

SEROTYPES OF ENTEROTOXIGENIC *Escherichia coli*
IN THAILAND AND THE PHILIPPINES

Principal Investigators : Peter Echeverria, LTC, MC
Frits Orskov, MD*
Ida Orskov, MD*
Duangratana Plianbangchang

OBJECTIVES : To determine the serotypes of enterotoxigenic *E. coli* in Thailand and the Philippines and compare the serotypes of these pathogens in Southeast Asia with other locations.

BACKGROUND : Enterotoxigenic *Escherichia coli* (ETEC) are 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 (18, 20, 21). These enteric pathogens have also been isolated from children and adults with milder diarrhea (3, 4, 6, 8, 10) as well as asymptomatic individuals (2, 4, 5). 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 transferrable between different *E. coli* (9, 23).

Orkov et al (16) first appreciated that ETEC from different geographical areas belonged to a relatively small number of serotypes. In Dacca, Bangladesh 86 percent 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 (11). Thirty-four ETEC which produced ST, however, belonged to 15 different O serogroups. Following this observation Merson et al (12) used pools of antisera against O serogroups to identify ETEC in 618 patients with acute diarrhea with greater than five percent dehydration in Dacca. Sixty-six percent of the ETEC and nine percent of the non-ETEC agglutinated in these antisera pools. Ninety-five percent 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, identifying ETEC by agglutination had a sensitivity of 64 percent, a specificity of 96 percent, and a predictive accuracy of 89 percent.

To determine whether observations made in Bangladesh were similar 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.

METHODS :

Source of specimens : *E. coli* were isolated either from children with diarrhea, the same children three weeks later (at which time none were

* WHO Collaborative Centre for Reference and Research on Escherichia, Statens Seruminstitut, Copenhagen, Denmark

infected with ETEC of the same serotype as during their episode of diarrhea), or age and sex matched controls in Bangkok. Five non-ETEC 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 (6). Non-ETEC (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 (5). The source 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, Cockeysville, MD) with 0.6% yeast extract (Difco, Detroit, MI) within two months of isolation. After 24 hours incubation 0.2 ml of this culture was inoculated into a 15x100 mm tube containing 5 mls of the same media and incubated on a rotating tissue culture apparatus at eight revolutions per minute for 24 hours at 37°C. The initial stationary culture was incubated for an additional 24 hours (48 hours total) and tested without centrifugation for LT by the Y-1 adrenal cell assay (19). The rotating culture was centrifuged at 2500 revolutions per minute for 15 minutes and the supernatant tested for ST by the infant suckling mouse assay (13). All enterotoxigenic organisms were identified as *E. coli* (7), and tested for resistance to the following antibiotics: ampicillin, chloramphenicol, gentamicin, kanamycin, neomycin, streptomycin, sulfonamides, sulfamethoxazole trimethoprim, and tetracycline by the Kirby Bauer method (1). ETEC and non-ETEC were tested for O, K, and H antigens as previously described (14, 15, 22).

RESULTS :

Serotypes of ETEC : One hundred and thirty-six *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 (06, 08, 025, or 078). The 196 LT+ST- *E. coli* (*E. coli* producing LT only) examined belonged to 35 different O groups and only 14 percent (28/196) belonged to the four O serogroups which were most common among LT+ST+ *E. coli*. Three O serogroups (020, 027, and 078) 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 078 were isolated from nine children only in Bangkok, 079 from two individuals only in the Philippines and 085 and 0148 from two and three American Peace Corps volunteers respectively only in Thailand.

Seventy-six percent (104/136) of LT+ST+ *E. coli* belonged to seven different serotypes (06:K-:H5, 06:K15:H16, 08:K25:H9, 08:K40:H9, 025:K?:H42, 078:H sp.aggl., and 078:H12). Forty-seven percent (64/136) of LT+ST+ *E. coli* belonged to four of seven "special" serotypes which have previously been associated with ETEC in diverse geographical locations (06:H16, 08:H9, 025:H42, and 078:H12) (16). Among LT+ST+ *E. coli* 08:H9, 16 had K antigen.

