Table 3. Genotypes of V. cholerae O139 strains based on the DNA sequence of the *ctxB* gene

Strain	No of stain	Nucleotide position				Amino acid position				*ctxB* gene Type
		83	115	138	203	28	39	46	68	
Classical 589B	1	A	C	T	C	D	H	F	T	1
El Tor Australia	1	A	C	G	C	D	H	L	T	2
El Tor N16961	1	A	T	T	T	D	Y	F	I	3
V. cholerae O139 (2001)	3	C	C	T	C	A	H	F	T	4
V. cholerae O139 (2004)	1	A	C	T	C	D	H	F	T	1
V. cholerae O139 (2004)	1	A	C	T	C	D	H	F	T	1
V. cholerae O139 (2005)	1	A	C	T	C	D	H	F	T	1
V. cholerae O139 (2006)	2	C	C	T	C	A	H	F	T	4

Table 4. Detection of *ctxB* classical allele among *V. cholerae* O1 strains isolated form different parts of India

Palace	Month/Year	No of strains received	No of strains tested for *ctxB* classical allele by MAMA-PCR#
Chennai	December 2007	29	6
Rohtak	December 2007	9	2
Pune	December 2007	8	2
Orissa*	July, August 2007	5	5
Assam*	October 2007	6	6
Mumbai	February 2008	4	4

*Strains isolated from cholera outbreaks
All the representative strains from each place were positive in the MAMA-PCR

Table 5. Resistance profile and other resistance genes of *Shigella* strains

Strain	Resistance gene	Resistance profile
S. dysenteriae type 1(2)	bla_{oxa-1}, *tet*B, *cat*1, *str*A	A Co T C Na S
S. dysenteriae type 1(16)	bla_{oxa-1}, *tet*B, *cat*1, *str*A	A Co T C Na Nf Cf Of S
S. dysenteriae type 5(2)	*tetA, catI, strA*	Co T C Na S
S. flexneri 1a	-	-
S. flexneri 1b	bla_{oxa-1}, *catI*, *tetB*, *str*A	A Co T Na S
S. flexneri 1b	*catI*, *tetB*	Co T C
S. flexneri 2a	bla_{oxa-1}, *catI*, *tetB*, *aadA1*	A Co T C Na S
S. flexneri 2a	bla_{oxa-1}, *catI*, *tet*B, *aadA1*	A Co C S
S. flexneri 2a	bla_{oxa-1}, *catI*, *tetB*, *str*A	A Co T C Na S
S. flexneri 2a	bla_{oxa-1}, *tetB*, *aadA1*	A Co T S
S. flexneri 2a	*catI*, *tetB*, *aadA1*	Co T C Na S
S. flexneri 2a	*catI*, *tetB*, *aadA1*	Co T C S
S. flexneri 2b	*tetB*, *aadA1*	Co T Na S
S. flexneri 2b	bla_{oxa-1}, *catI*, *tetB*, *str*A	A Co T C S
S. flexneri 2b	*tetB*, *str*A	A Co T C Na S
S. flexneri 3a	bla_{oxa-1}, *catI*, *tetB*, *str*A	A Co T C Na S
S. flexneri 6	bla_{oxa-1}, *tetB*, *cat*, *aadA1*	A Co T C S
S. flexneri 3a	*catI*, *tetB str*A	Co T C Na S
S. flexneri 3b	bla_{oxa-1}, bla_{TEM-1} *tetAB*, *str*A	A Co T Na Cf Nf Of Az S
S. boydii 1	*aadA1*	Co S
S. boydii 1	bla_{oxa-1}, *tetAB*,bla_{CTXM-3},, *aadA1*	A Co T C Na Nf Cf Of Cr AZ S
S. boydii 2	*tetB*, *cat*, *aadA1*	Co T C S
S. boydii 11	-	Co S
S. boydii 12 (2)	*tetA*, *strA*	Co T Na S
S. boydii 12	*tetB*, *aadA1*	Co T Na S
S. sonnei (12)	*tetA*, *strA*	Co T Na S
S. sonnei (2)	*strA*,	Co T Na S
S. sonnei (2)	-	Co T Na S
S. sonnei (1)	bla_{oxa-1}, *tetAB*,*catI*, *strA*, *aadA1*	A Co T C S

Number of strains indicated in parentheses. A, ampicillin; Co, Co-trimoxazole; T, tetracycline; C, chloramphenicol; Na, nalidixic acid; Nf, norfloxacin; Cf, ciprofloxacin; Of, ofloxacin; Cr, ceftriaxone; Az, azithromycin; S- steptomycin.

