

Prevalence of childhood diarrhoea-associated *Escherichia coli* in Thailand

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Escherichia coli isolates ($n = 2629$) were collected between 1996 and 2000 from 2100 Thai children less than 12 years of age with acute diarrhoea. Enterotoxigenic (ETEC), enteroinvasive (EIEC), Shiga-toxin-producing (STEC), enteropathogenic (EPEC) and enteroaggregative (EAEC) *E. coli* were identified by their virulence marker profiles, as determined by multiplex PCR, and HeLa cell-adherence patterns. Serogroups of isolates were determined using 43 monovalent O antisera. Of 2629 isolates, 16.9% were identified as diarrhoeagenic *E. coli*, and the mean isolation rates per year were 10.2% for EAEC (range 8–12.5%), 3.2% for EPEC (0–8%), 3.0% for ETEC (2–5.4%), 0.5% for EIEC (0–1%) and 0.04% for STEC (0–0.1%). The isolation rates of pathotypes from four different age groups (0–5 months, 6–11 months, 1–2 years and 2–12 years) in 905 children whose ages were recorded were respectively 19.3, 18.2, 9.1 and 8.1% for EAEC, 3.1, 4.3, 1.7 and 2.2% for EPEC and 2.6, 2.3, 1.3 and 5% for ETEC. About 38% of diarrhoeagenic *E. coli*, including 55.1, 66.7, 100, 45.9 and 29%, respectively, of ETEC, EIEC, STEC, EPEC and EAEC, and 24% of non-diarrhoeagenic *E. coli* were O-antigen typable. Only four serogroups (9.3%) were restricted to single pathotypes, whereas 27 serogroups (62.8%) were not restricted to any pathotype. This study shows that EAEC are the most prevalent diarrhoea-associated pathotype in Thai children.

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INTRODUCTION

Diarrhoea caused by *Escherichia coli* infection is one of the major health problems for children in many developing countries and travellers to those countries (Adachi *et al.*, 2001; Ogata *et al.*, 2002; Robins-Browne & Hartland, 2002). Diarrhoeagenic *E. coli* are recognized as five major pathotypes: enterotoxigenic (ETEC), enteropathogenic (EPEC), enteroinvasive (EIEC), Shiga-like toxin-producing (STEC) or enterohaemorrhagic (EHEC) and enteroaggregative (EAEC) *E. coli* (Nataro & Kaper, 1998). Diffuse-adherent *E. coli* (DAEC), also known as diarrhoea-associated haemolytic *E. coli* (DHEC), and cytolethal distending toxin-producing *E. coli* (CDT-EC) have also been described recently as diarrhoeagenic (Clarke, 2001; Nataro & Kaper, 1998). Their epidemiology and diarrhoeagenic potential are, however, not yet clear (Scaletsky *et al.*, 2002).

Standard methods currently used in the identification of major *E. coli* pathotypes are based on distinct sets of virulence markers, such as toxins [heat-labile and heat-stable enterotoxins LTh and STh or STp (ETEC) (Kuhnert *et al.*, 2000) and Shiga-like toxins SLT1 and SLT2 (STEC) (Nataro & Kaper, 1998)], adhesins [intimin (Cravioto *et al.*, 1996) and EPEC adherence factor (EAF) (Donnenberg *et al.*, 1997) (EPEC)] and invasins (EIEC) (Robins-Browne, 1987), and their cell-adherence characteristics (Clarke, 2001; Nataro & Kaper, 1998). None of these methods is available for use in routine clinical laboratories in developing countries such as Thailand. Although surveillance of diarrhoeagenic *E. coli* using standard identification methods had been carried out sporadically in Thailand in the past until 1986 (Chatkaeomorakot *et al.*, 1987; Sunthadvannich *et al.*, 1990), these surveillance studies were not designed to detect all major pathotypes.