25, while seven had K antigen 40. Of 17 LT+ST+ *E. coli* 025:H42, 14 had K antigens which could not be identified and three had K antigen seven. Only seven percent (14/196) of LT+ST- *E. coli* (10, 06:K15:H16; 3, 08:K25:H9; and 1, 025:K7:H42) and four percent (2/54) of LT-ST+ *E. coli* (2, 078:H12) belonged to these "special" serotypes, Table 3. The O, K, H 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 all similar (Table 3). Only one serotype, 06:K-;H51 which produced LT and ST was found in all three populations. *E. coli* 06:K15:H16 which produced LT and ST were found in both Peace Corps volunteers in rural Thailand and children in Bangkok. Sixteen other different serotypes were found among 32 LT+ST+, 53 among 182 LT only and nine among 52 ST only *E. coli*.

One hundred and eight ETEC isolated from 20 different Peace Corps volunteers (or during distinct episodes of diarrhea) comprised 22 distinct serotypes. Thirty-two different serotypes of 204 ETEC infected 39 children in Bangkok, while 20 different serotypes of 74 ETEC infected 23 hostesses and restaurant workers in the Philippines. 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 non-toxicogenic. There were, however, a few exceptions. Among children in Bangkok one LT+ST- *E. coli* 01:K-H45, isolated from a child with diarrhea, shared the same O and H but not K antigens with four non-toxicogenic strains (01:K95:H45) isolated from another child without diarrhea. Among Peace Corps volunteers in rural Thailand single LT-ST+ *E. coli* 021:K?:H4 and 058:K-:H40 were isolated from two different volunteers without diarrhea. Five non-ETEC 021:K1:H4 and four 058:K-:H40 were isolated from two other volunteers without diarrhea. None of the seven "special" serotypes found by Orskov et al (16) to be common among ETEC were present in non-ETEC in Thailand.

Eight individuals were infected with colonies of ETEC which produced LT and ST and others which made either LT or ST alone. One Peace Corps volunteer with diarrhea was infected with five colonies of *E. coli* of "special" serotype 06:K15:H16 which produced LT and ST and a sixth colony 012:K?:H- which produced ST alone. A child with diarrhea in Bangkok was simultaneously infected with three LT+ST+ *E. coli* of "special" serotype 025:K7:H42, one LT+ST- *E. coli* 025:K7:H42, and five other LT+ST- *E. coli* 02:H26 or 02:H-. The other six individuals were infected with a variety of *E. coli* serotypes which produced both LT and ST or either toxin alone.

Serotypes of ETEC isolated from individuals with diarrhea were compared to strains recovered from others without gastrointestinal symptoms in Thailand. Serotypes previously found to be common among ETEC (06:H16, 08:H9, 025:H42, and 078:H12) were found as frequently among ETEC isolated from children with or without diarrhea (23/150 vs 5/54) ($p > 0.3$). Three of 18 children with diarrhea from whom ETEC were isolated were infected with these "special" serotypes as compared to one of 17 children without diarrhea ($p > 0.95$). Among Peace Corps volunteers ETEC of these "special" serotypes which all produced LT and ST were more common among volunteers with than without diarrhea (35/78 vs 7/30 ($P < 0.025$)). ETEC of these serotypes were isolated from four of 13 volunteers with diarrhea infected with ETEC compared to only

one of eight volunteers without diarrhea infected with ETEC ($p > 0.90$). LT+ST+ *E. coli* 025:K-:H42 recovered from the volunteer without diarrhea was isolated five days after he had travelers' diarrhea during which time an LT+ST+ *E. coli* of the same serotype was isolated.

Forty-six percent (37/80) of ETEC of "special" serotypes 06:H16, 08:H9, 025:H42, and 078:H12 were resistant to two or more antibiotics in comparison to 68 percent (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.

