC06–170	
24	
94389	
20H183	
20H38	
4051–6	
CB10113	
CB9786	
CB9791	
HIPH08472	
E2675	
LMG20976	ABKX00000000
E. coli	
Sakai (EHEC O157:H7)	BA000007
11368 (EHEC O26:H11)	AP010953
11128 (EHEC O111:H-)	AP010960
12009 (EHEC O103:H2)	AP010958
K-12 MG1655	U00096
HS (O9)	CP000802
SE11	AP009240
SE15 (O150:H5)	AP009378
E24377A (ETEC O139:H28)	CP000800
B171 (EPEC O111:H-)	AAJX02000100
E2348/69 (EPEC O127:H6)	FM180568
O6:K2:H1, CFT073	AE014075
UTI89 (UPEC)	CP000243
APEC (O1:K1:H7)	CP000468
Shigella sonnei	
Ss046	CP000038
S. boydii	
Sb227	CP000036
BS512 CDC 3083–94	CP001063
S. flexneri	
2a 2457T	AE014073
2a 301	AE005674
S. dysenteriae	
Sd197	CP000034
E. fergusonii	
UMN026	CU928163
Salmonella enterica serovar Typhi	
CT18	AL513382



Technical Appendix Figure. A) Phylogenies of the *Escherichia albertii*–like OUT:HNM and *E. coli* O183:H18 strains determined by multilocus sequence analysis. Neighbor-joining tree constructed with concatenated partial nucleotide sequences of 7 housekeeping genes. The 49 strains (27 *E. albertii*, 20 *E. coli*/*Shigella* sp., 1 *E. fergusonii*, and 1 *Salmonella enterica* serovar Typhi) are included as references (online Technical Appendix Table). B) Enlarged view of the *E. albertii* lineage. Scale bars indicate nucleotide substitutions per site.