In this study, we have categorized *E. coli* isolates collected over a 5-year period (1996–2000) from Thai children with acute diarrhoea by examining their virulence markers and

Abbreviations: AA, aggregative adherence; EAEC, enteroaggregative *E. coli*; EAF, EPEC adherence factor; EIEC, enteroinvasive *E. coli*; EPEC, enteropathogenic *E. coli*; ETEC, enterotoxigenic *E. coli*; LA, localized adherence; STEC, Shiga-toxin-producing *E. coli*.

cell-adherence patterns. The isolates were further examined for their O antigens. The prevalence of diarrhoeagenic *E. coli* pathotypes, with their age distribution and the isolation rate per year, is presented.

METHODS

Clinical samples and bacterial strains. Rectal swabs (one per patient) were collected from 2100 Thai children less than 12 years of age with acute diarrhoea who attended 15 different hospitals across Thailand between 1 January 1996 and 31 December 2000.

Rectal swab samples collected in Cary-Blair transport medium were inoculated directly onto MacConkey agar, sorbitol MacConkey agar, thiosulfate/citrate/bile salt/sucrose agar, *Salmonella*-*Shigella* agar, xylose lysine desoxycholate agar, selenite broth and alkaline peptone water for culture overnight at 37 °C. Sorbitol non-fermenting colonies were again tested with O157:H7 antisera. One to three colonies of each sorbitol non-fermenting, lactose-fermenting and lactose non-fermenting isolate with typical *E. coli* morphology were initially selected and examined further biochemically following Edwards' and Ewing's identification methods (Ewing, 1986). *E. coli* isolates that had been biochemically confirmed at the hospitals concerned were submitted to our institute (National Institute of Health, Thailand), where they were stored on Dorset egg yolk agar (Nissui Pharmaceutical Inc.) until used. Samples derived from mixed infections with salmonellae, shigellae and vibrios were excluded from our study. A total of 2629 isolates thus obtained as single pathogens were included in this study.

E. coli strains 1298 (*invE*⁺), EDL931 (*stx1/2*⁺), 682 (*eltIA*⁺), 825 (*stIA*⁺, STp), 1296 (*stIA*⁺, STh) and 1228 (*eaeA*⁺, *bfpA*⁺, EAF⁺), which were kindly provided by the National Institute of Public Health, Tokyo,

Japan, were used as positive control strains and JM109 was used as a negative control strain for virulence markers. *E. coli* strains 28-3, 1228 and JM109 were respectively used as controls for aggregative adherence (AA), localized adherence (LA) and non-adherence on HeLa cells.

Identification of virulence markers by multiplex PCR. *E. coli* isolates were subjected to two PCR assays using two multiplex primer sets (Table 1). The first set was used to identify ETEC, EIEC and STEC (Itoh *et al.*, 1992), and the second set was used to detect EPEC (Franke *et al.*, 1994; Sueyoshi *et al.*, 1996). Boiled lysates from overnight-grown bacterial colonies on LB plates were used as PCR templates. PCR assays were carried out in 25 µl reaction mixtures consisting of 1 µl template DNA, 20 mM Tris/HCl, pH 8.4, 50 mM KCl, 1.5 mM MgCl₂, 0.25 mM dNTPs (New England Biolabs), 0.1 µM of each primer (0.2 µM for *stIA* primers) and 1 U *Taq* DNA polymerase (Gibco). The reaction mixtures were run in a thermal cycler (model 9700; Perkin-Elmer) with the following cycling profile: 94 °C for 5 min, 25 cycles of denaturation at 94 °C for 1 min, annealing at 48 °C for 1.5 min and primer extension at 72 °C for 2 min and a final extension at 72 °C for 5 min. The annealing temperature for EPEC PCR was 50 °C. Amplified products were resolved by 2% agarose gel electrophoresis and visualized under UV transillumination after ethidium bromide staining. DNA templates from positive and negative control strains for virulence markers and a minus-template sample were included in each PCR.

