Bacteriological and Epidemiological Characteristics of Enterotoxigenic *Escherichia coli* Isolated in Tokyo, Japan, between 1966 and 2009[▽]

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Enterotoxigenic *Escherichia coli* (ETEC) caused 131 outbreaks in Tokyo, Japan, between 1966 and 2009. The major serogroups were O6, O27, O148, and O159. The incidence of serogroups O25 and O169 recently increased. Heat-stable enterotoxin (ST) subtyping revealed that *E. coli* of serogroups O6, O15, O25, and O159 possessed the STh gene, whereas those serotyped as O27 and O169 possessed the STp gene.

Diarrheagenic *Escherichia coli* can be divided into at least 5 categories based on differences in the pathogenic mechanisms: enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enterohemorrhagic *E. coli* (EHEC), enteroinvasive *E. coli* (EIEC), and enteroaggregative *E. coli* (EAEC) (17). ETEC is a very important causal pathogen of diarrhea in children in developing countries (20, 21, 22) and the most frequent etiologic agent of diarrhea in individuals who have traveled to developing countries (4). Similar to EHEC, ETEC is largely responsible for food- and waterborne outbreaks in developed countries (5, 11, 18, 25).

ETEC produces either or both heat-labile enterotoxin (LT) and heat-stable enterotoxin (ST) (23). There are 2 types of ST (ST I and II), differentiated by their solubility in methanol, but only ST I is toxic in humans (16). The role of ST II in human diarrhea is unclear. ST I is classified into 2 subtypes, STh and STp, based on their homology to the ST I gene. STh is isolated from humans, and STp was originally isolated from pigs, but there has been no detailed report on the pathogenicity of STh- or STp-producing *E. coli*. The genes encoding ST are associated with plasmids (14, 15, 26, 29).

An *E. coli* serotyping system is used to screen for ETEC, because the main serotypes of ETEC are already known (7, 17, 19, 24). However, the relationships between serotypes and ST subtypes are unknown.

We investigated the serotypes and toxin types of ETEC isolated from outbreaks and sporadic cases of diarrhea in Tokyo, Japan, and examined the ST subtypes of the ST-producing *E. coli* isolates.

Heat-labile enterotoxin (LT) production was confirmed with an immunological method using reverse passive latex agglutination (RPLA), VET-RPLA (Denka Seiken Co., Tokyo, Japan), or PCR using primers reported by Ito et al. (9). Heat-stable enterotoxin (ST) production was examined using the ELISA detection kit, COLIST enzyme immunoassay (EIA)

(Denka Seiken). ST subtypes were determined by PCR using primers reported by Abe et al. (1). PCR was performed as follows. The reaction mixture was heated at 94°C for 4.5 min and then subjected to 30 cycles of amplification, with one cycle consisting of 30 s at 94°C, 30 s at 55°C, and 30 s at 72°C, followed by a final extension period of 2.5 min at 72°C. Amplified products were visualized by ethidium bromide staining after electrophoresis on 2% agarose gels. O and H serogroups were determined using immune sera prepared in our laboratory and commercial *E. coli* immune sera (Denka Seiken) (8).

We investigated 142 ETEC strains isolated from sporadic cases of diarrhea at hospitals in Tokyo, Japan, between 2006 and 2009. Of the 142 ETEC strains or cases, 114 (80.3%) were assumed to have been acquired during overseas travel, and 4 (2.8%) occurred in Japan; the location where the patient was infected was unclear in 24 cases (16.9%). The destinations of overseas travels encompassed 26 countries, mainly in Asia, including India (20 cases [14.1%]), Indonesia (19 cases [13.4%]), China (16 cases [11.3%]), and Thailand (12 cases [8.5%]).

