Jpn. J. Infect. Dis., 55, 14-18, 2002

Original Article

Prevalence of *Escherichia coli* Possessing the *eaeA* Gene of Enteropathogenic *E. coli* (EPEC) or the *aggR* Gene of Enteroaggregative *E. coli* (EAggEC) in Traveler's Diarrhea Diagnosed in Those Returning to Tama, Tokyo from Other Asian Countries

Kazue Ogata[1], Rei Kato[1], Kenichiro Ito[3] and Sumio Yamada[1,2]*

[1]*Tama Branch Laboratory, Tokyo Metropolitan Research Laboratory of Public Health, Shibazaki-cho 3-16-25, Tachikawa-shi, Tokyo 190-0023,*
[2]*Office of Quality Assurance, Tokyo Metropolitan Research Laboratory of Public Health, Hyakunin-cho 3-24-1, Shinjuku-ku, Tokyo 169-0073 and*
[3]*National Institute of Public Health, Shirokanedai 4-6-1, Minato-ku, Tokyo 108-8638, Japan*

(Received June 11, 2001. Accepted January 29, 2002)

SUMMARY: To elucidate the importance of enteropathogenic *Escherichia coli* (EPEC) and enteroaggregative *E. coli* (EAggEC) as etiological agents in traveler's diarrhea, the detection of the *eaeA* and *aggR* gene in *E. coli* strains isolated from overseas travelers with diarrhea in Tama, Tokyo was carried out using a PCR method. Of 192 travelers who were mostly adults and had visited Asian countries from April 1998 to March 1999, *aggR*-positive *E. coli* strains were detected in 26 (13.5%). These strains represent the second predominant enteropathogen following enterotoxigenic *E. coli* (ETEC), whereas *eaeA*-positive *E. coli* strains were confirmed in seven subjects (3.6%). In 13 cases with *aggR* and four cases with *eaeA*, the organisms were detected in stool samples of patients as the only potential enteric pathogen. The clinical symptoms of these patients were similar to those in patients with ETEC; however, the severity of illness was milder than that associated with ETEC alone. Three strains with *eaeA* and five strains with *aggR* were typed as six different kinds of O serogroups, of which four strains belonged to the classical EPEC serogroups (O55, O114, O119, and O127a). These findings suggest that *aggR*-positive *E. coli* (EAggEC) is a significant causative agent in traveler's diarrhea. In addition, it was demonstrated that *eaeA*-positive *E. coli* (EPEC) is markedly correlated with diarrhea in adults.

INTRODUCTION

Escherichia coli can cause gastrointestinal infections (1). Diarrheagenic *E. coli* can be grouped into five categories (2) on the basis of the types of virulence, i.e., production of toxin, invasiveness, patterns of bacterial attachment to host cells, and effects of attachment on host cells: enterotoxigenic *E. coli* (ETEC), enterohemorrhagic *E. coli* (EHEC), enteroinvasive *E. coli* (EIEC), enteropathogenic *E. coli* (EPEC), and enteroaggregative *E. coli* (EAggEC). In addition, it has been proposed that diffusely adhering *E. coli* (DAEC) is diarrheagenic, but its role in acute diarrheal disease is controversial (3). In Japan, strains belonging to classical EPEC serotypes have also been included as a group of enteropathogens (4).

EPEC strains usually exhibit localized adherence (LA) to epithelial cells and induce attaching and effacing (A/E) lesions on enterocytes (5,6). EPEC virulence genes *eaf* and *eae* are respectively associated with LA and A/E lesions (7-9), and recently, bundle-forming pilus (Bfp) has been reported as one adherence factor (10). EAggEC strains show aggregative adherence (AA) to HEp-2 or HeLa cells; such adherence is mediated by aggregative adherence fimbriae 1 (AAF/1) associated with specific *agg* operons (11,12). In recent studies, it was demonstrated that *agg* operons consisted of several potential reading frames, e.g., *aggA*, *aggB*, *aggC*, *aggD*, and *aggR* (13,14). Furthermore, it was revealed that certain EAggEC strains produce an ST-like toxin (enteroaggregative ST, EAST)(15).