HeLa cell-adherence assay. Cell-adherence patterns of *E. coli* isolates were examined using monolayers of HeLa cells (ATCC CCL-2) as described by Nataro *et al.* (1987). HeLa cells were grown to 70–80% confluence on circular coverslips (13 mm diameter) in 24-well tissue culture plates (Nalge Nunc) with DMEM supplemented with 10% fetal bovine serum in a 37 °C incubator with 5% CO₂. After washing three times with PBS, fresh DMEM containing 1% methyl α-D-mannoside

Table 1. Multiplex PCR primer sets used to identify recognized virulence markers of ETEC, EIEC, STEC and EPEC

LTh, Heat-labile enterotoxin; STh/STp, heat-stable enterotoxin (human/pig alleles); SLT1/2, Shiga-like toxins 1 and 2; BFP, bundle-forming pili. Primers for *stIA* can detect both STh and STp.

Virulence gene (factors)	Sequence (5'–3')	PCR product (bp)	Reference
Primer set 1			
ETEC			
<i>eltIA</i> (LTh)	(F) AGCAGGTTTCCCACCGGATCACCA (R) CGTGCTCAGATTCTGGGTCTC	132	Itoh <i>et al.</i> (1992)
<i>stIA</i> (STh/STp)	(F) ATTTCTGTATTGTCTTT (R) ATTACAACACAGTTCACAG	171	Itoh <i>et al.</i> (1992)
EIEC			
<i>invE</i> (regulator for cell invasion)	(F) ATATCTCTATTTCCAATCGCGT (R) GATGGCGAGAAATTATATCCCG	382	Itoh <i>et al.</i> (1992)
STEC			
<i>stx1/2</i> (SLT1/2)	(F) TTTACGATAGACTTCTCGAC (R) CACATATAAATTATTTGCTC	228	Itoh <i>et al.</i> (1992)
Primer set 2			
EPEC			
<i>eaeA</i> (intimin)	(F) GCTTAGTGCTGGTTTAGGAT (R) TCGCCGTTTACAGAGATCGC	488	Sueyoshi <i>et al.</i> (1996)
<i>bfpA</i> (BFP)	(F) GAAGTAATGAGCGCAACGTC (R) ACATGCCGCTTTATCCAACC	234	Sueyoshi <i>et al.</i> (1996)
EAF (EPEC adherence factor)	(F) CAGGGTAAAAGAAAGATGATAA (R) TATGGGGACCATGTATTATCA	397	Franke <i>et al.</i> (1994)

was added to the wells to inhibit type 1 fimbriae-mediated cell adherence. Cells were infected with bacteria (approx. 2×10^6 cells) that had been grown statically in LB broth at 37 °C for 18 h, to give an m.o.i. of 1 : 20. After 3 h incubation, cells were washed three times with PBS, fixed with 100 % methanol for 10 min and stained with 10 % Giemsa stain for 30 min. Cell-adherence patterns were determined using a light microscope (Nikon) following the criteria described by Nataro *et al.* (1987). Each assay was done in duplicate using positive and negative control strains.

Determination of diarrhoea-associated *E. coli* pathotypes. *E. coli* isolates were categorized using the following criteria: isolates positive for *eltIA*, *stIA* or both as ETEC; isolates positive for *invE* as EIEC; isolates positive for *stx1/2*, *eaeA* or both as STEC; isolates negative for *stx1/2* and positive for *eaeA* as EPEC; EPEC with LA pattern on HeLa cells as typical EPEC; EPEC with non-LA pattern on HeLa cells as atypical EPEC; isolates negative for all tested virulence markers but positive for AA pattern on HeLa cells as EAEC; and isolates negative for both tested virulence markers and AA pattern on HeLa cells as non-diarrhoeagenic *E. coli*. In this study, we used the AA phenotype as the marker for EAEC, because the known virulence-associated genes of EAEC, which have been widely used as genotypic markers in identifying EAEC, are also present in non-EAEC strains (Elias *et al.*, 2002).