Of 295 diarrheagenic *E. coli* outbreaks that occurred in Tokyo, Japan, in 1966 to 2009 (44 years), 131 (46.3%) were caused by ETEC: 5 outbreaks in 1966 to 1970, 26 in the 1970s, 30 in the 1980s, 42 in the 1990s, and 28 in 2001 to 2009. Many diarrheagenic *E. coli* outbreaks were caused by ETEC until the 1990s, but the incidence declined after 2000. Only 1 EHEC outbreak occurred in Tokyo, Japan, before the 1980s; however, 7 outbreaks occurred in the 1990s, and 42 outbreaks occurred between 2000 and 2009. Thus, the number of EHEC outbreaks has been increasing in recently.

Ninety-four (71.8%) of the 131 ETEC outbreaks were suspected to have originated from foods consumed while traveling domestically in 28 outbreaks (21.3%), food service meals at schools or companies in 17 outbreaks (12.9%), catered lunch boxes and meals at restaurants in 16 outbreaks each (12.2%), tap and well water in 10 outbreaks (7.6%), meals while traveling overseas in 3 outbreaks (2.3%), and meals at home and supplied in a dormitory in 2 outbreaks each (1.5%). The sources of infection and causative foods were unclear in 37 (28.2%) instances. ETEC could not be isolated from the implicated foods, because ETEC detection in food is very diffi-

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TABLE 1. Serotypes and toxin types of ETEC isolated from 131 outbreaks in Tokyo, Japan, from 1966 to 2009

Serotype	Toxin type(s)	No. of strains (%)
O6:H16	LT, ST	31 (19.5)
O6:NM	LT, ST	6 (3.8)
O169:H41	ST	23 (14.5)
O169:NM	ST	9 (5.7)
O27:H7	ST	19 (11.9)
O27:H20	ST	6 (3.8)
O148:H28	ST	17 (10.7)
O25:NM	ST	10 (6.3)
O25:NM	LT	4 (2.5)
O159:H20	ST	7 (4.4)
O159:NM	ST	2 (1.2)
O159:H34	ST	3 (1.9)
Others		22 (13.8)
Total		159 ^a

^a The total number of strains was greater than the number of outbreaks, because several types of ETEC were detected in some outbreaks.

cult. Moreover, food assumed to be the vehicle had been discarded by the patients during the period between ingestion and disease development. Tap or well water was also assumed to be the vehicle in many outbreaks, although detection of the pathogen is very difficult, because transiently contaminated tap or well water can cause diarrhea.

Of the isolates that caused 131 ETEC-induced diarrhea outbreaks, 159 representative strains were investigated. The total number of test strains was greater than the number of outbreaks, because several types of ETEC were detected in some outbreaks. The serotypes and toxin types were as follows: O6:H16/NM (LT and ST), 37 strains (23.3%); O169:H41/NM (ST), 32 strains (20.2%); O27:H7/20 (ST), 25 strains (15.7%); O148:H28 (ST), 17 strains (10.7%); O25:NM (ST), 10 strains (6.3%); O159:H20/34/NM (ST), 12 strains (7.5%); O25:NM (LT), 4 strains (2.5%); other serotypes and toxin types, 22 strains (Table 1).

Most outbreaks are caused by a single serotype and toxin type strain, but 14 outbreaks were assumed to be caused by

multiple types of ETEC (Table 2). Two serogroups were detected in patients in 8 outbreaks, 3 serogroups were detected in 1 outbreak, 4 serogroups were detected in 3 outbreaks, and 5 serogroups were detected in 1 outbreak. Six types of ETEC were detected in a food-borne outbreak that occurred in 2003 (outbreak 11). This was a large outbreak, and the number of patients exceeded 400. ETEC was detected in 48 of 84 fecal samples, and the serotypes and toxin types of isolated ETEC were O25:NM (LT), O25:NM (ST), O27:H20 (ST), O27:H7 (ST), O148:H28 (ST), and O78:NM (ST). Catered lunch boxes were assumed to be the causative food, but it was unclear where and how the lunch boxes were contaminated with ETEC (12). In outbreaks involving multiple sero- and toxin types, the most frequent suspected causative vehicle was meals consumed while traveling (10 outbreaks), and 3 of these occurred during overseas travel. In a previous survey of diarrhea in overseas travelers, ETEC was the most frequently isolated, followed by *Plesiomonas*, *Campylobacter*, *Aeromonas*, and *Salmonella* species (10, 13).