This study is complete.

Table 1. Source of enterotoxigenic and non-enterotoxigenic *Escherichia coli* serotyped

Source of ETEC	# of ETEC and non-EEC isolates			# of indi- vidual infected	Mean # isolates/ individual
	LT-ST	LT	ST non-ETEC		
<u>Thailand</u>					
Children's Hospital, Bangkok	73	-	-	12	6
	-	82	-	19	4
	-	-	49	8	6
	-	-	122	25 ⁺	5
American Peace Corps volunteers	54	-	-	7	8
	-	49	-	12	4
	-	-	5	4 ^A	1
	-	-	75	15 ^A	5
<u>Philippines*</u>					
Hostesses and restaurant workers, Angeles City	9	-	-	2	4
	-	65	-	21	3
	-	-	NT	NT	NT
TOTAL	136	196	54		

LT-ST = *E. coli* produced both heat-labile and heat-stable toxin

LT = *E. coli* produced only heat-labile toxin

ST = *E. coli* produced only heat-stable toxin

* *E. coli* isolated in the Philippines tested for LT-ST and LT but not ST alone + 11 children with diarrhea, 14 children without diarrhea

Δ 7 volunteers with diarrhea, 8 without diarrhea

Table 2. O serogroup of 386 enterotoxigenic *Escherichia coli* isolated in Thailand and the Philippines

O serogroup	No. of isolates producing enterotoxin types		
	LT-ST	LT only	ST only
01		1	
02		5	
04		1 (3) ^a	
06	26 (1)	12	
08	16 (7)	4 (5)	
09		3 (3)	
012			1
015		1	
018	2 ^b	4	
020	2		31
021		(4)	1
023		1	
025	20	5 (1)	
027		1	10
039	1	1	
051		1	
058			1
059		1	
070		(1)	
075		(2)	
077	3	14 ^c (25) ^d	
078	43		10
079		(6)	
080		3 (2)	
082	1	2	
085		12	
086	1		
088		3	
0109		(1)	
0110		(1)	
0114		13 (1)	
0115		3	
0119		4	
0127	1		
0140		(1)	
0146		1	
0148		14	
0154	1	2	
OX2		3 (1)	
OX3		(1)	
OX8		1	
Untypable	10 (1)	15 (7)	

a = includes three 04, 0133:K?:H LT+ST- isolates; b = includes two 018ac, 077, 0106:K?LH8 LT+ST+ isolates; c = includes one 077, 0106 LT+ST- isolate; d = includes 12 077, 0106:K13:H45 LT+ST- isolates; () = isolates from the Philippines.

Table 3. Serotype of enterotoxigenic *Escherichia coli* isolated in Thailand and the Philippines

<u>LT-ST</u>	<u>PCV*</u>	<u>PI**</u>	<u>B***</u>	<u>Total</u>
1. 06:K-:H51	2	1	7	10
2. <u>06:K15:H16</u>	12	0	5	17
3. <u>08:K25:H9</u>	16	0	0	16
4. <u>08:K40:H9</u>	0	7	0	7
5. 018ac, 077, 0106:K?:H18	2	0	0	2
6. 020:K?:H-	2	0	0	2
7. <u>025:K?:H42</u>	14	0	0	14
8. 025:K?:H-	3	0	0	3
9. <u>025:K7:H42</u>	0	0	3	3
10. 039:K?:H9	1	0	0	1
11. 077, 0106:H18	0	0	2	2
12. 077, 0106:K?:H?	0	0	1	1
13. 078:H sp. aggl.	0	0	33	33
14. <u>078:H12</u>	0	0	7	7
15. 078, 0110:H sp. aggl.	0	0	3	3
16. 086:K1:H-	1	0	0	1
17. 0154:K?:H8	1	0	0	1
<u>0 only</u>				
18. 077, 0106	0	0	1	1
Untypable	0	1	10	11
Total	54	9	73	136

