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Serotypes of Enterotoxigenic *Escherichia coli* in Thailand and the Philippines

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The serotypes of 386 enterotoxigenic *Escherichia coli* (ETEC) isolated from 82 individuals with and without diarrhea in Thailand and the Philippines were determined. The 136 strains producing both heat-labile toxin (LT) and heat-stable toxin (ST) belonged to 12 different O serogroups; however, 83% (113/136) were of one of four serogroups (O6, O8, O25, and O78), and 76% (104/136) belonged to one of seven O:K:H serotypes. Only 14% (28/196) of LT-only-producing ETEC belonged to serogroups most common among LT and ST strains, and these 196 strains belonged to 35 different O:K:H serotypes. Three O serogroups (O20, O27, and O78) accounted for 94% (51/54) of strains producing only ST. Although only 4% (2/54) of ST-only ETEC belonged to the seven serotypes most commonly found among strains which produced LT and ST, 85% of ETEC belonged to three other serotypes, O20:K?:H21, O27:K?:H7, and O78:H-. A total of 46% (37/80) of ETEC of serotypes O6:H16, O8:H9, O25:H42, and O78:H12 were resistant to two or more antibiotics in comparison to 68% (208/306) of ETEC of other serotypes ($P < 0.001$). In Thailand and the Philippines, *E. coli* which produced LT and ST or ST alone, but not those which produced LT alone, were restricted in their O:K:H serotypes.

Enterotoxigenic *Escherichia coli* (ETEC) is an important cause of diarrhea in tropical, developing countries. In Bangladesh and India, these organisms are frequently isolated from patients with severe watery diarrhea similar to cholera (20, 21, 23). These enteric pathogens have also been isolated from children and adults with milder diarrhea (2–4, 8, 11, 12) as well as asymptomatic individuals (4–6). The ability of ETEC to produce either a heat-labile toxin (LT), a heat-stable toxin (ST), or both is plasmid mediated and therefore theoretically transferable between different *E. coli* (10, 25).

Orskov et al. (18) first showed that ETEC from different geographical areas belonged to a relatively small number of serotypes. In Dacca, Bangladesh, 86% of 69 ETEC which produced LT and ST belonged to one of four O serogroups, and 81% belonged to one of six O:K:H serotypes (13). Thirty-four ETEC which produced ST, however, belonged to 15 different O serogroups. Following this observation, Merson et al. (14) used pools of antisera against O serogroups to identify ETEC in 618 patients with acute diarrhea with more than 5% dehydration in Dacca. A total of 66% of the ETEC and 9% of the non-ETEC agglutinated in these antiserum pools. A total of 95% of LT^+ST^+ (*E. coli* producing LT and ST) but only 42% of LT^-ST^+ (*E. coli* producing ST only) strains belonged to O serogroups included in these pools. In a comparison with standard enterotoxin assays, identification of ETEC by agglutination had a sensitivity of 64%, a specificity of 96%, and a predictive accuracy of 89%.

To determine whether observations made in Bangladesh were similar to those in Thailand and the Philippines, serotypes of ETEC isolated from individuals with and without diarrhea were examined. Furthermore, the relationship between antibiotic resistance, serotype, and toxin production among ETEC was reviewed.

MATERIALS AND METHODS

Source of specimens. *E. coli* were isolated from children with diarrhea, the same children 3 weeks later (at which time none were infected with ETEC of the same serotype as that during their episode of diarrhea), and age- and sex-matched controls in Bangkok (11). Five non-ETEC strains from children with diarrhea or controls without gastrointestinal symptoms were selected randomly for serotyping. ETEC from American Peace Corps volunteers were isolated from individuals with or without diarrhea (2). Non-ETEC

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TABLE 1. Source of ETEC and non-ETEC serotypes

Source of ETEC	No. of ETEC isolates producing:			No. of non-ETEC isolates	No. of individuals infected	Mean no. of isolates/individual
	LT-ST	LT	ST			
Thailand						
Children's Hospital, Bangkok	73				12	6
		82			19	4
			49		8	6
				122	25[a]	5
American Peace Corps volunteers	54				7	8
		49			12	4
			5		4	1
				75	15[b]	5
Philippines[c]						
Hostesses and restaurant workers, Angeles City	9				2	4
		65			21	3
			NT[d]		NT	NT

[a] Eleven children with diarrhea, fourteen children without diarrhea.
[b] Seven volunteers with diarrhea, eight without diarrhea.
[c] *E. coli* isolated in the Philippines was tested for LT-ST and LT but not ST alone.
[d] NT, Not tested.

strains (five/individual) were also selected at random from volunteers with or without diarrhea. ETEC were also isolated either from hostesses or restaurant workers without gastrointestinal symptoms in Angeles City (6). The sources of ETEC serotyped in this study are summarized in Table 1.