The main serogroups and toxin types of ETEC isolated from outbreaks in Tokyo, Japan, are shown over time in Table 3. Serogroups O6, O27, O148, and O159 were continuously detected after the 1970s. Eleven and 15 outbreaks of serogroup O6 were detected in the 1970s and 1980s, respectively, but the number of outbreaks decreased over the years, and only 5 outbreaks were detected after 2001. In contrast, serogroups O25 and O169 were not detected before 1990 but were detected in the 1990s, showing the presence of serotype trends over time. Wolf reported that serogroups O6, O78, O8, and O128 were frequently isolated from samples collected from various regions throughout the world (28). In our survey, O6 was frequently isolated after the 1970s, suggesting that serogroup O6 of ETEC is prevalent worldwide (2, 27). Although some serogroups, such as O6, O27, and O148, were detected in all periods, O25 and O169 were isolated after the 1990s. During the 8 years between 1996 and 2003, 10 outbreaks caused by serogroup O169 were reported in Tokyo, Japan. Before 1996, only one outbreak caused by serogroup O169 was reported in

TABLE 2. Outbreaks caused by several types of ETEC

Outbreak	Yr	No. of patients	Suspected causative vehicle	No. of serogroups	Serogroups of isolates
1	1979	19	Meals while traveling	2	O6, O148
2	1982	23	Meals while traveling?	5	O27, O148, O70, O85, OUT
3	1985	54 ^a	Meals while traveling	2	O6, O159
4	1990	129	Meals while traveling	2	O6, O148
5	1992	391	Meals while traveling	2	O6, O27
6	1992	385	Meals while traveling	2	O6, OUT
7	1999	342	Meals at cafeteria at a company	3	O6, O27, O169
8	2001	107	Meals while traveling ^b	4	O6, ^d OUT ^d
9	2001	219	Drinking water	2	O159, O169
10	2002	Unknown	Meals while traveling ^b	4	O6, O25, O169, OUT
11	2003	>400	Catered lunch box ^c	6	O25, ^d O27, ^d O148, O78
12	2004	Unknown	Meals while traveling ^b	4	O25, O27, O148, OUT
13	2005	1 ^a	Meal while traveling	2	O159, O169
14	2007	72	Catered lunch box ^c	2	O6, O15

^a Number of patients only in Tokyo, Japan.

^b Travel abroad.

^c ETEC was not detected from food samples. Catered lunch boxes were suspected to be the vehicle food by epidemiological analysis.

^d Two kinds of ETEC with different toxin types or H serogroups were isolated.

TABLE 3. Main serogroup and toxin type of ETEC isolated from outbreaks in Tokyo, Japan, over time

Serogroup	Toxin type(s)	No. of outbreaks in the following time period:					Total no. of outbreaks
		1966–1970	1971–1980	1981–1990	1991–2000	2001–2009	
O6	LT, ST		11	15	6	5	37
O27	ST	4	6	6	3	6	25
O148	ST		2	8	5	2	17
O159	ST		7	1		4	12
O25	LT				3	1	4
O25	ST				4	6	10
O169	ST				21	11	32

the United States (3, 6), suggesting that O169 is a relatively new epidemic serotype.

We determined the ST subtypes of ST-producing *E. coli* to investigate the relationships between serotypes and ST subtypes. Strains such as serotype O6:H16, O25:NM, and O148:H28 possessed the STh-encoding gene, whereas strains such as O169:H41/NM, O27:H7/NM, O159:HUT, and O167:H41 pos-

sessed the STp-encoding gene, which is interesting. Both subtypes were detected in O159:H34 and O8:H9 serotypes (Table 4). The details of the relationship between these serotypes and ST subtypes remains unknown, but it is clear that the ST type is determined to some extent by the serotype. Further investigations are necessary to characterize the mechanistic relationship between serotype and ST subtype of ETEC.