Table 6. Fluoroquinolone resistance and amino acid substitutions in the QRDR of *Shigella* strains

Strain No	Year	Place	MIC (μg/ml)				Amino acid substitution		
			NA	CIP	NOR	OFX	GyrA		ParC
HU8 *S. dyesnteriae* type 1	1988	Tripura outbreak	1	0.08	0.027	0.047	S_{83}	S_{87}	S_{80}
BCH518 *S. dyesnteriae* type 1	1995	Calcutta sporadic case	>256	0.094	0.19	0.038	S_{83} - L	D_{87}	S_{80}
NK2678 *S. dyesnteriae* type 1	2002	Calcutta sporadic case	>256	4	12	12	S_{83} - L	D_{87} - G	S_{80}-I
H16576 *S. dyesnteriae* type 1	2002	Calcutta sporadic case	>256	6	16	8	S_{83} - L	D_{87} - G	S_{80}-I
D2 *S. dyesnteriae* type 1	2002	Diamond Harbor outbreak	>256	4	12	8	S_{83} - L	D_{87} - G	S_{80}-I
21 *S. dyesnteriae* type 1	2002	Siliguri outbreak	>256	6	16	12	S_{83} - L	D_{87} - G	S_{80}-I
12567 *S. dyesnteriae* type 1	2002	Goa sporadic case	>256	4	8	12	S_{83} - L	D_{87} - G	S_{80}-I
AZ11 *S. dyesnteriae* type 1	2003	Aizal outbreak	>256	3	6	16	S_{83} - L	D_{87} - N	S_{80}-I
115 *S. dyesnteriae* type 1	2003	Chandigarh outbreak	>256	3	8	8	S_{83} - L	D_{87} - G	S_{80}-I
NK2379 *S. boydii* type 1	2002	Calcutta sporadic case	1	0.006	0.032	0.047	S_{83}	S_{87}	S_{80}
G24371 *S. boydii* type 1	2001	Calcutta sporadic case	>256	200	500	>32	S_{83} - L	D_{87} - N	S_{80}-I
NK2788 *S. flexneri* type 3b	2002	Calcutta sporadic case	>256	200	250	>32	S_{83} - L	D_{87} - N	S_{80}-I
C15320 *S. flexneri* type 3b	1997	Calcutta sporadic case	1.25	0.012	0.023	0.064	S_{83}	S_{87}	S_{80}
NK2017 *S. sonnei*	2002	Calcutta sporadic case	>256	0.064	0.014	0.012	S_{83} - L	S_{87}	S_{80}

Table 7. Accumulation of norfloxacin and ciprofloxacin in clinical isolates of *Shigella* strains

Strains	Serotype	Accumulation of norfloxacin* (μg /mg of cells)		Accumulation of ciprofloxacin* (μg /mg of cells)	
		Before addition of CCCP	After addition of CCCP	Before addition of CCCP	After addition of CCCP
S. dysenteriae C152	1	0.220 ± 0.003	0.320 ± 0.004	0.053 ± 0.007	0.079 ± 0.001
S. dysenteriae 12567	1	0.096 ± 0.006	0.310 ± 0.001	0.026 ± 0.009	0.076 ± 0.002
S. boydii NK2379	1	0.250 ± 0.009	0.350 ± 0.001	0.057 ± 0.008	0.084 ± 0.002
S. boydii G24371	1	0.066 ± 0.005	0.330 ± 0.002	0.012 ± 0.003	0.081 ± 0.002
S. flexneri C15320	3b	0.260 ± 0.005	0.340 ± 0.007	0.059 ± 0.008	0.082 ± 0.001
S. flexneri NK2788	3b	0.087 ± 0.001	0.320 ± 0.002	0.019 ± 0.001	0.080 ± 0.003

* Data represent the means ± standard deviations of three determinations.