In some case-control studies of infant and childhood diarrhea, the pathogenic role of EPEC in infants younger than 12 months old has been well described (16-20); EAggEC has been associated with persistent diarrhea among children (20-22). In contrast, the significance of these organisms as etiological agents in adult disease remains unclear. EAggEC was evaluated as a significant etiological agent in recent studies of diarrhea diseases in North American travelers to Mexico (23) and among Spanish travelers to developing countries (24). However, the role of EPEC in traveler's diarrhea has not been thoroughly investigated. In Japan, traveler's diarrhea has also been a public health problem and has affected the actual states of gastrointestinal infection (25). To determine the role played by EPEC and EAggEC as causative agents of traveler's diarrhea in Japanese travelers to developing countries, the presence or absence of the *eaeA* or *aggR* gene of *E. coli* strains isolated from overseas travelers in Tama, Tokyo, was examined by PCR. In addition, the clinical symptoms of travelers infected with EPEC and EAggEC were investigated.

*Corresponding author: Mailing address: Office of Quality Assurance, Tokyo Metropolitan Research Laboratory of Public Health, Hyakunin-cho 3-24-1, Shinjuku-ku, Tokyo 169-0073. Tel: +81-3-3363-3231, Fax: +81-3-3368-4060, E-mail: yamada@tokyo-eiken.go.jp

MATERIALS AND METHODS

Stool samples: A total of 192 fecal specimens obtained from travelers returning to Japan from other countries, mostly Asian countries, during the period from April 1998 to March 1999 were examined. Stool specimens collected from persons under quarantine or receiving medical care were received in Cary and Blair-transportation medium at public health centers in Tama, Tokyo, and were transferred within 2 days to our laboratory. Health cards of the travelers accompanied the specimens; these cards included information such as countries visited and clinical symptoms. Information on the cards was given by the traveler at the time of stool sample collection.

Standard procedures: Stool samples were cultured by previously described methods for bacterial pathogens (26). For the diagnosis of diarrheagenic *E. coli*, three suspected colonies on DHL agar (Nissui Pharmaceutical Co., Ltd., Tokyo) plates were randomly selected and screened biochemically. The *E. coli* strains thus identified were tested for a classical EPEC serotype, ETEC, and Shiga-toxin (STX) producing-*E. coli* (STEC). In brief, boiled cells of *E. coli* strains were serogrouped by a slide agglutination test with the following types of commercial antisera from Denka-Seiken Co., Ltd. (Tokyo): O26, O44, O55, O86, O111, O114, O119, O125, O126, O127a, O128, O146, and O166. For the toxin tests, *E. coli* strains were inoculated into casamino acid-yeast extract broth (Nissui) and were shaken at 37°C for overnight. The culture was centrifuged for 20 min at 23,000 × g, and the supernatant was examined for heat-labile toxin (LT) by CT-RPLA (Denka-Seiken), for heat-stable toxin (ST) by COLIST EIA (Denka-Seiken), and for STX by VT-RPLA (Denka-Seiken).

PCR assay: The various primers used for the detection of the virulent genes of EPEC and EAggEC are listed in Table 1 (27,28). The determination of EPEC and EAggEC in the strains tested was performed by the identification of the *eaeA* gene, and *aggR* gene. Furthermore, the *eaeA*- or *aggR*-positive strains were tested for the presence of the *eaf* and *bfpA* genes, or the *astA* gene. In brief, stock cultures were inoculated onto nutrient agar slants and incubated at 37°C overnight. A small cluster of bacteria was suspended in 100 μl of distilled water, and boiled at 94°C for 5 min. After a short centrifugation (1 min, 18,000 × g), 4.8 μl of template were mixed with 5 μl of 10 ×PCR buffer (Takara Shuzo Co. Ltd., Kyoto), 3 μl of 25 mM MgCl, 2 μl of 2.5 mM dNTP (Takara Shuzo), 0.1 μl of 50 uM sense primer and antisense primer, 0.2 μl of Taq DNA polimerase (Takara Shuzo), and 35 μl of distilled water. The mixture was amplified by 25 cycles of denaturation at 94°C for 0.5 min, annealing at 50°C for 1.0 min, and extension at 72°C for 1.5 min with Program Temp Control System PC-700 (ASTEC Co., Ltd., Tokyo). Ten microliters of the reaction mixture were then analyzed by electrophoresis on 1.5% agarose gels, and the reaction products were visualized by staining with ethidium bromide.