Processing of specimens. Stool or rectal swabs were plated on MacConkey agar and incubated overnight at 37°C. Ten lactose-positive colonies with the typical appearance of *E. coli* were selected from each plate and stored on nutrient agar slants. Isolates were inoculated into 1 ml of Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) with 0.6% yeast extract (Difco Laboratories, Detroit, Mich.) within 2 months of isolation. After 24 h of incubation, 0.2 ml of this culture was inoculated into a tube (15 by 100 mm) containing 5 ml of the same medium and incubated on a rotating tissue culture apparatus at 8 rpm for 24 h at 37°C. The initial stationary culture was incubated for an additional 24 h (48 h total) and tested without centrifugation for LT by the Y-1 adrenal cell assay (22). The rotating culture was centrifuged at 2,500 rpm for 15 min, and the supernatant was tested for ST by the infant suckling mouse assay (15). All enterotoxigenic organisms were identified as *E. coli* (7), and tested for resistance to the following antibiotics: ampicillin, chloramphenicol, gentamicin, kanamycin, neomycin, streptomycin, sulfonamides, sulfamethoxalzole trimethoprim, and tetracycline by the Kirby-Bauer method (1). ETEC and non-ETEC were tested for O, K, and H antigens as previously described (16, 17, 24).

RESULTS

Serotypes of ETEC. A total of 136 *E. coli* producing LT and ST, 196 producing LT only, and 54 producing ST only were serotyped. The O serogroups of these ETEC are shown in Table 2. The 136 LT^+ST^+ *E. coli* examined belonged to 12 O groups, and 113 (83%) were of one of four serogroups (O6, O8, O25, or O78). The 196 LT^+ST^- *E. coli* (*E. coli* producing LT only) examined belonged to 35 different O groups and only 14% (28/196) belonged to the four O serogroups which were most common among LT^+ST^+ *E. coli*. Three O serogroups (O20, O27, and O78) accounted for 51 of 54 (94%) LT^-ST^+ *E. coli* examined. Certain O serogroups were isolated more frequently from one population than another. ETEC serogroup O78 was isolated from nine children only in Bangkok; O79 was from two individuals only in the Philippines; and O85 and O148 were from two and three American Peace Corps volunteers, respectively, only in Thailand.

A total of 76% (104/136) of LT^+ST^+ *E. coli* belonged to seven different serotypes (O6:K-:H5, O6:K15:H16, O8:K25:H9, O8:K40:H9, O25:K?:H42, O78:H sp. aggl. [spontaneous agglutination], and O78:H12). A total of 47% (64/136) of LT^+ST^+ *E. coli* belonged to four of seven special serotypes which have been previously associated with ETEC in diverse geographical locations (O6:H16, O8:H9, O25:H42, and O78:H12) (18). Among LT^+ST^+ *E. coli* O8:H9, 16 had K antigen 25, whereas 7 had K antigen 40. Of 17 LT^+ST^+ *E. coli* O25:H42, 14 had K antigens which could not be identified, and 3 had K antigen 7. Only 7% (14/196) of LT^+ST^- *E. coli* (10, O6:K15:H16; 3, O8:K25:H9; and 1, O25:K7:H42) and 4% (2/54) of LT^-ST^+ *E. coli* (O78:H12) belonged to these special serotypes (Table 3). The O, K, and H

TABLE 2. O serogroup of 386 ETEC isolated in Thailand and the Philippines

Serogroup	No. of isolates producing enterotoxin types:		
	LT-ST	LT only	ST only
O1		1	
O2		5	
O4		1 (3)[a]	
O6	26 (1)	12	
O8	16 (7)	4 (5)	
O9		3 (3)	
O12			1
O15		1	
O18	2[b]	4	
O20	2		31
O21		(4)	1
O23		1	
O25	20	5 (1)	
O27		1	10
O39	1	1	
O51		1	
O58			1
O59		1	
O70		(1)	
O75		(2)	
O77	3	14[c] (25)[d]	
O78	43		10
O79		(6)	
O80		3 (2)	
O82	1	2	
O85		12	
O86	1		
O88		3	
O109		(1)	
O110		(1)	
O114		13 (1)	
O115		3	
O119		4	
O127	1		
O140		(1)	
O146		1	
O148		14	
O154	1	2	
OX2		3 (1)	
OX3		(1)	
OX8		1	
Untypable	10 (1)	15 (7)	

