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Original Article

Diarrheagenic *Escherichia coli* Isolated from Stools of Sporadic Cases of Diarrheal Illness in Osaka City, Japan between 1997 and 2000: Prevalence of Enteroaggregative *E. coli* Heat-Stable Enterotoxin 1 Gene-Possessing *E. coli*

Yoshikazu Nishikawa*, Zhijiang Zhou*[1], Atsushi Hase, Jun Ogasawara, Teruyo Kitase, Niichiro Abe, Hiromi Nakamura, Takayuki Wada, Eiji Ishii, Kosuke Haruki and the Surveillance Team*[2]

Osaka City Institute of Public Health and Environmental Sciences, Tojo-cho 8-34, Tennoji-ku, Osaka 543-0026, Japan

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SUMMARY: Diarrheagenic *Escherichia coli* (DEC) represents an elusive target, since they are not easily distinguished from fecal coliforms. To clarify if DEC are prevalent among sporadic cases of diarrheal illness in Osaka City, Japan, diarrheal specimens were examined for *E. coli* that were enterohemorrhagic (EHEC), enteropathogenic (EPEC), enterotoxigenic (ETEC), enteroinvasive (EIEC), or enteroaggregative (EAggEC). EAST1EC, a strain of *E. coli* that does not possess any diarrheagenic characteristics except the EAggEC heat-stable toxin 1 (EAST1) gene, was also included as a possible DEC. A total of 924 specimens were examined between July 1997 and March 2000. DEC and *Salmonella* were isolated from 7.3% (67/924) and 6.8% (63/924) of specimens, respectively. DEC was therefore as prevalent as *Salmonella* among sporadic cases. The 67 strains were composed of 17 EPEC (26%), 10 EHEC (15%), four ETEC (6%), 13 EAggEC (20%), and 23 EAST1EC (35%), including two strains of EAST1EC O166:H15. Although PCR and tissue culture adhesion tests were useful to detect DEC, the effectiveness of serotyping was limited: only 40 strains (17.5%) out of 229 isolates that had been assumed to be enterovirulent on the basis of their O antigen were recognized to be diarrheagenic. In conclusion, not only EHEC but also the other subgroups of DEC, including EAST1EC, seem to play an important role in causing sporadic diarrheal illnesses. Methods to detect and unified criteria to identify various kinds of DEC are strongly desired.

INTRODUCTION

During 1996, a marked increase in sporadic cases of enteritis due to enterohemorrhagic *Escherichia coli* (EHEC) of serogroup O157 occurred in Osaka City, Japan. Epidemiological investigation revealed that the majority of the cases identified in this year were due to a huge outbreak (1). Compared with the years before 1996, however, the number of sporadic cases due to EHEC O157 has stayed high since the incident. We could not determine whether the increased number of patients diagnosed with infection with O157 organisms reflected a real increase in cases or was attributable to clinical practitioners paying more attention to this pathogen. Etiological data concerning diarrheagenic *E. coli* (DEC) among sporadic patients with diarrheal illness in Osaka City have not been available because clinical laboratories cannot easily distinguish most types of DEC from normal fecal coliforms, although EHEC O157 can now be detected using special agar plates.

To accumulate precise information on the prevalence of DEC among sporadic cases and to prepare for the possibility of future outbreaks, a surveillance team was organized. Diarrheal specimens were examined for DEC, including EHEC, enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), and enteroaggregative *E. coli* (EAggEC). *E. coli* that did not have any diarrheagenic characteristics except the EAggEC heat-stable toxin 1 (EAST1) gene were defined as EAST1EC and were also included as a group of DEC in this investigation. Although EAST1 was reported as a diarrheagenic toxin of EAggEC (2-4), it has not been well accepted as a virulence factor (5,6). However, we have focused our attention on EAST1EC because an outbreak due to EAST1EC O166:H15 occurred in Osaka City in 1996 (7,8). *Salmonella* was examined as a representative enteric pathogen for comparison with DEC.

MATERIALS AND METHODS

Specimens: Between July 1997 and March 2000, diarrheal stool specimens were taken from patients diagnosed with infectious gastroenteritis by pediatricians or physicians who belonged to the surveillance team. Specimens were stored in Labo-Swab tubes (Labotec Science, Osaka) and were transported to our laboratory three times a week by the staff of the Disinfection Office and Infectious Disease Prevention Division, Public Health and Welfare Bureau, Osaka Municipal Government.