Table 1. PCR primers used in this study

Gene	Primer	Sequence	Location
eaeA	eaek1*	GCTTAGTGCTGGTTTAGGAT	66-85
	eaek4*	TCGCCGTTCAGAGATCGC	554-537
EAF	EAF-B**	TGGATCGCCAATGTTCTTGG	141-160
	EAF-SR**	ATGGGGACCATGTATTATCA	922-941
bfpA	bfps**	GAAGTAATGAGCGCAACGTC	154-173
	bfpas**	ACATGCCGCTTTATCCAACC	387-368
aggR	aggRks1**	GTATACACAAAAGAAGGAAGC	100-120
	aggRkas2**	ACAGAATCGTCAGCATCAGC	353-334
astA	EASTOS1**	GCCATCAACACAGTATATCCG	3-23
	EASTOAS2**	CGCGAGTGACGGCTTTGTAG	111-92

* : Reference 28.
**: Reference 27.

O-serotyping of *E. coli* isolates and *eaeA*- or *aggR*- positive strains: O antigens were determined by slide agglutination tests with kits using *E. coli* antisera (I) (Denka-Seiken).

Adherence assay: For the *eaeA*- or *aggR*- positive *E. coli* isolates, mannose-resistant adhesion to HEp-2 cells was tested by the method described by Cravioto et al. (29).

RESULTS

The travelers examined were mostly adults, and were acute or convalescent-phase patients. According to the standard procedure (4), enteropathogens were detected in 87 of 192 cases (45.3%). As shown in Table 2, by the introduction of PCR assays to detect *eaeA* or *aggR* genes of EPEC or EAggEC, the *aggR*-positive *E. coli* was detected in 26 cases (13.5%), whereas *eaeA*-positive *E. coli* was confirmed in only seven cases (3.6%). Three cases with *eaeA-E. coli* and 13 with *aggR-E. coli* involved a possible mixed infection. Consequently, a total of 104 cases were pathogen-positive, and the positivity ratio increased to 54.2%. Number of enteropathogens isolated were 162 strains, in which most prevalent one was the ETEC. Next most prevalent pathogen isolated was the *aggR*-positive *E. coli*, followed by *Plesiomonas shigelloides*, *Aeromonas* spp., *Campylobacter* spp., classical EPEC serotype, *Salmonella* spp., *Vibrio parahaemolyticus*, and *eaeA*-positive *E. coli*.

Three of the patients with *eaeA*-positive *E. coli* had mixed infection with *Plesiomonas*, *Aeromonas* spp. and *Campylobacter* spp., or *Aeromonas* spp. and *aggR*-positive *E. coli*. One-half (13 cases) of all *aggR*-positive *E. coli* cases were detected together with one to four other enteropathogens. They were classified in ten patterns, and involved combinations of *Camplyobacter* spp., *Plesiomonas*, *Salmonella* spp., ETEC, *Aeromonas* spp., *V. parahaemolyticus*, and *eaeA*-positive *E. coli*.

The suspected countries where infection with *eaeA*-positive

Table 2. Detection of enteropathogens from overseas travelers with diarrhea in Tama, Tokyo (April 1998-March 1999)

Number examined	192
Number positive for pathogens (%)	104 (54.2)
No. of cases with (%):	
Enterotoxigenic *E. coli*	36 (18.7)
aggR gene-positive *E. coli*	26 (13.5)
Plesiomonas shigelloides	25 (13.0)
Aeromonas spp.	18 (9.4)
Campylobacter spp.	12 (6.2)
Classical EPEC serotype	11 (5.7)*
Salmonella spp.	10 (5.2)
V. parahaemolyticus	10 (5.2)
eaeA gene-positive *E. coli*	7 (3.6)
Shigella spp.	3 (1.6)
Verotoxin-producing *E. coli*	2 (1.0)
V. cholerae O-1 (tox+)	1 (0.5)
V. cholerae O-1 (tox–)	1 (0.5)

*: One *eaeA* gene-positive *E. coli* and three *aggR* gene-positive *E. coli* were observed.