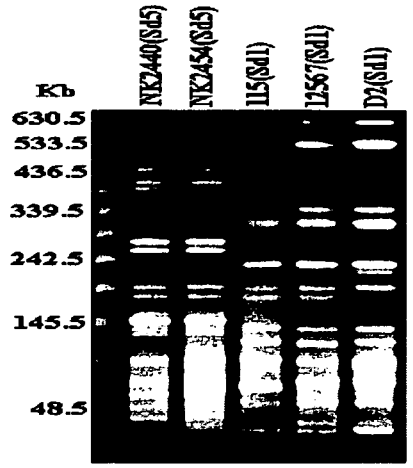

Fig. 1a

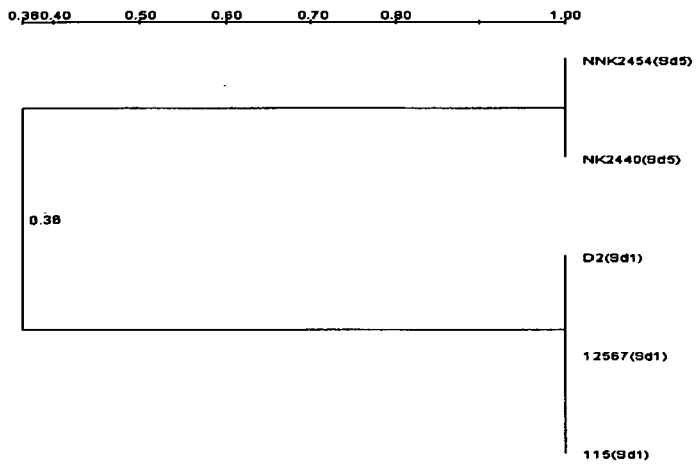

Fig. 1b

Fig.1a PFGE profile of *S. dysenteriae* (Sd) strains after digestion with *XbaI* . Serotypes are mentioned in the parenthesis.

Fig.1b Dendrogram analysis of the *S. dysenteriae* strains based on the PFGE profiles with Diversity Database software (Bio-Rad) employing the UPGMA method.

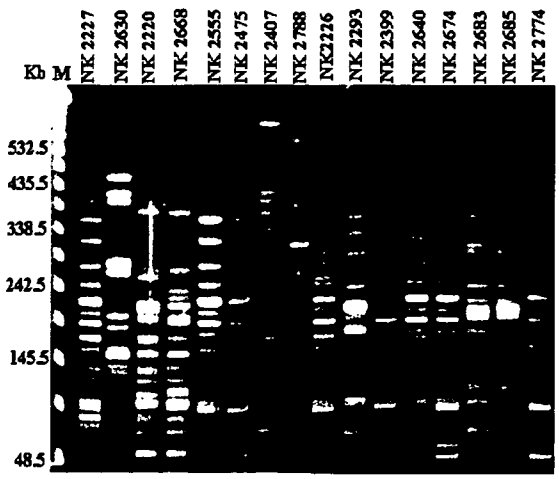

Fig. 2a

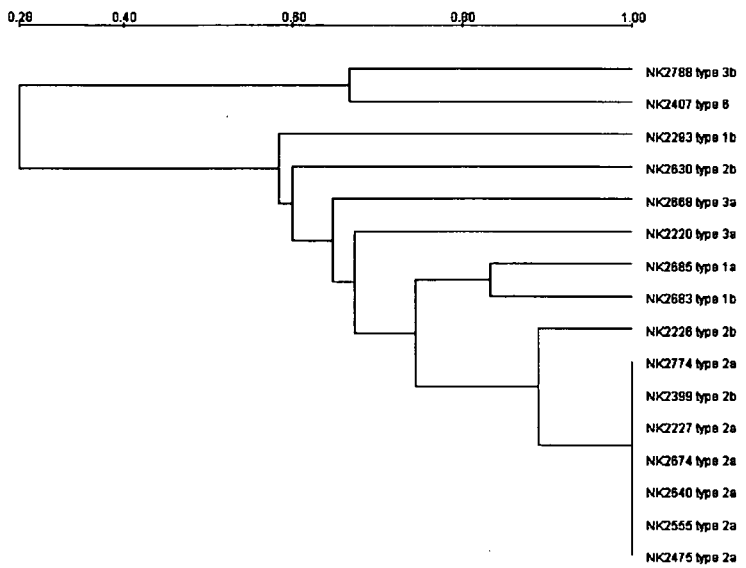

Fig. 2b

Fig. 2a PFGE profile of *S. flexneri* strains after digestion with *Xba*I.

Fig. 2b Dendrogram analysis of the *S. flexneri* strains based on the PFGE profiles with Diversity Database software (Bio-Rad) employing the UPGMA method.