*[1]Present address: Changchun University of Agriculture and Animal Sciences, Changchun, China.

*[2]Members of the Surveillance Team are listed in the Appendix.

*Corresponding author: Mailing address: Graduate School of Human Life Science, Osaka City University, Sugimoto 3-3-138, Sumiyoshi-ku, Osaka 558-8585, Japan. Tel & Fax: +81-6-6605-2883, E-mail: nisikawa@life.osaka-cu.ac.jp

Bacterial examination: Fecal specimens were examined for the presence of *E. coli*, *Salmonella*, and *Shigella* with desoxycholate hydrogen sulfide lactose agar (Nissui Pharmaceutical, Tokyo) and SSK agar (Kyokuto Pharmaceutical, Tokyo). Three to five suspicious colonies were tested in TSI (Nissui) and LIM media (Nissui), and they were identified on the basis of biochemical tests. *E. coli* and *Salmonella* were serotyped with their antisera (Denka Seiken Co. Ltd., Tokyo).

Examination of diarrhea-associated genes and the production of enterotoxin: PCR was used to examine the presence of enterotoxin genes as previously described (9). The primer sets and amplification conditions used are shown in Table 1 (10-14).

Heat-stable enterotoxin (ST), heat-labile enterotoxin (LT), and Shiga toxin (Stx) production were examined by using the *E. coli* ST EIA (Denka Seiken), a competitive ELISA for ST I (15), and VET-RPLA (Denka Seiken) and Verotox-F (Denka Seiken), which are reverse passive latex agglutination tests for LT and Stx (16-18).

Tissue culture adhesion tests: Adhesion tests to HEp-2 cells in culture were performed over 6-h periods as described previously (19). Monolayers of HEp-2 cells grown on cover slips (diameter 13 mm) in 24-well plates were prepared in the absence of antibiotics. Two-day-old monolayers of HEp-2 cells were used for the tests. Bacterial strains were grown overnight statically at 37°C in 1% casitone water. Before the test, monolayers were washed once with Dulbecco's PBS. One ml of Basal Eagle's medium containing D-mannose (1% w/v) without antibiotics or sera was added to each well. The overnight bacterial culture (20 μl) was inoculated into each well, and the plates were incubated at 37°C for 3 h. The monolayers were washed three times with PBS, and 1 ml of the medium was added to each well. After a further 3 h incubation period, the monolayers were washed thoroughly three times with PBS, fixed with absolute methanol, and stained with 10% (v/v) Giemsa.

Susceptibility to antimicrobial agents: Sensitivity of *E. coli* strains to the following antibiotics was examined using Sensi Disk (Becton-Dickinson Microbiology Systems, Cockeysville, Md., USA) according to the manufacturer's instructions: ampicillin, chloramphenicol, cefotaxime, ciprofloxacin, fosfomycin, gentamycin, kanamycin, nalidixic acid, streptomycin, sulfamethoxazole/trimethoprim, tetracycline, and trimethoprim. The plates were incubated at 37°C and assayed for inhibition of bacterial growth.

Pulsed-field gel electrophoresis (PFGE): PFGE was performed by the method of Izumiya et al. (20).

RESULTS

Characteristics of DEC: A total of 924 specimens were examined between July 1997 and March 2000. Sixty-seven strains of *E. coli* isolated from 66 specimens (7.1%) were identified as putative diarrheagenic and causal agents.

Properties of DEC strains isolated through this surveillance are shown in Table 2. Possession of *eaeA* and localized adhesion to HEp-2 cells were sought as the primary conditions to define an isolate as EPEC in this investigation. Consequently, eight strains for which serotypes could not be determined with the set of antisera provided by Denka Seiken were also included as EPEC along with organisms of well-established serotypes such as O55. Each strain of O86a and O146 was identified as class II EPEC because the organisms showed typical diffuse adhesion to HEp-2 cells, and their O serotypes fitted into a scheme of EPEC (21); however, the strains did not react with primers for *eaeA* in PCR.

Thirteen strains of EAggEC produced DNA fragments of characteristic size with the PCR primers, although two strains (Nos. 508 and 597) did not show typical aggregative adherence to HEp-2 cells. Only three strains of EAggEC (3/13, 23%) were found to possess the EAST1 gene in PCR tests.