E. coli occurred were Thailand (2 cases), Indonesia (2 cases), Viet Nam (1 case), and others (2 cases). Those countries where *aggR*-positive *E. coli* infection occurred were Thailand (8 cases), Indonesia (5 cases), India (5 cases), Viet Nam (2 cases), Philippines (2 cases), Iran (1 case), and South Africa (1 case). The remaining two cases were detected in travelers who had visited other Asian countries. There was no special seasonal variation associated with either of the *E. coli* isolations.

The *eaeA*- and *aggR*-positive strains were characterized serologically, phenotypically, and genetically; only three *eaeA*-positive strains and five *aggR*-positive strains were typed to particular serogroups, namely, O153 (2 strains) and O55 (1 strain) in the former, and O15 (2 strains), O114, O119, or O127a (each 1 strain) in the latter. Among serotyped strains, *eaeA*-positive *E. coli* O55, and *aggR*-positive *E. coli* O114, O119, and O127a had already been determined as one of the classic EPEC groups. In the HEp-2 cell adherence assay, a typical LA pattern was observed in only two of the *eaeA*-positive strains, whereas 14 *aggR*-positive strains showed the typical AA pattern. Furthermore, none of the *eaeA*-positive strains possessed the *eaf* and *bfpA* gene, but 12 *aggR*-positive strains were *astA*-positives.

To clarify the severity of illness and the clinical symptoms of patients infected with *eaeA*-, or *aggR*-positive *E. coli*, retrospective investigations were carried out on 16 patients, among whom either of the two strains was found as the only potential enteric pathogen; these cases were compared with those in which patterns were infected with ETEC alone. As shown in Table 3, the severity of the illness and the clinical symptoms of patients infected with *aggR*-positive *E. coli* and those with ETEC were almost identical, although a predominance of mild cases with diarrhea occurring less than four times per day was observed in *aggR*-positive *E. coli* infections. In addition, abdominal cramps were observed in 69% of the cases; a few of the cases were accompanied by fever (31%), nausea, and/or vomiting (38%). A few patients infected with *eaeA*-positive *E. coli* also experienced mildly watery diarrhea and headache or fever, but none reported abdominal cramps.

Classic EPEC serotype strains were detected in 11 cases; these which serotypes belonged to serogroups O114 and O128 (2 strains each), and O55, O119, O125, O126, O127a, O142, or O146 (1 strain each). Four of the strains mentioned above were positive for either the *eaeA* or *aggR* gene. In contrast, the ETEC and verotoxin-producing *E. coli* (VTEC) strains that were isolated did not possess either of the genes.

Table 3. Symptoms and severity of traveler's diarrhea in patients from whom *eaeA* or *aggR* gene positive *E. coli* was isolated as the only potential enteric pathogen

Characteristics of patients	% of symptoms with:		
	eaeA-E. coli (n = 4)	*aggR-E. coli* (n = 13)	ETEC* (n = 25)
Character of stool			
Watery	100	61	96
Loose	0	31	4
Bloody	0	8	0
Maximum number of diarrhea episodes per day			
Mild (1-3)	100	54	24
Moderrate (4-6)	0	38	52
Severe (>7)	0	8	24
Abdominal cramps	0	69	64
Nausea/vomiting	50	39	36
Fever	50	31	28
Headache	50	15	16

*: Patients infected with enterotoxigenic *E. coli* (ETEC) alone during the same period.

DISCUSSION

Enteric infection due to EPEC or EAggEC is characterized by acute diarrhea in infants or chronic diarrhea in children in developing countries. In previous studies (23,24), EAggEC strains have been involved in the etiology of traveler's diarrhea in North American travelers to Mexico and in Spaniards traveling to developing countries. However, an association between the presence of EAggEC or EPEC strains in stool samples of diarrhea in adults has not been conclusively demonstrated. In order to determine the potentially causal role of the EAggEC and EPEC strains in traveler's diarrhea, genetic diagnosis by PCR assay was performed on the *E. coli* strains isolated from overseas travelers presenting with diarrhea in Tama, Tokyo. The *aggR*-positive *E. coli* was detected in 26 (13.5%) of the 192 cases of traveler's diarrhea; thus, this strain was the second most prevalent strain after ETEC. Similarly, Mathewson et al. (23) reported that *E. coli* strains showing mannose-resistant focal adherence were detected in 17 cases (9.0%) of the North American travelers studied. Recent work by Gascon et al. (24) showed that the plasmid DNA for EAggEC was present in 23 cases (13.9%) among the Spanish travelers included in that study. Typically, *aggR*-positive *E. coli* strains were detected among travelers to Southeast Asian countries or to the Indian subcontinent, and geographic distribution was similar to that observed in the case of ETEC. On the other hand, EAggEC strains confirmed in the study of Spanish travelers were isolated in travelers returning from all geographic areas, especially from the Indian subcontinent, West Africa, and Central America.