Serogrouping. O antigen determination was done by slide agglutination test using a heated suspension (100 °C for 1 h) of bacterial cells and eight polyvalent and 43 monovalent O antisera (Denka Seiken Co.) that are targeted against common O-serogroups of EPEC, ETEC, EIEC and STEC. The following 43 serogroups were determined: O1, O6, O8, O15, O18, O20, O25, O26, O27, O28ac, O29, O44, O55, O63, O78, O86a, O111, O112ac, O114, O115, O119, O124, O125, O126, O127a, O128, O136, O142, O143, O144, O146, O148, O151, O152, O153, O157, O158, O159, O164, O166, O167, O168 and O169. Results were confirmed by the test-tube agglutination test (Ewing, 1986).

Statistical analysis. The χ^2 and Fisher's exact tests were used to report the significance of differences between variables.

RESULTS AND DISCUSSION

E. coli isolates ($n = 2629$) were initially examined for their virulence markers by two multiplex PCR assays. Strains negative for all tested virulence markers ($n = 2453$) were further examined for their HeLa cell-adherence patterns. Additionally, 85 EPEC strains were included in the HeLa cell-adherence assay to differentiate typical EPEC from atypical EPEC. We did not investigate DAEC or CDT-EC in this study as their role in diarrhoea is still not clear (Scaletsky *et al.*, 2002).

Prevalence of diarrhoea-associated *E. coli*

Tables 2 and 3 show the characteristics and isolation frequency of diarrhoea-associated *E. coli* during the 5-year study period. The mean isolation rates per year were 16.9 % (range 13.3–22.6 %) for total diarrhoeagenic *E. coli*, 10.2 % (8–12.5 %) for EAEC, 3.2 % (0–8 %) for EPEC, 3.0 % (2–5.4 %) for ETEC, 0.5 % (0–1 %) for EIEC and 0.04 % (0–0.1 %) for STEC. EAEC, EPEC, ETEC, EIEC and STEC respectively accounted for 60.4 % (269/445), 19.1 % (85/445), 17.5 % (78/445), 2.7 % (12/445) and 0.2 % (1/445) of diarrhoeagenic *E. coli*.

The relatively high prevalence of EAEC in this study (10.2 %) and in previous studies from Calcutta (9%; Dutta *et al.*, 1999), northern India (12.3 % in acute diarrhoea and 34.5 % in persistent diarrhoea; Bhan *et al.*, 1989) and southern Israel

Table 2. Determination of pathotypes among 2629 childhood diarrhoea-associated *E. coli* isolates on the basis of virulence marker profiles and HeLa cell-adherence patterns

Virulence markers were examined by multiplex PCR.

Pathotype	<i>n</i> (%)	Virulence marker profile							<i>n</i>
		<i>eltIA</i>	<i>stIA</i>	<i>invE</i>	<i>stx1/2</i>	<i>eaeA</i>	<i>bfpA</i>	EAFC	
ETEC	78 (3)	–	+	–	–	–	–	–	42
		+	–	–	–	–	–	–	30
		+	+	–	–	–	–	–	6
EIEC	12 (0.5)	–	–	+	–	–	–	–	12
STEC	1 (0.04)	–	–	–	+	+	–	–	1
EPEC	85 (3.2)	–	–	–	–	+	–	–	61*
		–	–	–	–	+	+	–	12†
		–	–	–	–	+	–	+	7†
		–	–	–	–	+	+	+	5†
EAEC	269 (10.2)	–	–	–	–	–	–	–	269‡
Non-diarrhoeagenic	2184 (83.1)	–	–	–	–	–	–	–	2184§

*Non-adherent to HeLa cells; classified as atypical EPEC.

†LA pattern on HeLa cells; classified as typical EPEC.

‡Negative for virulence markers but positive for AA phenotype in HeLa cell-adherence assay.

§Negative for both virulence markers and AA phenotype in HeLa cell-adherence assay.