A total of 149 strains showed adhesion to HEp-2 cells. Eighty strains adhered in a localized adhesion-like manner, and 21 strains showed an EAggEC-like adhesion. Forty-six strains attached to HEp-2 cells with diffuse adhesion, and the adherence of two isolates looked like a chain stitch or chain works. However, none of these adherent strains reacted with the primer sets used in this study, and thus none were included in the DEC for analysis in this study.

Epidemiology of DEC in Osaka City: The organisms comprised 17 strains of EPEC, 10 strains of EHEC, four strains of ETEC, 13 strains of EAggEC, and 23 strains of EAST1EC (Figs. 1 and 2). The isolation rates except for that of EAggEC showed a rising tendency in summer (Fig. 3). EPEC obviously tended to infect children, with these strains isolated from 14 children and three teenagers (Table 3). On

Table 1. PCR used in this study

Target	Primer (5′----3′)	Condition	Product	Reference
LT	AGCAGGTTTCCCACCGGATCACCA GTGCTCAGATTCTGGGTCTC	94°C 30 s, 47°C 1 min, 72°C 1.5 min 25 cycles	132 bp	11
ST	TTTATTTCTGTATTGTCTTT ATTACAACACAGTTCACAG	94°C 30 s, 47°C 1 min, 72°C 1.5 min 25 cycles	171 bp	11
VT	TTTACGATAGACTTCTCGAC CACATATAAATTATTTCGCTC	94°C 30 s, 47°C 1 min, 72°C 1.5 min 25 cycles	228 bp	11
*inv*E	ATATCTCTATTTCCAATCGCGT GATGGCGAGAAATTATATCCCG	94°C 30 s, 47°C 1 min, 72°C 1.5 min 25 cycles	382 bp	11
*eae*A	ACGTTGCAGCATGGGTAACTC GATCGGCAACAGTTTCACCTG	94°C 1 min, 55°C 1 min, 72°C 2 min 35 cycles	815 bp	10
bfp	GATTGAATCTGCAATGGTGC GGATTACTGTCCTCACATAT	94°C 1 min, 57°C 1 min, 72°C 80 s 30 cycles	597 bp	13
Aggregative Adhesion	CAATGTATAGAAATCCGCTGTT CTGGCGAAAGACTGTATCAT	94°C 1 min, 55°C 1 min, 72°C 2 min 35 cycles	630 bp	12
EAST1(1a,b)	CCATCAACACAGTATATCCGA GGTCGCGAGTGACGGCTTTGT	94°C 1 min, 55°C 1 min, 72°C 2 min 35 cycles	111 bp	14

Table 2. List of EHEC, ETEC, EPEC, EAggEC, and EAST1EC strains isolated through the surveillance