Among the cases in which *aggR*-positive *E. coli* was detected, 13 cases involved this organism as the only potential enteropathogen. The clinical symptoms associated with the *aggR*-positive strain were watery diarrhea (61%), abdominal cramps (69%), fever (31%), and nausea or vomiting (39%). However, the severity of illness was milder than that observed in patients with ETEC infection alone. In the investigation of Spanish travelers, Gascon et al. reported that five of 23 EAggEC strains caused chronic diarrhea (>14 days); the clinical symptoms of patients with other EAggEC strains were similar to those reported in our results. The present and previous findings indicate that EAggEC is important as an etiological agent in travelers returning with gastrointestinal illness from a developing country.

As shown in the report by Albert et al. (20), EPEC is a significant enteric pathogen in infants of up to 12 months of age; the prevalence of this pathogen tends to diminish with age. In the present study, it was suggested that the *eaeA*-positive *E. coli* strain is markedly correlated with adult diarrhea, although the occurrence of such strains was lower than that of EAggEC, which accounted for seven cases (3.6%). Four of seven *eaeA*-positive *E. coli* strains were identified as only potential enteropathogens, and their patients had mild cases with watery diarrhea occurring less than four times per day. Additional clinical symptoms were nausea and/or vomiting, and fever (2 cases each); however, no patients in this group experienced abdominal cramps.

Although it is of interest to study the correlations between

certain serotypes and particular gene-positive *E. coli* strains, only three *eaeA*-positive strains and five *aggR*-positive strains could be typed in the present study. Of which one *eaeA*-positive *E. coli* strain and three *aggR*-positive *E. coli* strains belonged to the classic EPEC serogroups; O55 in the case of *eaeA* and O114, O119, and O127a in *aggR*. Among the remaining four strains, two *eaeA*-positive strains were O153, and two *aggR*-positive strains were O15. These results agree with those of Mathewson et al. (23) and Gascon et al. (24), who also described that only two of 28 enteroadherent *E. coli* strains isolated from North American travelers belonged to the classical serogroup O44, and none of the EAggEC strains isolated from the Spanish cases was positive for the classic serotypes. On the other hand, in a previous study on acute infantile diarrhea (18), 70% of LA-*E. coli* strains belonged to the classical EPEC O serogroups. Moreover, Chan et al. (30) demonstrated that the predominant serogroups of AA-*E. coli* strains from acute and chronic diarrhea were O44, O111, and O126. These differences may indicate that there are broader serogroups of EPEC or EAggEC than are currently recognized and that differences in the specific O serogroups are predominant in infant, childhood, and adult types of diarrhea.

The identification of EPEC and EAggEC has been performed by observing the adherence patterns to HEp-2 cells, or by a combination of cell assay and hybridization with DNA probes of virulence factors. However, several studies have demonstrated that 37% of adherent strains of *E. coli* exhibit atypical or non-characteristic patterns (17), and that there are differences among the results of cell assays and DNA-hybridization tests as regards LA-*E. coli* strains (17,19,20). In present study, two *eaeA*-positive *E. coli* strains and 14 *aggR*-positive *E. coli* strains showed typical adherence patterns, but the other strains could not be identified. In addition, none of the *eaeA*-positive *E. coli* strains possessed the *bfpA* or *eaf* gene, whereas 12 *aggR*-positive *E. coli* strains were *astA*-positive. Further studies will be necessary in order to determine whether EPEC or EAggEC strains play a significant role in infantile and childhood diarrhea, or in gastroenteritis in adults.