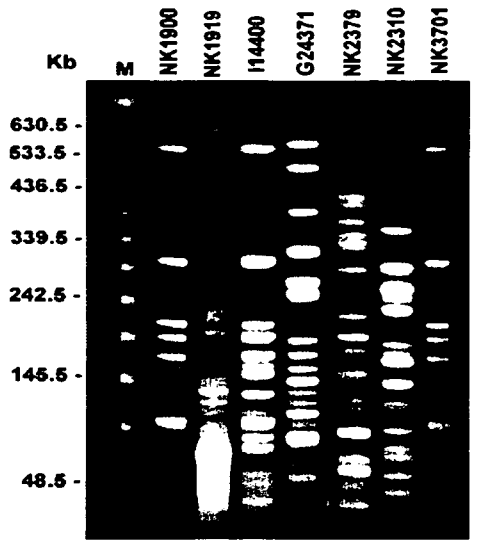

Fig. 3a

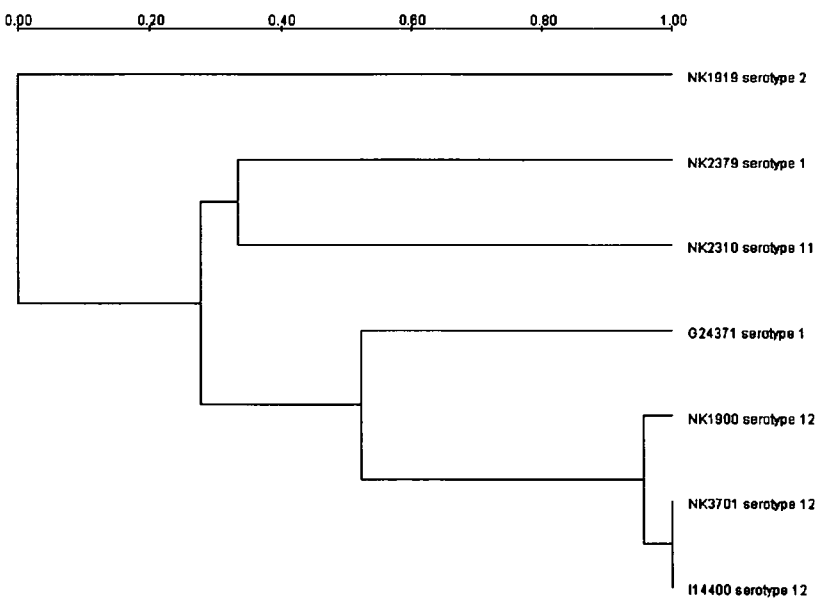

Fig. 3b

Fig. 3a PFGE profile of *S. boydii* strains after digestion with *Xba*I.

Fig. 3b Dendrogram analysis of the *S. boydii* strains based on the PFGE profiles with Diversity Database software (Bio-Rad) employing the UPGMA method.