Strain No.	Date	Sex	Age	Serotype	Stx	ST	LT	*invE*	EAST1	*eae*A	EAgg	Adhesion	Remarks
59	7/15/97	M	12	O111:H-	1	–	–	–	–	+	–	LA	
122	8/12/97	F	4	O26:H11	1	–	–	–	–	+	–	LA/DA	
131	8/13/97	M	1	OUT:HUT	2	–	–	–	–	+	–	LA/DA	
207	10/20/97	M	4	O157	1,2	–	–	–	–	+	–	–	
424	8/31/98	M	2	O157	1,2	–	–	–	–	+	–	LA	
438	9/10/98	M	39	O157	1,2	–	–	–	–	+	–	LA	
687	6/16/99	F	3	O157	1,2	–	–	–	–	+	–	–	
785	8/25/99	M	13	O157	2	–	–	–	–	+	–	LA	
791	8/31/99	M	3	O157	1,2	–	–	–	–	+	–	LA	
831	10/6/99	F	2	O157	2	–	–	–	–	+	–	–	
27	7/7/97	F	27	O6:H16	–	+	+	–	+	–	–	–	Tourist to HongKong
80	7/28/97	M	49	O6:H16	–	+	+	–	+	–	–	–	Tourist to Indonesia
96	8/4/97	M	39	O25	–	+	–	–	+	–	–	–	Tourist to China
635a	5/10/99	F	24	O8	–	+	+	–	+	–	–	LAL	Tourist to Philippines
121	8/12/97	M	1	O55:H7	–	–	–	–	–	+	–	LA/DA	
139	8/18/97	M	10	O63:HUT	–	–	–	–	–	+	–	LAL	*C. jejuni**
145	8/19/97	M	2	OUT:HUT	–	–	–	–	–	+	–	LA	
146	8/19/97	F	2	O119:H2	–	–	–	–	–	+	–	LA	
148	8/20/97	M	2	O153:H7	–	–	–	–	–	+	–	DA/LA	
189	9/30/97	F	3	OUT:HUT	–	–	–	–	–	+	–	LA/Eagg	
228	11/11/97	M	7	O153:HUT	–	–	–	–	–	+	–	DA	
241	11/19/97	M	12	O119:HUT	–	–	–	–	–	+	–	LA	
261	12/10/97	F	0	O26:HUT	–	–	–	–	–	+	–	–	
273	1/13/98	M	13	OUT	–	–	–	–	–	+	–	LA	
304	4/22/98	M	3	OUT	–	–	–	–	–	+	–	LAL	*bfp* +
394	7/27/98	F	1	OUT	–	–	–	–	+	+	–	LA	
396	7/29/98	F	3	OUT	–	–	–	–	–	+	–	LA	EAggFimI+
397	7/29/98	F	7	OUT	–	–	–	–	–	+	–	LA	EAggFimI+
411	8/12/98	M	1	OUT	–	–	–	–	–	+	+	LA/Eagg	
422	8/26/98	F	6	O146	–	–	–	–	–	–	–	DA	
827	10/6/99	M		O86a	–	–	–	–	–	–	–	DA	
88	7/30/97	M	1	O126	–	–	–	–	+	–	+	EAgg	
196	10/6/97	F	24	OUT:HUT	–	–	–	–	–	–	+	EAgg	Tourist to Indonesia
343	6/8/98	F	71	O126	–	–	–	–	+	–	+	EAgg	
467	10/7/98	F	7	O126	–	–	–	–	+	–	+	EAgg	
508	11/20/98	F	4	OUT	–	–	–	–	–	–	+	–	
516	12/2/98	F	8	O128	–	–	–	–	–	–	+	EAgg	
546	1/25/99	M	2	OUT	–	–	–	–	–	–	+	EAgg	
551	1/27/99	F	2	OUT	–	–	–	–	–	–	+	EAgg	
566	2/10/99	F	2	O86a	–	–	–	–	–	–	+	EAgg	*C. jejuni*
579	3/1/99	F	29	O86a	–	–	–	–	–	–	+	EAgg	
597	3/23/99	M	6	OUT	–	–	–	–	–	–	+	LA	
635b	5/10/99	F	24	O15	–	–	–	–	–	–	+	EAgg	Tourist to Philippines
885	12/22/99	M	8M	O111	–	–	–	–	–	–	+	EAgg	
91	8/4/97	M	0	O119:H4	–	–	–	–	+	–	–	LA	
199	10/7/97	F	2	O25	–	–	–	–	+	–	–	LA	
255	12/3/97	M	2	O166	–	–	–	–	+	–	–	LA	
287	2/24/98	F	18	OUT	–	–	–	–	+	–	–	EAgg	
340	6/1/98	M	22	O25	–	–	–	–	+	–	–	–	Tourist to China
348	6/10/98	M	0	OUT	–	–	–	–	+	–	–	LAL	
381	7/15/98	F	3	O25	–	–	–	–	+	–	–	LA	
398	7/29/98	F	2	OUT	–	–	–	–	+	–	–	LAL	
448	9/28/98	M	2	OUT	–	–	–	–	+	–	–	–	
462	10/5/98	M	31	OUT	–	–	–	–	+	–	–	–	
470	10/12/98	M	24	OUT	–	–	–	–	+	–	–	–	
471	10/12/98	F	26	O166	–	–	–	–	+	–	–	–	
556	2/3/99	F	4	O143	–	–	–	–	+	–	–	–	
594	3/15/99	M	48	O28ac	–	–	–	–	+	–	–	–	
613	4/7/99	M	3	UT	–	–	–	–	+	–	–	–	
638	5/12/99	M	3	O1	–	–	–	–	+	–	–	–	
697	6/28/99	M	4	UT	–	–	–	–	+	–	–	–	*C. jejuni*
703	6/28/99	M	48	UT	–	–	–	–	+	–	–	EAgg	
716	7/14/99	F	9	UT	–	–	–	–	+	–	–	–	
717	7/14/99	M	6	UT	–	–	–	–	+	–	–	–	*C. jejuni*
718	7/14/99	M	43	O25	–	–	–	–	+	–	–	–	Tourist to Indonesia
821	9/29/99	F	15	UT	–	–	–	–	+	–	–	–	
915	3/8/00	F	8M	UT	–	–	–	–	+	–	–	LA	