[a] Includes three O4,O133:K?:H LT^+ST^- isolates.
[b] Includes two O18ac,O77,O106:K?:H8 LT^+ST^+ isolates.
[c] Includes one O77,O106 LT^+ST^- isolate.
[d] Includes 12 O77,O106:K13:H45 LT^+ST^- isolates; number of isolates shown within parentheses were from the Philippines.

antigens of ETEC isolated from children in Bangkok, from Peace Corps volunteers in rural Thailand, and hostesses and restaurant workers in Angeles City, the Philippines, were not at all similar (Table 3). Only one serotype, O6:K-:H51 which produced LT and ST was found in all three populations. *E. coli* O6:K15:H16, which produced LT and ST, was found in both Peace Corps volunteers in rural Thailand and children in Bangkok. A total of 16 other different serotypes were found among 32 LT^+ST^+ *E. coli*, 53 were found among 182 LT-only *E. coli*, and 9 were found among 52 ST-only *E. coli*.

Eight individuals were infected with ETEC which produced LT and ST and LT or ST alone. One Peace Corps volunteer with diarrhea was infected with five *E. coli* of special serotype O6:K15:H16, which produced LT and ST and a sixth serotype, O12:K?:H-, which produced ST alone. A child with diarrhea in Bangkok was simultaneously infected with three LT^+ST^+ *E. coli* of special serotype O25:K7:H42, one LT^+ST^- *E. coli* O25:K7:H42, and five other LT^+ST^- *E. coli* O2:H26 or O2:H-. The other six individuals were infected with a variety of *E. coli* serotypes which produced LT and ST and either toxin alone.

Serotypes of non-ETEC. When serotypes of ETEC and non-ETEC were compared in the same population serotypes of isolates which produced toxin were almost always different from isolates which were nontoxigenic (Table 3). There were, however, a few exceptions. Among children in Bangkok, one LT^+ST^- *E. coli*, O1:K?:H45, isolated from a child with diarrhea, shared the same O and H but not K antigens with four nontoxigenic strains (O1:K95:H45) isolated from another child without diarrhea. Among Peace Corps volunteers in rural Thailand, single LT^-ST^+ *E. coli* O21:K?:H4 and O58:K-:H40 were isolated from two different volunteers without diarrhea. Five non-ETEC O21:K1:H4 and four O58:K-:H40 were isolated from two other volunteers without diarrhea. None of the seven special serotypes found by Orskov et al. (18) to be common among ETEC was present in non-ETEC in Thailand.

Antibiotic resistance of ETEC. Of ETEC special serotypes O6:H16, O8:H9, O25:H42, and O78:H12, 46% (37/80) were resistant to two or more antibiotics in comparison to 68% (208/306) of ETEC of other serotypes ($P < 0.001$). Four patients were infected with ETEC of special serotypes and ETEC of at least one other serotype. The serotypes included in the special group were usually resistant to less antibiotics than ETEC of other serotypes which produced the same toxin (Table 4).

DISCUSSION

Orskov et al. (18) and Rowe et al. (19) previously recognized that ETEC were often of a limited number of serotypes. Our experience in Thailand and the Philippines agrees with the observations of Merson et al. (13, 14) in Bangladesh that *E. coli* which produce LT and ST are restricted to a limited number of serovars. In