REFERENCES

1. Gray, L. D. (1995): *Escherichia*, *Salmonella*, *Shigella*, and *Yersinia*. p.450-456. *In* P.R. Murray, E. J. Baron, M. A. Pfaller, et al. (eds.), Manual of Clinical Microbilogy. 6th ed. AMS Press, Washington.
2. Salyers, A. A. and Whitt, D. D. (1994): *Escherichia coli* gastrointestinal infection. p.190-204. *In* A. A. Salyers and D. D Whitt (eds.), Bacterial Pathogenesis-a molecular approach. AMS Press, Washington.
3. Gomes, T. A., Blake, P. A. and Trabulsi, L. R. (1989): Prevalence of *Escherichia coli* strains with localized, diffuse, and aggregative adherence to Hela cells in infants with diarrhea and matched controls. J. Clin. Microbiol., 27, 266-269.
4. Zen-Yozi, H., Sakai, S., Terayama, T., Kudoh, Y. and Ito, T. (1985): Manual for Examination of Enteropathogenic Bacteria. 4th ed. Igakushoin, Tokyo (in Japanese).
5. Nataro, J. P., Kaper, J. B., Robins-Browne, R., Prado, V., Vial, P. and Levine, M. M. (1987): Patterns of adherence of diarrheagenic *Escherichia coli* to HEp-2 cells. Pediatr. Infect. Dis., 6, 829-831.
6. Knutton, S., Lloyd, D. R. and McNeish, A. S. (1992): Adhesion of enteropathogenic *Escherichia coli* to human intestinal enterocytes and cultured human intestinal mucosa. Infect. Immun., 55, 69-77.
7. Donnenberg, M. S. and Kaper, J. (1992): Enteropathogenic *Escherichia coli*. Infect. Immun., 60, 3953-3961.
8. Giron, J. A., Suc Yue Ho, A. and Schoolnik, G. K. (1991): An inducible bundle-forming pilus of enteropathogenic *Escherichia coli*. Science, 254, 710-713.
9. Jerse, A. E., Yu, J., Tall, B. and Kaper, J. B. (1990): A genetic locus of enteropathogenic *Escherichia coli* necessary for the production of attaching and effacing lesions on tissue culture cells. Proc. Nath. Acad. Sci., 87, 7839-7843.
10. Grion, J. A., Donnenberg, M. S., Martin, W. C., Jarvis, K. G. and Kaper, J. B. (1993): Distribution of the bundle-forming pilus structural gene (*bfpA*) among enteropathogenic *Escherichia coli*. J. Infect. Dis., 168, 1037-1041.
11. Vial, P. A., Robins-Browne, R., Lior, H., Prado, V., Kaper, J. B., Nataro, J. P., Maneval, D., Elsayed, A. E. and Levine, M. M. (1988): Characterization of enteroadherent aggregative *Escherichia coli*, a putative agent of diarrheal disease. J. Infect. Dis., 158, 70-79.
12. Nataro, J. P., Deng, Y., Maneval, D. R., German, A. L., Martin, W. C. and Levine, M. M. (1992): Aggregative adherence fimbriae I of enteroaggregative *Escherichia coli* mediate adherence to HEp-2 cells and hemagglutination of human erythrocytes. Infect. Immun., 60, 2297-2304.
13. Savarino, S. J., Fox, P., Yikang, D. and Nataro, J. P. (1994): Identification and characterization of a gene cluster mediating enteroaggregative *Escherichia coli* aggregative adherence fimbria I biogenesis. J. Bacteriol., 176, 4949-4957.
14. Nataro, J. P., Yikang, D., Yingkang, D. and Walker, K. (1994): AggR, a transcriptional activator of aggregative adherence fimbria I expression in enteroaggregative *Escherichia coli*. J. Bacteriol., 176, 4691-4699.
15. Savarino, S. J., Fasano, A., Robertson, D. C. and Levine, M. M. (1991): Enteroaggregative *Escherichia coli* elaborate a heat-stable enterotoxin demonstrable in an in vitro rabbit intestinal model. J. Clin. Invest., 87, 1450-1455.
16. Gomes, T. A. T., Rassi, V., MacDonald, K. L., Romos, S. R. T. S., Trabulsi, L. R., Vieira, M. A. M., Guth, B. E. C., Candieas, J. A. N., Ivery, C., Toledo, M. R. F. and Blake, P. A. (1991): Enteropathogens associated with acute diarrheal disease in urban infantis in São Paulo, Brazil. J. Infec. Dis., 164, 331-337.
17. Forestier, C., Meyer, M., Favre-Bonte, S., Rich, C., Malpuech, G., Le Bougenec, C., Sirot, J., Joly, B. and de Champs, C. (1996): Enteroadherent *Escherichia coli* and diarrhea in children: a prospective case-control study. J. Clin. Microbiol., 34, 2897-2903.
18. Gonzalez, R., Diaz, C., Marino, M., Cloralt, R., Pequeneze, M. and Perez-Schael, I. (1997): Age-specific prevalence of *Escherichia coli* with localized and aggregative adherence in Venezuelan infants with acute diarrhea. J. Clin. Microbiol., 35, 1103-1107.
19. Gomes, T. A. T., Vieira, M. A. M., Abe, C. M., Rodrigus, D., Griffin, P. M. and Ramos, S. R. T. S. (1998): Adherence patterns and adherence-related DNA sequences in *Escherichia coli* isolates from children with and without diarrhea in São Paulo city, Brazil. J. Clin. Microbiol., 36, 3609-3613.
20. Albert, M. J., Faruque, A. S. G., Sack, R. B. and Mahalanabis, D. (1999): Case-control study of enteropathogens associated with childhood diarrhea in