**Campylobacter jejuni* was isolated from the specimen simultaneously.

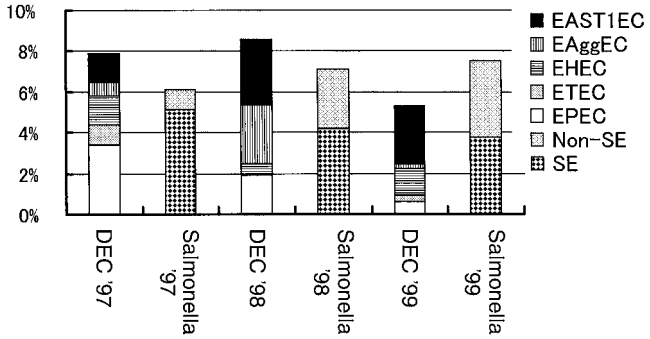

Fig. 1. Isolation rates of diarrheagenic *E. coli* and *Salmonella* in this study.

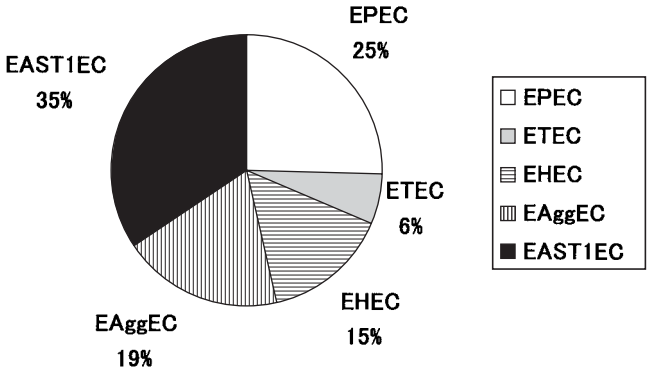

Fig. 2. Proportion of each subgroup of diarrheagenic *E. coli* isolates.

the other hand, all four strains of ETEC were isolated from adults who had traveled abroad (Table 2). Both ETEC O8 and EAggEC O15 were isolated from the stool of a patient who had made a tour of Cebu Island, the Philippines. Seventy-four percent (34/46) of the other DEC isolates (EHEC, EAggEC, and EAST1EC) were from the stools of children. However, their isolation rate in adults (9.5%, 12/126) was higher than that in children (4.3%, 34/798). Although 229 strains were

Table 3. Prevalence of diarrheagenic *E. coli* (DEC) in each age group of patients

Pathogens	No. of positives / no. of examined (%)			
	<10 yr	10-19 yr	Adults	Total
EPEC	14/707 (2.0)	3/91 (3.3)	0/126 (0)	17/924 (1.8)
ETEC	0/707 (0)	0/91 (0)	4/126 (3.2)	4/924 (0.4)
EHEC	7/707 (1.0)	2/91 (2.2)	1/126 (0.8)	10/924 (1.1)
EAggEC	9/707 (1.3)	0/91 (0)	4/126 (3.2)	13/924 (1.4)
EAST1EC	14/707 (2.0)	2/91 (2.2)	7/126 (5.6)	23/924 (2.5)
All DEC	44/707 (6.2)	7/91 (7.7)	16/126 (12.7)	67/924 (7.3)
Salmonella	49/707 (6.9)	4/91 (4.4)	10/126 (7.8)	63/924 (6.8)

assumed to be enterovirulent on the basis of their O antigens, strains that possessed diarrheagenic properties were in the minority in each serogroup, especially in serogroups O1, O8, and O18 (Fig. 4). Only 40 strains (17.5%) were recognized to be diarrheagenic.

In December 2000, an outbreak of diarrheal illness due to O126 occurred at a day-care center in Osaka City. Three strains of O126 isolated during this surveillance study were compared with the outbreak strains; all O126 strains reacted with PCR primers for EAggEC and EAST1. They were resistant to ampicillin and streptomycin except for one strain and showed similar PFGE patterns (Table 4, Fig. 5).