TABLE 3. Serotypes of ETEC and non-ETEC isolated in Thailand and the Philippines

Serotype[a]	PCV[b]	PI[c]	B[d]
ETEC			
LT-ST			
O6:K-:H51	2	1	7
O6:K15:H16	12	0	5
O8:K25:H9	16	0	0
O8:K40:H9	0	7	0
O18ac,O77,O106:K?:H18	2	0	0
O20:K?:H-	2	0	0
O25:K?:H42	14	0	0
O25:K?:H-	3	0	0
O25:K7:H42	0	0	3
O39:K?:H9	1	0	0
O77,O106:H18	0	0	2
O77,O106:K?:H?	0	0	1
O78:H sp. aggl.	0	0	33
O78:H12	0	0	7
O78,O110:H sp. aggl.	0	0	3
O86:K1:H-	1	0	0
O154:K?:H8	1	0	0
O only			
O77,O106	0	0	1
Untypable	0	1	10
LT			
O1:K-:H45	0	0	1
O2:H-	0	0	1
O2:H26	0	0	4
O4,O7:K?:H-	1	0	0
O6:H51	0	0	1
O6:K15:H16	0	0	10
O6:K?:H1	1	0	0
O8:K9:H11	0	0	4
O8:K25:H9	0	3	0
O8:K?:H-	0	1	0
O8:K?:H sp. aggl.	0	1	0
O9:K28:H-	0	0	3
O9:K?:H10	0	1	0
O9:K?:H-	0	2	0
O15:K2:H18	1	0	0
O18abOX8:K:H15	0	0	1
O18ac:K-:H16	0	0	2
O18ac:K1:H-	0	0	1
O21:K-:H5	0	3	0
O21:K?:H-	0	1	0
O23:K?:H16	0	0	1
O25:K?:H-	2	0	0
O25:K-:H10	0	1	0
O25:K7:H42	0	0	1
O25 (O18ac):K?:H-	2	0	0
O27:K-:H20	1	0	0
O39:K?:H9	1	0	0
O51:K12:H52	0	0	1
O59:K?:H19	0	0	1
O70:K?:H-	0	1	0
O77:K?:H18	0	0	12
O77:K13:H45	0	13	0
O77,O106:K13:H45	0	12	0
O79:K-:H10	0	6	0
O80:H19	0	0	3
O80:K-:H40	0	2	0
O82:H8	0	0	2
O85:K?:H5 or H sp. aggl.	12	0	0
O88:H25	0	0	3
(O109):K?:H21	0	1	0
O110:K?:H28	0	1	0
O114:H-	0	0	12
O114:H16	0	0	1
O115:H49	0	1	0
O115:K-:H28	3	0	0
(O119):H-	0	0	3
O119:H6	0	0	1
O140:H4	0	1	0
O146:H10	0	0	1
O148:K-:H8	14	0	0
O154:K-:H8	2	0	0
O4,O133:K?:H-	0	3	0
O only			
O75:K?:H?	0	2	0
O77	1	0	
O77,O106	0	0	1
Untypable	4	7	11
ST			
O12:K?:H-	1	0	0
O20:K?:H21	0	0	30
O20:H7	0	0	1
O21:K?:H4	1	0	0
O27:K?:H7	0	0	8
O27:K-:H20	2	0	0
O58:K-:H40	1	0	0
O78:H-	0	0	8
O78:H12	0	0	2
Non-ETEC			
O1:K95:H45	0		4
O1:K?:H4	0		5
O4:K12:H40	0		5
O4:K-:H40	0		1
O6:K?:H-	0		5
O7:K16:H rough	5		0
O8:K47:H10	5		0
O8:K?:H1,12	0		4
O8:K?:H25	0		1
O8:K?:H sp. aggl.	0		5
O9:K?:H-	0		2
O9:K35:H-	0		1
O10:K-:H43	1		0
O11:K4:H-	0		2
O15:K54:H33	5		5
O15:K15:H18	5		2
O15:K16:H18	0		1
O15:K?:H-	0		2
O18ac:K1:H-	0		2
O19,O133:K?:H-	0		1
O20:K?:H?	0		7
O21:K1:H4	5		0
O25:K-H-	0		2
O29:K-:H-	0		1
O39:K-:H16	0		5
O39:K?:H9	0		2
(O40):K-:H-	0		1
O44:K74:H18	3		0
O46:K-:H18	4		0
O51:K12:H52	1		0
O51:K-:H36	0		1
O55:K?:H-	0		5
O58:K-:H40	4		0

TABLE 3—*Continued*

Serotype[a]	PCV[b]	PI[c]	B[d]
O60:K-:H rough			
O64:K?:H-	0		1
O74:K-:H28	1		0
O75:K?:H5	0		1
O77,O106:K-:H38	5		0
O79,O120:K-:H7	1		0
O88:K-:H-	0		3
O89:K-:H45	0		1
O95:K?:H10	2		0
O100:K?:H19	0		1
O101:K?:H-	0		2
O103:K-:H-	0		5
O107:K-:H1	0		1
O111:K4:H-	0		2
O114:K-:H4	4		0
O115:K?:H-	0		1
O116:K-:H33	0		1
O128:K-:H-	0		1
O140:K-:H42	1		0
O142:K-:H6	0		5
O155:K-:H-	2		0
O163:K-:H19	0		1
OX8:K2:H15	0		1
Untypable	20		23

[a] Special serotypes are shown in boldface type.
[b] PCV, American Peace Corps volunteers in Thailand.
[c] PI, Hostesses and restaurant workers in Angeles City, the Philippines.
[d] B, Children at Children's Hospital, Bangkok, Thailand.