Table 3. Prevalence of childhood diarrhoea-associated *E. coli* in Thailand, 1996–2000

Category	Number of isolates (%)					
	Total	1996	1997	1998	1999	2000
EAEC	269 (10.2)	29 (8)	55 (8.1)	26 (12.5)	30 (12.4)	129 (11.3)
EPEC	85 (3.2)	9 (2.5)	23 (3.4)	17 (8)	0 (0)	36 (3.1)
ETEC	78 (3.0)	9 (2.5)	20 (3)	4 (2)	13 (5.4)	32 (2.8)
EIEC	12 (0.5)	1 (0.3)	7 (1)	0 (0)	0 (0)	4 (0.3)
STEC	1 (0.04)	0 (0)	1 (0.1)	0 (0)	0 (0)	0 (0)
Diarrhoeagenic	445 (16.9)	48 (13.3)	106 (15.7)	47 (22.6)	43 (17.8)	201 (17.6)
Non-diarrhoeagenic	2184 (83.1)	313 (86.7)	570 (84.3)	161 (77.4)	198 (82.2)	942 (82.4)
Total	2629	361	676	208	241	1143

(25.9%; Porat *et al.*, 1998) is consistent with reports that EAEC is the pathotype responsible for persistent diarrhoea among international travellers who visit developing countries (Adachi *et al.*, 2001; Jiang *et al.*, 2002; Vargas *et al.*, 1998).

By the definition adopted at the Second International Symposium on EPEC in Sao Paulo in 1995, typical EPEC possess the bundle-forming pili (BFP)-producing EAF plasmid with the LA pattern on cultured cells, whereas atypical EPEC lack this plasmid and the LA pattern (Nataro & Kaper, 1998; Trabulsi *et al.*, 2002). We used the LA pattern on HeLa cells as the only marker for classifying typical EPEC, because the methods commonly used to detect the BFP-producing EAF plasmid, such as hybridization with EAF and *bfpA* probes (Nataro & Kaper, 1998) and PCR amplification of the EAF region and *bfpA* gene (Franke *et al.*, 1994), can be misleading, as some LA-expressing EPEC strains, which are positive for either EAF or *bfpA*, may not carry the true BFP-producing EAF plasmid (Nataro & Kaper, 1998; Trabulsi *et al.*, 2002). Accordingly, 71.8% (61/85) of our EPEC strains were non-adherent to HeLa cells and therefore classified as atypical EPEC, whereas the remaining 24 EPEC strains exhibited the classical LA pattern on HeLa cells (Table 2). In addition, we also observed that 50% (12/24) of LA-positive EPEC strains were positive for *eaeA* and *bfpA* but negative for EAF and that 29% (7/24) of LA-positive EPEC strains were positive for *eaeA* and EAF but negative for *bfpA*. Only 20.8% (5/24) were positive for *eaeA*, *bfpA* and EAF. Some atypical EPEC strains have been shown to express the intimin-mediated LA-like pattern (Pelayo *et al.*, 1999; Trabulsi *et al.*, 2002), but all our atypical EPEC strains were non-adherent. Although geographical specificities may exist, the high presentation of atypical EPEC in this study and in previous reports from Brazil (Gomes *et al.*, 1989) and other industrialized countries (Nataro & Kaper, 1998) underscores the emergence of atypical EPEC strains worldwide.

Among ETEC isolates, 53.8% (42/78) and 38.5% (30/78) were respectively positive for *stIA* and *eltIA* and 7.7% (6/78) were positive for both *eltIA* and *stIA*. Taken together, 61.5% (48/78) of ETEC were *stIA* positive. ETEC are responsible for

20–40% of diarrhoeal cases amongst travellers and ST-producing ETEC, in particular, are known to be associated with the majority of endemic cases (Nataro & Kaper, 1998). Although the occurrence of ETEC in Thai children had been rather steady during the last 5 years, most strains (61.5%) in this study were *stIA* positive, indicating that there is a persistent risk of ETEC-associated outbreak in Thailand. On the other hand, similar to reports from south-western Nigeria (Okeke *et al.*, 2000), southern Israel (Porat *et al.*, 1998) and Bangladesh (Albert *et al.*, 1995), EIEC and STEC seem to be only minor diarrhoeagenic pathogens for children in Thailand, since their occurrence was negligible among diarrhoea-associated *E. coli* in three studies, including this one, from Thailand (Chatkaemorakot *et al.*, 1987; Sunthadvanich *et al.*, 1990).