Twenty-three strains of EAST1EC were isolated as a group of DEC in this investigation. Nine strains of EAST1EC were observed to be adhesive to HEp-2 cells, although they did not react with primers for *eae*A or EAggEC-associated gene. Thirteen of the strains of EAST1EC could not be typed with the set of antisera. Two strains (Nos. 255 and 471) of EAST1EC O166:H15 were isolated in 1997 and 1998, respectively, although their PFGE patterns were distinct from that of the 1996 outbreak strain (7). Although the other strains that belonged to the same group of DEC and possessed the same O antigen were also analyzed with PFGE, they were clearly distinguishable from each other, with the exception of the previously mentioned O126 strains.

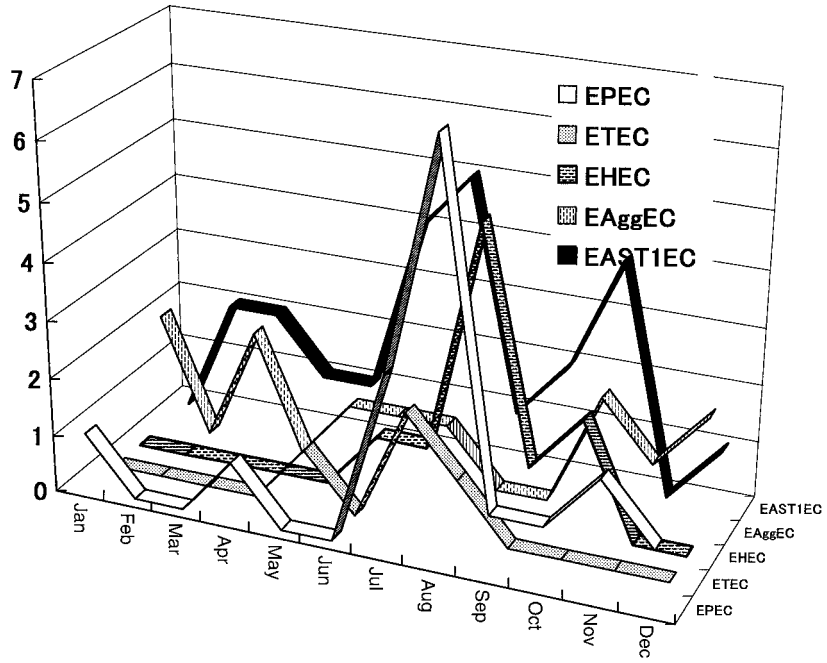

Fig. 3. Cumulative number of strains of each subgroup of diarrheagenic *E. coli* isolated during this study by month.

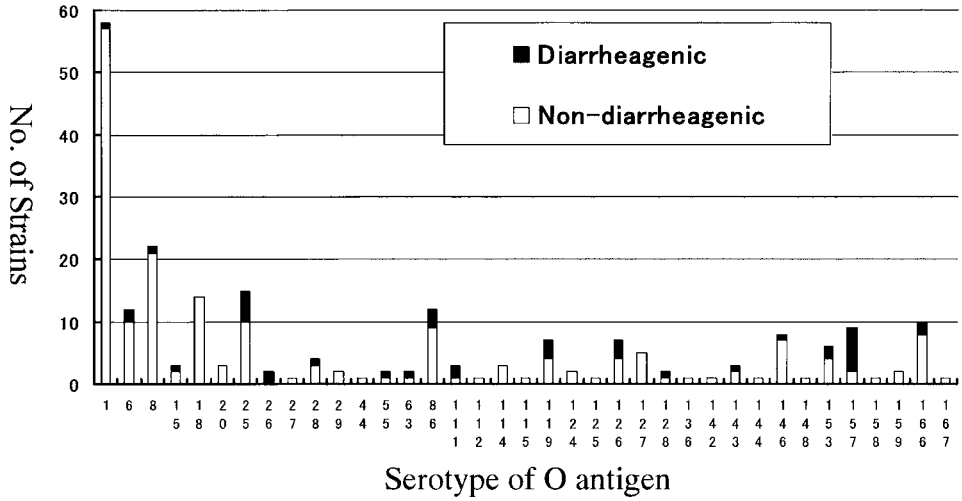

Fig. 4. Cumulative number of *E. coli* strains isolated throughout this study by O serotype. The number of strains that were confirmed to be diarrheagenic is indicated by the shaded portion of the column.