Thailand and the Philippines, 76% of LT^+ST^+ *E. coli* belonged to one of seven different serotypes (O6:K-:H5, O6:K15:H16, O8:K25:H9, O8:K40:H9, O25:K?:H42, O78:H sp. aggl., and O78:H12), whereas in Dacca, 81% belonged to one of six serotypes (O6:K15:H16, O8:K40:H9, O78:K-:H11, O78:K-:H12, O115:K-:H40, and O115:K-:H51). ETEC strains producing LT only were not limited to certain serotypes either in the Philippines, Thailand, or in Bangladesh (11). Serotypes O20:K?:H21, O27:K?:H7, and O78:H- accounted for 85% of ST-only-producing ETEC in Thailand.

The grouping of special serotypes among LT^+ST^+ in comparison to LT-only or ST-only *E. coli* as well as the isolation of LT^+ST^+ *E. coli* of special serotypes and other serotypes of *E. coli* which produce either LT or ST alone from the same patient suggested that *E. coli* belonging to special serotypes are better able to accept or retain the plasmids coding for LT and ST than other serotypes.

Although we did not examine the stability of ETEC which produced LT and ST, LT alone, and ST alone after storage systematically, LT-only ETEC were often negative for toxin production after either shipment to Copenhagen or storage in Bangkok. The instability of LT-only ETEC which were of diverse serotypes agrees with Evan's observation (9) and further suggests that enterotoxin coding for LT is unstable in *E. coli* which are not of special serotypes.

The prevalence of antibiotic resistance among ETEC appears to vary geographically. Orskov et al. (18) found that ETEC strains from a variety of sources were usually sensitive to multiple antibiotics. In Thailand, however, 83% of ETEC are resistant to more than one antibiotic, and resistance is usually R factor mediated (11). It is unknown whether the regional differences in antibiotic resistance among ETEC reflect the use of antibiotics or compatability between plasmids coding for enterotoxins and R factors. ETEC of special serotypes were less resistant to multiple antibiotics than other serotypes of *E. coli*; however, geographical differ-

TABLE 4. Antibiotic resistance of ETEC of different serotypes infecting four individuals

Source	No. of ETEC	Toxin produced	Serotype[a]	Antibiotic resistance[b]
PCV with travelers' diarrhea	8	LT-ST	**O8:K25:H9**	Tc
	1	LT-ST	**O8:K25:H9**	Ap Cm Km Nm Sm Su Tc
	1	LT-ST	**O20:K?:H-**	Ap Cm Km Nm Sm Su Tc
PCV with travelers diarrhea[c]	10	LT-ST	O25:K?:H42	Multiple sensitive
	1	LT-ST	O20:K?:H-	Ap Cm Sm Su Tc
	1	LT-ST	O39:K?:H9	Ap Cm Sm Su Tc
Child with diarrhea in Bangkok	7	LT-ST	**O78:H12**	Cm Sm Su Tc
	1	LT-ST	O127:H46	Ap Cm Km Nm Sm Su Tc
Hostess without diarrhea in the Philippines	1	LT	**O8:K25:H9**	Sm Su
	2	LT	O75:K?:H?	Sm Su Tc

[a] Special serotypes are shown in boldface.
[b] Ap, ampicillin; Cm, chloramphenicol; Km, kanamycin; Nm, neomycin; Sm, streptomycin; Su, sulfonamides; Tc, tetracycline.
[c] Cultured on 2 successive days; PCV, Peace Corps volunteer.

ences in the prevalence of antibiotic resistance among ETEC do not appear to be due to regional differences in serovars.

Usage of the pools of antisera employed in Dacca (14) would probably be nearly as effective in identifying *E. coli* which produce LT and ST in Thailand and the Philippines as in Bangladesh. It is unlikely, however, that this technique would identify *E. coli* which produced LT only. Plasmids coding for LT or ST or both can be carried by a variety of different serotypes of *E. coli*, and serological techniques would not be effective in defining either the epidemiology of these enteric pathogens or in identifying all *E. coli* which carry plasmids coding for enterotoxins.

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