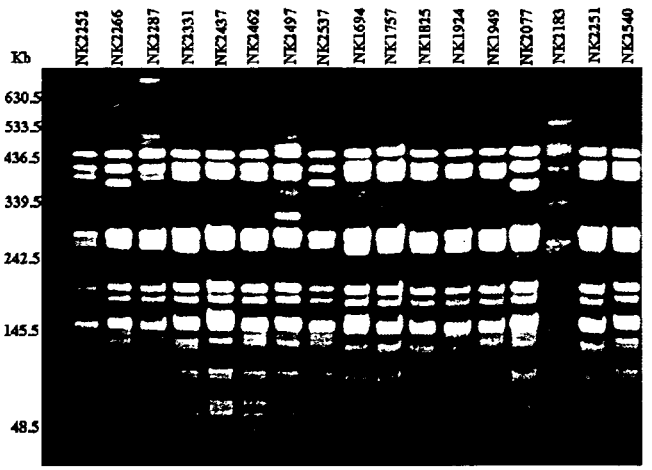

Fig. 4a

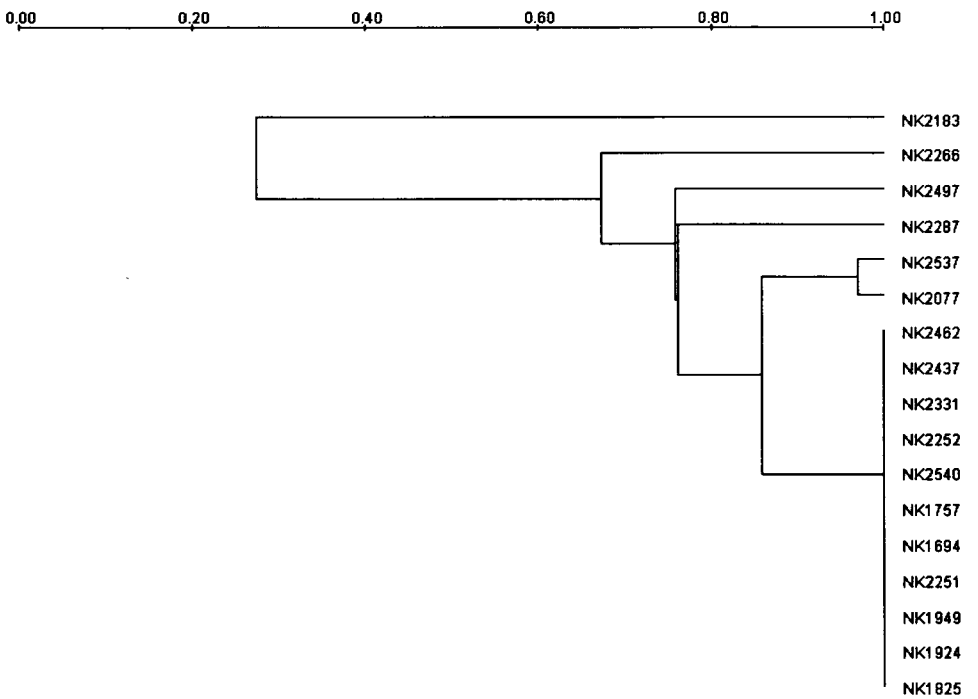

Fig. 4b

Fig. 4a PFGE profile of S. sonnei strains after digestion with XbaI

Fig. 4b Dendrogram analysis of the S. sonnei strains based on the PFGE profiles with Diversity Database software (Bio-Rad) employing the UPGMA method.

10. Publication list for this work:

1. Chakraborty, R., S. Sinha, A. K. Mukhopadhyay, M. Asakura, S. Yamasaki, S. K. Bhattacharya, G. B. Nair, and T. Ramamurthy. 2006. Species-specific identification of *Vibrio fluvialis* by PCR targeted to the conserved transcriptional activation and variable membrane tether regions of *toxR* gene. J. Med. Microbiol. 55: 805-808.

2. Saha, A., R. Deb, S. Shah, T. Ramamurthy, S. Shinoda, A. K. Mukhophadyay, and R. K. Bhadra. 2006. A PCR based identification of *Vibrio cholerae*, its biotypes and closely related *Vibrio mimicus* targeting the chromosomal origin of replication (*oriCI*$_{VC}$) of *V. cholerae*. FEMS Micrbiol. Lett. 257: 84-91.

3. Dutta, B., R. Ghosh, N. C. Sharma, G. P. Pazhani, N. Taneja, Raychowdhuri, B. L. Sarkar, S. K. Mondal, A. K. Mukhopadhyay, R. K. Nandy, M. K. Bhattacharya, S. K. Bhattacharya, and T. Ramamurthy. 2006. Spread of Cholera with new clone of *Vibrio cholerae* O1 El Tor, serotype Inaba in India. J. Clin. Microbiol. 44:3391-3393.

4. Sen, B., B. Dutta, S. Chatterjee, M. K. Bhattacharya, R. K. Nandy, A. K. Mukhopadhyay, B. D. N. Gangopadhyay, S. K. Bhattacharya, and T. Ramamurthy. 2007. The first outbreak of acute diarrhea due to pandemic strain of *Vibrio parahaemolyticus* O3:K6 in Kolkata, India. Internal. Int J Infect Dis.11(2):185-187.

5. Raychowdhuri A., S. Chatterjee, G. P. Pazhani, R. K. Nandy, M. K. Bhattacharya, S. K. Bhattacharya, T. Ramamurthy and A. K. Mukhopadhyay. 2007. Molecular characterization of recent *Vibrio cholerae* O1, El Tor, Inaba strains isolated from hospitalized patients in Kolkata, India during 2004-05. J Infect. 2007 Nov;55(5):431-438.

6. Pazhani, G. P., S. K. Niyogi, A. K. Singh, B. Sen, N. Taneja, M. Kundu, S. Yamasaki, and T. Ramamurthy. 2008. Molecular characterization of multi-drug resistant *Shigella* spp isolated from epidemic and endemic cases of shigellosis. J. Med. Microbiol. (Accepted for publicaion).

Title: Virulence-associated genes and pulsed-field gel electrophoresis of Enteroaggregative *Escherichia coli* strains in Thailand

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Summary: 300~500 words:

Enteroaggregative *Escherichia coli* (EAEC) have been reported as causative agents of persistent diarrhea in infants and young children in developing countries, outbreaks of food borne illness in industrialized countries. A growing number of reports and the rising proportion of diarrheal cases in which EAEC are implicated suggest that EAEC are important emerging agents of pediatric diarrhea. In this study, a total of 3,668 *E. coli* isolates obtained from patients with diarrheic symptoms in Thailand during years 2006-2007 were identified for EAEC by HeLa cell adherence assay. EAEC strains were then characterized for virulence associated genes of EAEC, including aggregative adherence fimbriae (AAF) types I (*aggA*), II (*aaf*A), and III (*agg3*A) genes, EAEC heat stable enterotoxin (EAST1) genes (*ast*A) and Plasmid encoded enterotoxin (Pet) genes (*pet*) by a multiplex PCR. EAEC were also tested for O-serogroups with 43 commercial available antisera by slide agglutination test. In addition, EAEC strains collected from outbreak of food-borne disease during year 2006-2007 were characterized for genetic relationships by *xba*I digested PFGE patterns.