Table 4. Properties of enteroaggregative *E. coli* O126 isolated from sporadic cases in this study and from patients of an outbreak that occurred in December 2000

Strain	Origin	Date of Isolation	Serotype	Resistant to
88	Surveillance	7/'97	O126:H27	Amp
343	Surveillance	6/'98	O126:H–	Amp, SM
467	Surveillance	10/'98	O126:H27	Amp, SM
377-24	Outbreak	12/'00	O126:H27	Amp, SM
377-26	Outbreak	12/'00	O126:H27	Amp, SM
377-35	Outbreak	12/'00	O126:H27	Amp, SM

Amp: ampicillin
SM: streptomycin

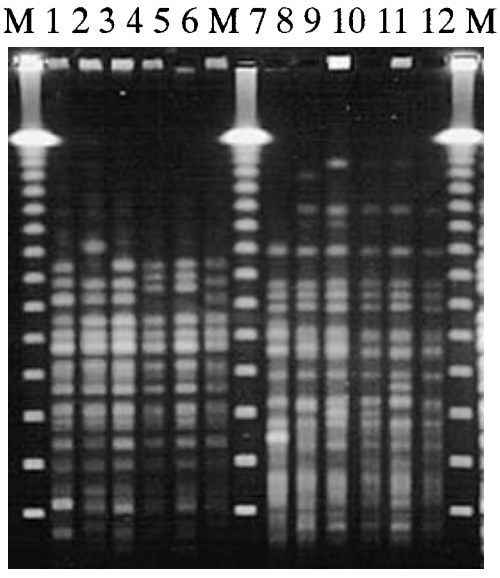

Fig. 5. PFGE patterns of enteroaggregative *E. coli* O126 strains. Lanes M, Marker; 1&7, no. 88; 2&8, no. 343; 3&9, no. 467; 4&10, no. 377-24; 5&11, no. 377-26; 6&12, no. 377-35. DNA on lanes 1-6 was digested by *Xba*I, and DNA on lanes 7-12 was digested by *Sfi*I.

Salmonella: *Salmonella* was detected in 63 stools (6.8%) throughout this investigation (Table 3). *Salmonella* Enteritidis was the most predominant serotype (61.9%, 39/63) among the *Salmonella* strains (Fig. 1), while the other 24 strains were assigned into 17 serotypes (data not shown).

DISCUSSION

The present study revealed that DEC was as prevalent as *Salmonella* among sporadic diarrheal patients in Osaka City (Fig. 2). DEC and *Salmonella* were isolated from 7.3% (67/924) and 6.8% (63/924) of stool specimens, respectively. However, if EAggEC and EAST1EC were not included as DEC, the rate would be 3.4% (31/924), half that of *Salmonella*. The decision as to whether to include EAggEC and EAST1EC as DEC will have an impact on our understanding of the etiology of sporadic diarrheal illnesses.

In 1996, an outbreak due to EAST1EC O166:H15 (8) occurred in Osaka City. Although these organisms were isolated from 27 of 31 patients examined, the organism did not have any diarrheagenic virulence traits except the EAST1 gene (*astA*) (7). Considering this experience, we included EAST1EC as a possible DEC in this study. During this study, two strains of EAST1EC O166:H15 were isolated from sporadic patients. These EAST1EC strains were isolated most often in summer, like other enteric pathogens (Fig. 3). Reports suggesting the diarrheagenicity of EAST1 gene-possessing *E. coli* have gradually increased (22-27). Thus, the circumstantial evidence suggests that EAST1EC is a possible enteric pathogen, and EAST1EC strains constituted 35% of the DEC isolates in this study. Some microbiologists seem to be suspicious of EAST1. There is no convenient assay to prove the elaboration of EAST1 by organisms. As with ETEC, some organisms possessing genes for the enterotoxins may not be pathogenic for humans. It is likely that only a group of EAST1EC that possesses a set of genes for particular colonization factors with the EAST1 gene can be diarrheagenic for humans. Further, we do not have data showing at what rate healthy carriers of EAST1EC are found in Osaka City. We think, however, that the present data show the importance of studying EAST1EC as a putative diarrheagenic agent.