In two previous studies from Thailand in 1985 and 1986 (Chatkaemorakot *et al.*, 1987; Sunthadvanich *et al.*, 1990), ETEC (6 and 7%, respectively), EIEC (< 1 and 2%), STEC (0 and 0%) and EAF-positive EPEC (4 and 6%), as determined by probe hybridization, colony hybridization and HeLa adherence-assay, were recovered from 393 children in 16 district hospitals (Sunthadvanich *et al.*, 1990) and 278 children in the Children's Hospital in Bangkok (Chatkaemorakot *et al.*, 1987). Compared with these data, the prevalence of ETEC (3%) and EAF-positive EPEC (0.5%) in this study was significantly lower ($P < 0.002$ and $P < 0.0001$, respectively). Nonetheless, the overall prevalence of major diarrhoeagenic *E. coli* pathotypes in Thailand did not change much during the 5-year study period.

Age distribution of diarrhoea-associated *E. coli*

The prevalence of diarrhoeagenic *E. coli* in four different age groups among 905 children whose ages were recorded was 25% (48/192) in those aged 0–5 months, 24.8% (64/258) in those aged 6–11 months, 12.1% (28/232) in those aged 1–2 years and 16.1% (36/223) in those aged 2–12 years (Fig. 1). The isolation rate of diarrhoeagenic *E. coli* was significantly higher in children less than 1 year of age, compared with children older than 1 year ($P < 0.0001$). The prevalence of EAEC was 19.3% (37/192) in 0–5 months, 18.2% (47/258)

Table 4. Distribution of O serogroups in *E. coli* isolates of this study

Values in parentheses are percentages.

Serogroup	Diarrhoeagenic (<i>n</i> = 445)					Non-diarrhoeagenic
	ETEC	EIEC	STEC	EPEC	EAEC	
O1	—	—	—	—	—	70
O6*	10	—	—	2	1	73
O8*	2	—	1	—	3	34
O15*	1	—	—	—	16	38
O18*	—	—	—	1	1	16
O20*	1	—	—	—	—	—
O25*	7	—	—	—	11	15
O26	—	—	—	3	—	18
O27	1	—	—	—	1	—
O28ac	—	2	—	—	—	3
O44	—	—	—	—	4	18
O55	—	—	—	10	—	10
O63	—	—	—	1	—	1
O78	1	—	—	—	—	1
O86a*	3	—	—	1	12	59
O111	—	—	—	1	—	10
O112ac	—	—	—	—	—	3
O114	—	—	—	2	—	9
O115	—	—	—	—	—	1
O119*	—	—	—	12	1	10
O124†	—	1	—	—	—	—
O125	—	—	—	1	—	15
O126*	4	—	—	—	15	28
O127a*	2	—	—	1	4	6
O128*	2	—	—	1	—	17
O142	—	—	—	—	—	2
O144	—	—	—	—	—	1
O146*	1	—	—	—	2	11
O148	—	—	—	—	—	2
O152†	—	1	—	—	—	—
O153	—	—	—	2	1	22
O158	—	—	—	—	1	8
O159*	2	—	—	—	1	1
O164†	—	4	—	—	—	—
O166*	—	—	—	1	1	18
O167	—	—	—	—	—	1
O168	—	—	—	—	2	1
O169	6	—	—	—	1	—
Typable	43 (55.1)	8 (66.7)	1 (100)	39 (45.9)	78 (29)	522 (23.9)
Non-typable	35 (44.9)	4 (33.3)	0 (0)	46 (54.1)	191 (71)	1662 (76.1)
Total	78	12	1	85	269	2184

*Identified in three or more categories.