Of the 3,668 *E. coli* isolates, 466 isolates (12.7%) were identified as EAEC. Of the 466 EAEC strains, 74 (15.8 %) and 392 (84.2%) strains were AAF genes positive and negative, respectively. About 128 (27.5%) strains of EAEC were *astA* positive, and 27 (5.8%) strains were *pet* positive. The AAF and enterotoxin genes negative strains which accounted for 59% of EAEC were found the most frequent. About 33% of EAEC strains agglutinated with 23 O-serogroups which are the most predominant in O15 (6%), O6 (5.4%), O44 (2.4%), and O86a (1.9%). IN 2006, an outbreak of food-borne illness related with EAEC happened in a military camp. EAEC O6:Huntyble, AAF genes negative, but both *astA* and *pet* genes positive were isolated from 12 out of 30 patients, and 7 food handlers. *Xba*I-digested PFGE patterns of EAEC strains

isolated from patients and food handlers showed identical PFGE pattern. These result indicated that EAEC strains in Thailand are heterogeneous with regard to their virulence factor profile. The expression of aggregative adherence (AA) phenotype can be mediated by as-yet-unidentified, alternative adherence factor(s). Toxins or factors other than EAST1 and Pet may play a role in pathogenesis of EAEC.

Purpose:

EAEC are defined as *E. coli* strains that do not secrete heat-labile (LT) and heat-stable (ST) enterotoxins, and adhere to HEp-2 or HeLa cells in an aggregative adherence (AA) pattern. Since Nataro *et al* [1] first described EAEC as an agent of pediatric diarrhea in 1987, a growing number of studies have supported the association of EAEC with persistent diarrhea in infants and young children in developing countries. Recent outbreaks of EAEC as a cause of food borne illness in industrialized countries have been reported. In 1993, a massive outbreak of EAEC diarrhea occurred in the Gifu Prefecture, Japan, in which 2,697 children at 16 schools became ill after consuming contaminated school lunches [2]. Four outbreaks of EAEC-related diarrhea occurred in the United Kingdom in 1994 [1]. A growing number of reports and the rising proportion of diarrheal cases in which EAEC are implicated suggest that EAEC are important emerging agents of pediatric diarrhea. The pathogenesis of EAEC infection is not well understood. Histopathologic examination of tissues from infected patients and animal models have shown that EAEC strains enhance mucus secretion from the mucosa, with bacteria trapped in a bacteria-mucus biofilm. Human volunteers who ingested EAEC strains excrete mucoid stools. The role of excess mucus production in EAEC is unknown. Three types of aggregative adherence fimbriae types I (AAF/I), II (AAF/II), III (AAF/III) have been shown to be required for expression of an AA pattern on the surfaces of HEp-2 and HeLa cells [3,4,5]. In addition, a variety of other virulence factors have been described, including the heat-stable enterotoxin (EAST1) [6] , and the plasmid-encoded toxin (Pet) [7]. The role of EAST1 and Pet in mucus secretion has not yet been determined.

The study of prevalence of childhood diarrhea-associated *E. coli* in Thailand, during 1996 – 2000 showed that EAEC was the pathotype most frequently isolated in Thai children in every year, indicating the important of EAEC in Thai children [8]. Little is known about the

pathogenesis of EAEC in Thailand. Therefore, analysis of virulence factors of EAEC is required to better understand their role in diarrhea disease that may lead to the improvement of laboratory diagnosis, prevention and control of EAEC infections.

This study has been desired to investigate the virulence associated genes of EAEC isolated from patients with diarrhea by multiplex PCR.

Methods:

1.1. Bacterial strains

E. coli isolates obtained from patients with acute diarrhea who attended hospitals across Thailand during years 2006-2007 were included in this study. *E. coli* isolates were confirmed by standard methods[9].