EAggEC has been recognized as DEC, since epidemiological information from case-control studies and outbreaks due to the organisms has accumulated and supported its significance (6, 28-33). Four serotypes (O86a, O111, O126, O128) of *E. coli* were assigned into EAggEC in this study

irrespective of whether their O antigens fitted into the scheme of EPEC. These serotypes have been reported to include not only EPEC but also EAggEC (34-39) or unusual EHEC (40). Three strains of O126 showed similar PFGE patterns (Fig. 5). We have paid close attention to this organism since the clonal expansion of O126 in Osaka City was presumed. In December 2000, an outbreak of diarrheal illness occurred at a day-care center in Osaka City. As summarized in Table 4, the O126 strains from the outbreak and those from sporadic cases were similar in serotype, sensitivity to antibiotics, and PFGE patterns. Although the PFGE patterns of isolates from sporadic cases were not identical to those of the outbreak strains, they were very similar, particularly to outbreak strain No. 377-35. It seems possible that these strains were the results of a clonal expansion.

In this investigation we found more putative EAggEC strains beyond the abovementioned 13 strains on the basis of adhesion tests. We did not include them, however, because they did not show a positive reaction in our PCR tests, and their adhesion patterns were somewhat atypical. *E. coli* showing diffuse adhesion were also not included as DEC strains, since their etiological significance is still very controversial. It was difficult to assess their role etiologically in sporadic cases. If these strains were included as DEC, the isolation rate of DEC would be twice as high as that of *Salmonella*.

Throughout this study, serotyping of O antigens was not useful for detection of DEC strains (Fig. 4), although it worked for some particular serotypes – such as O26, O157 and so on – as Giammanco et al. suggested (38). We had difficulty deciding whether the strains that did not fit into the existing scheme were EPEC. On the other hand, commercial kits to detect enterotoxins and Stx are available and were useful to identify EHEC and ETEC strains. Methods to detect each subgroup of DEC and criteria to identify the strains as EPEC or EAggEC have not yet been standardized in a convenient manner. A unified analytical manual for DEC should be provided as soon as possible for clinical laboratories. Throughout this study we did not regard strains as DEC if they did not react with each PCR primer set, even if the strains showed adherence to HEp-2 cells. Further analyses are underway to identify diarrheagenic strains from among the HEp-2 adherent isolates.

In 1996 it became obligatory for physicians to report the occurrence of patients infected with EHEC to regional health centers, and clinical laboratories are already well prepared for detection of EHEC O157. We therefore compared the number of persons infected with EHEC O157 in Osaka City and the number of patients found in this study to estimate the coverage of our surveillance. It was shown that the number of infected persons in Osaka City was about 72 times (288/4) as many as the number detected through this investigation. If the number of patients due to all DEC were extrapolated on the basis of this ratio, the morbidity would be about 198 per 100,000 individuals per year, at least in Osaka City.

In conclusion, it was suggested that not only EHEC but also the other subgroups of DEC, including EAST1EC, play important roles in sporadic diarrheal illness in Osaka City. Further epidemiological, ecological, and etiological studies are needed to clarify the significance of unusual EPEC, EAST1EC, EAggEC, and HEp-2 adherent *E. coli*.

APPENDIX

Eleven hospitals were selected as sentinels in order to cover the Osaka City area. The participants collaborating in this study were as follows:

Hidenori Imaishi, Akira Fukumoto and Akihiko Hori at NTT West Osaka Hospital; Isao Yamamori, Kenji Suzuki and Shigeko Saitoh at Nagayoshi General Hospital; Tetsuji Goto, Hideki Yoshida, Hironari Niimi, Eiji Ikeda and Yoshihiro Sakaue at Osaka City General Medical Center; Atsushi Ono and Tetsuro Yoshida at Saiseikai-Izuo Hospital; Ryouji Ebina and Katsushi Kaji at Nakano Children Hospital; Gen Unishi, Mayumi Ideno, Miyoko Tsuji, Toshiaki Hayashi and Hiroaki Sudo at Saiseikai-Noe Hospital; Yuriko Tsubakio, Chiyo Iwamura and Akitomo Yoshimura at Osaka City Juso Hospital; Yoshiro Morikawa and Miyoshi Kitazato at Yodogawa Christian Hospital; Hiroyuki Ichiba at Sumiyoshi Citizen's Hospital; Tomoyuki Kawamura at University Hospital, Osaka City University; Takeshi Goto and Michiko Suzuki at Kita Citizen's Hospital; and the staff at Infectious Disease Prevention Division, Public Health and Welfare Bureau, Osaka Municipal Government.

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