Dhaka, Bangladesh. J. Clin. Microbiol., 37, 3458-3464.
21. Baqui, A. H., Sack, R. B., Black, R. E., Haider, K., Hossain, A., Alim, A. R. M. A., Yunus, M., Chowdhury, H. R. and Siddique, A. K. (1992): Enteropathogens associated with acute and persistent diarrhea in Bangladish children <5 years of age. J. Infec. Dis., 166, 792-796.
22. Fang, G. D., Lima, A. A. M., Martins, C. V., Nataro, J. P. and Guerrant, R. L. (1995): Etiology and epidemiology of persistent diarrhea in northeastern Brazil: a hospital-based, prospective, case-control study. J. Pediatr. Gastroenterol. Nutr., 21, 137-144.
23. Mathewson, J. J., Johnson, P. C., DuPont, H. L., Morgan, D. R., Thornton, S. A., Wood, L. V. and Ericsson, C. D. (1985): A new recognized cause of travelers' diarrhea: enteroadherent *Escherichia coli*. J. Infec. Dis., 151, 471-475.
24. Gascon, J., Vargas, M., Quinto, L., Corachan, M., Jimenez de Anta, M. T. and Vila, J. (1998): Enteroaggregative *Escherichia coli* strains as a cause of traveler's diarrhea: a case-control study. J. Infec. Dis., 177, 1409-1412.
25. National Institute of Health and Infectious Diseases Control Division, Ministry of Health and Welfare (1995): Traveler's diarrhea, Japan, January 1992-July 1995. Infect. Agents Surveillance Rep., 16, 217'-218'.
26. Yamada, S., Matsushita, S., Dejsirilert, S. and Kudoh, Y. (1997): Incidence and clinical symptoms of *Aeromonas*-associated travellers' diarrhea in Tokyo. Epidemiol. Infect., 119, 121-126.
27. Ratchtrachenchai, O-A., Subpasu, S. and Ito, K. (1997): Investigation on enteroaggregative *Escherichia coli* infection by multiplex PCR. Bull. Dept. Med. Sci., 39, 211-220.
28. Yamazaki, M., Saito, M., Inuzuka, K., Shima, S., Taniwaki, H. and Ito, K. (1997): *eaeA* genes in *Escherichia coli* derived from Japanese patients with sporadic diarrhea. J. Jpn. Assoc. Infect. Dis., 71, 1059-1065.
29. Cravioto, A., Tello, A., Navarro, A., Ruiz, J., Villajan, H., Uribe, F. and Eslava, C. (1979): An adhesive factor found in strains of *Escherichia coli* belonging to the traditional infantile enteropathogenic serotypes. Curr. Microbiol., 3, 95-99.
30. Chan, K. N., Phillips, A. D., Knutton, S., Smith, H. R. and Walker-Smith, J. A. (1994): Enteroaggregative *Escherichia coli*: another cause of acute and chronic diarrhoea in England? J. Pediatr. Gastroenterol. Nutr., 18, 87-91.