†Identified exclusively in a single pathotype.

in 6–11 months, 9.1 % (21/232) in 1–2 years and 8.1 % (18/223) in 2–12 years. EAEC were significantly more common in children less than 1 year old, compared with the older children ($P < 0.0001$). EPEC were isolated from 3.1 % (6/

192), 4.3 % (11/258), 1.7 % (4/232) and 2.2 % (5/223), respectively, of children in age groups 0–5 months, 6–11 months, 1–2 years and 2–12 years, whereas ETEC were isolated from 2.6 % (5/192), 2.3 % (6/258), 1.3 % (3/232) and

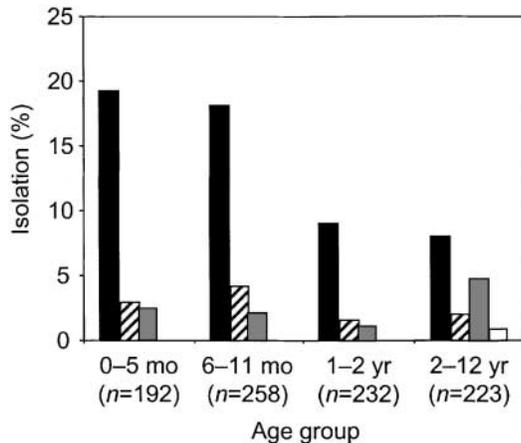


Fig. 1. Isolation frequencies of EAEC (filled bars), EPEC (hatched bars), ETEC (shaded bars) and EIEC (open bars) among diarrhoea-associated *E. coli* from 905 Thai children with diarrhoea. mo, Months; yr, years.

5% (11/223) of children in the corresponding age groups. ETEC were more common in children older than 2 years, compared with the younger children ($P < 0.05$). We did not find any significant difference in the age distribution of EPEC. EIEC were isolated from only two children in age group 2–12 years. STEC were not identified in children of known age.

Serogroups

With the 43 monovalent antisera used in this study, 38% (169/445) of diarrhoeagenic *E. coli* and 23.9% (522/2184) of non-diarrhoeagenic *E. coli* were typable for their O antigens. In addition, 55.1% (43/78), 66.7% (8/12), 100% (1/1), 45.9% (39/85) and 29% (78/269) of ETEC, EIEC, STEC, EPEC and EAEC, respectively, were O-antigen typable and they were distributed respectively into 14, 4, 1, 14 and 18 different serogroups. O antigen-typable non-diarrhoeagenic *E. coli* were distributed into 32 serogroups. Twenty-seven serogroups were observed in more than one category, accounting for 62.8% of total serogroups tested: 13 serogroups (O6, O8, O15, O18, O25, 86a, O119, O126, O127a, O128, O146, O159 and O166) were identified in three or more different categories, including non-diarrhoeagenic *E. coli*. There were only four serogroups that were exclusively associated with a single pathotype: O20 (ETEC), O124, O152 and O164 (all EIEC).

That about 71% of EAEC, 54% of EPEC, 45% of ETEC and 33% of EIEC strains were non-typable while 24% of non-diarrhoeagenic *E. coli* strains were typable indicates that a significant number of isolates would have been misidentified by serogroup-based diagnosis. Besides, although only about 9% of serogroups were identified exclusively in single

pathotypes, more than 60% of serogroups tested were not restricted to any pathotype, signifying the unrestricted nature of serogroups among different pathotypes.

Taken together, EAEC were the most common pathotype in every age group examined and in every year during the 5-year study period. Inasmuch as this is the first study to report EAEC isolates from Thailand, further characterization of these strains is required in order to understand better their role in diarrhoeal diseases in Thailand. The findings in this study will be of great importance in implementing management guidelines for *E. coli*-associated diarrhoea in Thailand. Given that Thailand is one of the popular tourist destinations in Asia, hosting 7–8 million international travellers every year, the results presented here would also have a significant impact on the management of *E. coli*-associated acute and persistent diarrhoea among travellers.

In conclusion, this study highlights that O antigen examination alone is of little value in epidemiological studies related to diarrhoeagenic *E. coli* and that the comprehensive surveillance of diarrhoeagenic *E. coli* in developing countries remains an important part of preventative and control measures in reducing the overall incidence of diarrhoeal diseases around the world.

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