REFERENCES

- Abe, A., et al. 1992. A sensitive method for the detection of enterotoxigenic *Escherichia coli* by the polymerase chain reaction using multiple primer pairs. *Zentralbl. Bakteriol.* **277**:170–178.
- Beatty, M. E., et al. 2006. Epidemic diarrhea due to enterotoxigenic *Escherichia coli*. *Clin. Infect. Dis.* **42**:329–334.
- Beatty, M. E., et al. 2004. Enterotoxin-producing *Escherichia coli* O169:H41, United States. *Emerg. Infect. Dis.* **10**:518–521.
- Bölin, L., et al. 2006. Enterotoxigenic *Escherichia coli* with STh and STp genotypes is associated with diarrhea both in children in areas of endemicity and in travelers. *J. Clin. Microbiol.* **44**:3872–3877.
- Dalton, C. B., E. D. Mintz, J. G. Wells, C. A. Bopp, and R. V. Tauxe. 1999. Outbreak of enterotoxigenic *Escherichia coli* infection in American adults: a clinical and epidemiologic profile. *Epidemiol. Infect.* **123**:9–19.
- Devasia, R. A., et al. 2006. Endemically acquired foodborne outbreak of enterotoxin producing *Escherichia coli* serotype O169:H41. *Am. J. Med.* **119**:168.e7–168.e10.
- Ethelberg, S., et al. 2010. Outbreaks of gastroenteritis linked to lettuce, Denmark, January 2010. *Eurosurveillance* **15**:pii=19484.
- Ewing, W. H. 1986. The genus *Escherichia*, p. 93–134. In *Edwards and Ewing's identification of Enterobacteriaceae*, 4th ed. Elsevier Science Publishing, New York, NY.
- Ito, F., T. Ogino, K. Ito, and H. Watanabe. 1992. Differentiation and detection of pathogenic determinants among diarrheagenic *Escherichia coli* by PCR using mixed primers. *Nippon Rinsho*. **50**:343–347.
- Kai, A. 2006. Traveler's diarrhea. *J. Med. Technol.* **50**:1245–1250.
- Kimura, T., et al. 2006. Enterotoxigenic *Escherichia coli* O6:H16 food poisoning outbreak in prison. *Jpn. J. Infect. Dis.* **59**:410–411.
- Konishi, N., et al. 2009. Colony-sweep PCR efficacy in food-borne outbreak associated with six types of enterotoxigenic *Escherichia coli*. *Jpn. J. Infect. Dis.* **83**:490–495.
- Kudoh, Y., and S. Yamada. 1997. Etiological survey of travellers' diarrhea in Tokyo, p. 99–104. In *S. Irimajiri, M. Saito, R. Nakaya, and Y. Matsubara (ed.), Infectious enteritis in Japan II*. Saikon Syuppan, Tokyo, Japan.
- McConnell, M. M., H. R. Smith, G. A. Willshaw, S. M. Scotland, and B. Rowe. 1980. Plasmids coding for heat-labile enterotoxin production isolated from *Escherichia coli* O78: comparison of properties. *J. Bacteriol.* **143**:158–167.
- Moseley, S. L., M. Samadpour-Motalebi, and S. Falkow. 1983. Plasmid association and nucleotide sequence relationships of two genes encoding heat-stable enterotoxin production in *Escherichia coli* H-10407. *J. Bacteriol.* **156**:441–443.
- Moseley, S. L., J. W. Hardy, M. H. Imdadul, P. Echeverria, and S. Falkow. 1983. Isolation and nucleotide sequence determination of a gene encoding a heat-stable enterotoxin of *Escherichia coli*. *Infect. Immun.* **39**:1167–1174.
- Nataro, J. P., and J. B. Kaper. 1998. Diarrheagenic *Escherichia coli*. *Clin. Microbiol. Rev.* **11**:142–201.
- Pakalinskiene, J., et al. 2009. A foodborne outbreak of enterotoxigenic *E. coli* and *Salmonella* Anatum infection after a high-school dinner in Denmark, November 2006. *Epidemiol. Infect.* **137**:396–401.
- Peruski, L. F., et al. 1999. Phenotypic diversity of enterotoxigenic *Escherichia coli* strains from a community-based study of pediatric diarrhea in periurban Egypt. *J. Clin. Microbiol.* **37**:2974–2978.
- Qadri, F., A. M. Svennerholm, A. S. G. Faruque, and R. B. Sack. 2005.