1.2 HeLa Cells adherence assay

E. coli isolates were identified for EAEC by HeLa cell-adherence assay as described by Nataro *et al* [10]. HeLa cells were grown to 70-80% confluence in DMEM with 10 % fetal bovine serum and antibacterial-antimycotics (100 U of penicillin, 100 μg of streptomycin, and 0.25 μg/ml of amphotericin B in 0.85% saline) on circular cover slips of 13 mm diameter in 24-well tissue culture plates at 37°C in 5% CO_2. Cells were washed twice with 500 μl of phosphate-buffered saline (PBS), and 500 μl of fresh DMEM with 1% alpha-D-mannoside were added into each well. Ten microlitre of overnight-grown *E. coli* in LB-broth ($2x10^6$ cells) were inoculated into the wells. After 3 h incubation, the wells were washed thoroughly three times with PBS to remove non-adherent bacteria and air-dried completely. The cover slips in the wells were fixed with 100% methanol for 10 min, stained with 10% Giemsa stain for 30 min, and then air-dried completely. The cover slips were mounted on glass slides and examined under a light microscope. Each assay was done in duplicate. The adherence pattern was observed under light microscope. Three distinct adherence patterns were identified according to the descriptions of Nataro. Agregative adherence pattern (AA) was characterized by the appearance of stacked-brick clumps of bacteria on the surface of HeLa cells as well as on the cover slip. Localized adherence pattern (LA) was characterized by the formation of microcolonies on the cell surface but rarely on the cover slip. Diffuse adherence pattern (DA), the surface of cells were covered with individual bacteria. Non-adherence, only few bacteria adhered to the cells. *E. coli*

isolates that adhered to HeLa cells in an AA pattern were identified as EAEC.

1.3 *E. coli* isolates were also ruled out of ETEC, EIEC, STEC, and EPEC by multiplex PCR as described by Fumiaki *el al* [11].

1.4 Determination of virulence-associated genes of EAEC by PCR.

Bacterial cell suspension (10^5 to 10^6 organisms per ml), which prepared by suspending colonies on overnight-grown LB agar plate in 100 μl of sterile distilled water, were boiled for 20 min and spin down to obtain template DNA in the supernatant. The PCR assay was carried out in 0.2 ml microcentrifuge tubes with 25 μl of reaction mixture consisting of PCR buffer, dNTPs, primers specific for genes encoding AAF/I (*agg*A), AAF/II (*aaf*A), AAF/III (*agg3*A), EAST1 (*ast*A) and Pet (*pet*), *Taq* DNA Polymerase, and template DNA. The reaction mixtures were run in a thermal cycler (model 9700, Perkin-Elmer Corp). The amplified DNA products were resolved by agarose gel electrophoresis at a constant voltage of 100 until the front dye had migrated approximately two-thirds of the length of the gel. The gel was stained with ethidium bromide (0.5 μg/ml) for 15 min, then rinsed twice with water for 10 min each. DNA fragments were visualized under UV light at 320 nm and photographed.

1.5 Pulsed-field gel electrophoresis

DNA fingerprint of EAEC obtained from outbreak of food-borne diseases was performed by using the one day (24-28 h) standardized protocol for pulsed-field gel electrophoresis of *E. coli* O157:H7. PFGEs were run on CHEF-DR III and then PFGE patterns were analyzed by Syngene Gene Directory Application version 1.02.0.

Results:

A total of 3,668 *E. coli* isolates obtained from 1,487 patients during the year 2006 and 2007 were examined in this study as shown in table1. EAEC strains were identified by HeLa cells adherence assay. The isolation rates of EAEC were about 14% and 11% in year 2006 and 2007 (Figure1). The mean isolation rate was about 12.3%. The mean isolation rate per year of EPEC and ETEC were about 3.2 and 1.2 %, respectively, but STEC and EIEC were very rare.

EAEC strains were then determined for the presence of genes encoding AAF/I (*agg*A), AAF/II (*aaf*A), AAF/III (*agg3*A), EAEC heat stable enterotoxin (EAST1, *ast*A) and Plasmid encoded enterotoxin (Pet, *pet*) by a multiplex PCR. About 12%, 2.3%, and 0.8% of EAEC were

aggA, *aafA*, and *agg3A* positive, respectively; whereas, 84% of EAEC were AAF genes negative. Regarding the prevalence of enterotoxin genes, *astA* and *pet* genes were found in about 22%, and 1 % of EAEC, 4.7% of EAEC were both *astA* and *pet* genes positive. Taken together, about 59% of EAEC were both aggregative adherence and enterotoxin genes negative. Data was shown in Table 2. No single virulence marker was observed among EAEC.

About 33% of EAEC strains agglutinated with 23 O-serogroups which are the most predominant in O15 (6%), O6 (5.4%), O44 (2.4%), O86a (1.9%), and other 19 O serogroups (13%) as shown in Table 3. About 66.8% of EAEC were O-untypable.

During this study, an outbreak of EAEC happened in a military camp in year 2006. EAEC serotype O6:Huntyble were isolated from 12 out of 30 patients, and 7 food handlers. They were AAF genes negative, but both *astA* and *pet* genes positive. The *Xba*I digested PFGE patterns of EAEC strains isolated from patients and food handlers showed identical PFGE pattern as shown in Figure 2.

Discussion:

In this study, EAEC strains were investigated to determine the carriage of genes that encode for virulence-associated factors, such as *aggA* (AAF/I) and *aafA* (AAF/II), *agg3A* (AAF/III), *astA* (EAST1) and *pet* (Pet), by multiplex PCR. It was found that 15.1% were AAF genes positive. The majority of EAEC (~85%) were AAF genes negative. In addition, *astA* and *pet* were found in about 22% and 1% of EAEC strains, and 4.7% of EAEC were both *astA* and *pet* genes positive. Taken together, the majority (59%) of EAEC from this study were both AAF and enterotoxin genes negative.

These results were somewhat different from other studies. Bouzari *et al.* [12] had shown by hybridization that among 98 EAEC strains from Iranian children, 8% were positive with AAF/I probe, 25% with AAF/II probe, and 5% with AAF/I and AAF/II probes. Ellis *et al.* [13] detected AAF/I and AAF/II in 19% and 8% of 115 EAEC strains associated with childhood diarrhea in Sao Paulo, Brazil. In contrast, Okeke *et al.* [14] detected AAF/I and AAF/II genes in 63% and 35% of 131 EAEC strains isolated from children in southwestern Nigeria. Czeczulin *et al.* [15] found that 32% of EAEC strains from various epidemiologic studies hybridized with AAF/I probe, and 18% hybridized with AAF/II probe. In addition,Vila *et al.* [16] showed that AAF/I and AAF/II were detected, respectively, in 0% and 8.7% of 23

diarrhea-associated EAEC strains from Spanish travelers to developing countries. Thus, the prevalence of AAF in EAEC appears to vary substantially as it ranges from 0 to 47.2% for AAF/I and 8 to 35% for AAF/II. That ~85% of EAEC in this study were negative for *aggA*, *aafA*, and *agg3A*, is in agreement with those reports of Vila [16] and Ellis [13] that the majority of EAEC strains were negative to AAF/I and AAF/II probes. Therefore, these results strongly suggest the presence of alternative adherence factor(s) in the expression of AA phenotype.

The role of EAST1 (*astA*) and Pet (*pet*) in intestinal secretion and enterotoxicity has not yet been determined in humans. In this study, *astA* and *pet* were found in about 22% and 1% of EAEC strains. *astA* was found in 38% of EAEC strains associated with diarrhoea in Iranian children [12]. Savarino et al. [6] demonstrated that *astA* was found in approximately 40% of EAEC and in the similar proportion of non-pathogenic *E. coli* strains. Other *E. coli* categories, most notably EHEC, elaborate EAST1 with a higher frequency than EAEC [6]. Vila et al. [16] demonstrated that *astA* was detected in EAEC, diffuse adherent *E. coli*, and non diarrhoeagenic *E. coli* with more frequently in strains from the control group than from the case group. Thus, the potential of EAST1 to cause the disease is controversial. It is possibly that unless additional virulence factor(s) is present, EAST1 alone is insufficient to cause diarrhea.

In an outbreak of EAEC diarrhoea in Mexico [17], Pet was identified in sera from patients, suggesting its potential role in causing disease. Isolation rate of *pet* was variable geographically. Therefore, significant questions persist in understanding of EAEC epidemiology and pathogenesis. It has led some investigators to doubt the pathogenicity of EAEC.

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Table 1. *E. coli* isolates obtained from patients during year 2006-2007.

Year	No. of *E. coli* isolates	No. of patients
2006	2,009	770
2007	1,659	717
Total	3,668	1,487

Table 2. Aggregative adherence fimbriae genes and enterotoxin genes of EAEC strains as determined by PCR.

Aggregative fimbriae genes			Enterotoxin genes		No.(%)
*agg*A	*aaf*A	*agg3*A	*ast*A	*pet*	of strains
+	-	-	-	-	53(11.4)
+	-	-	+	-	6 (1.3)
-	+	-	+	-	2 (0.4)
-	+	-	+	+	9 (1.9)
-	-	+	+	-	2 (0.4)
-	-	+	-	+	2 (0.4)
-	-	-	+	-	97 (20.8)
-	-	-	+	+	13 (2.8)
-	-	-	-	+	3 (0.6)
-	-	-	-	-	279 (59.9)
59(12.7)	11(2.3)	4(0.8)	129(27.6)	27(5.7)	466(100)

Table 3. Distribution of O serogroups among 465 EAEC strains.

O-serogroups	No. of strains	%
O15	28	6.0
O6	25	5.4
O44	11	2.4
O86a	9	1.9
O153	8	1.7
O126	7	1.5
O25	6	1.3
O152	6	1.3
O18	5	1.1
O1	5	1.1
O78	5	1.1
Other		
12		
O-serogroups	18	3.9
Rough	21	4.5
Un-typable	311	66.8
Total	465	100