TABLE 4. Serotype and ST I subtypes of ETEC isolated from outbreaks and sporadic cases in Tokyo, Japan, from 2006 to 2009^a

Serotype	Toxin type(s)	No. of ETEC isolates with the indicated ST subtype from:			
		Outbreaks		Sporadic cases	
		STh	STp	STh	STp
O6:H16	LT, ST			12	
O6:H16	ST			4	
O6:NM	LT, ST	3		3	
O6:NM	ST			2	
O8:HUT	LT, ST			1	
O15:H11	ST	1		7	
O15:NM	ST			1	
O25:NM	ST	3		8	
O28:H10	ST			1	
O63:NM	ST			1	
O126:HUT	ST			3	
O126:NM	ST			2	
O128:H45	ST			2	
O128:HUT	ST			1	
O148:H20	ST			1	
O148:H28	ST			5	
O153:H1/2	ST			1	
O159:H20	ST	1			
OUT:H11	ST			1	
OUT:H18	ST			1	
OUT:H28	LT, ST			1	
OUT:H10	ST			1	
OUT:NM	ST			1	
O27:H7	ST		4		16
O27:NM	ST				1
O159:HUT	ST				1
O167:H41	ST				1
O169:H41	ST		4		34
O169:NM	ST		4		2
O8:H9	LT, ST			1	1
O159:H34	ST	1	2	2	4
Total		9	14	63	60

^a There were a total of 23 isolates from outbreaks and a total of 123 isolates from sporadic cases.

- Enterotoxigenic *Escherichia coli* in developing countries: epidemiology, microbiology, clinical features, treatment, and prevention. *Clin. Microbiol. Rev.* **18**:465–483.
21. **Rao, M. R., et al.** 2003. High disease burden of diarrhea due to enterotoxigenic *Escherichia coli* among rural Egyptian infants and young children. *J. Clin. Microbiol.* **41**:4862–4864.
 22. **Richie, E., et al.** 1997. Enterotoxigenic *Escherichia coli* diarrhea among young children in Jakarta, Indonesia. *Am. J. Trop. Med. Hyg.* **57**:85–90.
 23. **Sack, R. B.** 1980. Enterotoxigenic *Escherichia coli*: identification and characterization. *J. Infect. Dis.* **142**:279–289.
 24. **Shaheen, H. A., et al.** 2004. Phenotype profiles of enterotoxigenic *Escherichia coli* associated with early childhood diarrhea in rural Egypt. *J. Clin. Microbiol.* **42**:5588–5595.
 25. **Wang, S., H. Duan, W. Zhang, and J. Li.** 2007. Analysis of bacterial food-borne disease outbreaks in China between 1994 and 2005. *FEMS Immunol. Med. Microbiol.* **51**:8–13.
 26. **Willshaw, G. A., E. A. Barclay, H. R. Smith, M. M. McConnell, and B. Rowe.** 1980. Molecular comparison of plasmids encoding heat-labile enterotoxin isolated from *Escherichia coli* strains of human origin. *J. Bacteriol.* **143**:168–175.
 27. **Wolf, M. K., et al.** 1993. Characterization of enterotoxigenic *Escherichia coli* isolated from U.S. troops deployed to the Middle East. *J. Clin. Microbiol.* **31**:851–856.
 28. **Wolf, M. K.** 1997. Occurrence, distribution, and associations of O and H serogroups, colonization factor antigens, and toxins of enterotoxigenic *Escherichia coli*. *Clin. Microbiol. Rev.* **10**:569–584.
 29. **Yamamoto, T., and T. Yokota.** 1983. Plasmids of enterotoxigenic *Escherichia coli* H10407: evidence for two heat-stable enterotoxin genes and a conjugal transfer system. *J. Bacteriol.* **153**:1352–1360.