<u>LT</u>	<u>PCV</u>	<u>PI</u>	<u>B</u>	<u>Total</u>
1. 01:K-:H45	0	0	1	1
2. 02:H-	0	0	1	1
3. 02:H26	0	0	4	4
4. 04, 07:K?:H-	1	0	0	1
5. 06:H51	0	0	1	1
6. <u>06:K15:H16</u>	0	0	10	10
7. 06:K?:H1	1	0	0	1
8. 08:K9:H11	0	0	4	4
9. <u>08:K25:H9</u>	0	3	0	3
10. 08:K?:H-	0	1	0	1
11. 08:K?:H sp. aggl.	0	1	0	1
12. 09:K28:H-	0	0	3	3
13. 09:K?:H10	0	1	0	1
14. 09:K?:H-	0	2	0	2
15. 015:K2:H18	1	0	0	1
16. 018abOX8:K:H15	0	0	1	1
17. 018ac:K-:H16	0	0	2	2
18. 018ac:K1:H-	0	0	1	1
19. 021:K-:H5	0	3	0	3
20. 021:K?:H-	0	1	0	1
21. 023:K?:H16	0	0	1	1
22. 025:K?:H-	2	0	0	2
23. 025:K-:H10	0	1	0	1
24. <u>025:K7:H42</u>	0	0	1	1
25. 025 (018ac):K?:H-	2	0	0	2
26. 027:K-:H20	1	0	0	1
27. 039:K?:H9	1	0	0	1
28. 051:K12:H52	0	0	1	1
29. 059:K?:H19	0	0	1	1
30. 070:K?:H-	0	1	0	1
31. 077:K?:H18	0	0	12	12
32. 077:K13:H45	0	13	0	13
33. 077, 0106:K13:H45	0	12	0	12

<u>LT</u>	<u>PCV</u>	<u>PI</u>	<u>B</u>	<u>Total</u>
34. 079:K-:H10	0	6	0	6
35. 080:H19	0	0	3	3
36. 080:K-:H40	0	2	0	2
37. 082:H8	0	0	2	2
38. 085:K?:H5 or H sp. aggl.	12	0	0	12
39. 088:H25	0	0	3	3
40. (0109):K?:H21	0	1	0	1
41. 0110:K?:H28	0	1	0	1
42. 0114:H-	0	0	12	12
43. 0114:H16	0	0	1	1
44. 0115:H49	0	1	0	1
45. 0115:K-:H28	3	0	0	3
46. (0119):H-	0	0	3	3
47. 0119:H6	0	0	1	1
48. 0140:H4	0	1	0	1
49. 0146:H10	0	0	1	1
50. 0148:K-:H8	14	0	0	14
51. 0154:K-:H8	2	0	0	2
52. 04, 0133:K?:H-	0	3	0	3
<u>0 only</u>				
53. 075:K?:H?	0	2	0	2
54. 077	1	0	0	1
55. 077, 0106	0	0	1	1
untypable	4	7	11	22

<u>ST</u>	<u>PCV</u>	<u>PI</u>	<u>B</u>	<u>Total</u>
1. 012:K?:H-	1	0	0	1
2. 020:K?:H21	0	0	30	30
3. 020:H7	0	0	1	1
4. 021:K?:H4	1	0	0	1
5. 027:K?:H7	0	0	8	8
6. 027:K--:H20	2	0	0	2
7. 058:K--:H40	1	0	0	1
8. 078:H-	0	0	8	8
9. <u>078:H12</u>	0	0	2	2

"special serotypes" underlined

PCV*= American Peace Corps volunteers in Thailand

PI**= hostesses and restaurant workers in Angeles City, The Philippines

B***= children at Children's Hospital, Bangkok, Thailand

Table 4. Antibiotic resistance of enterotoxigenic *Escherichia coli* of different serotypes infecting four individuals.

<u>Source</u>	<u># of ETEC</u>	<u>Toxin produced</u>	<u>Serotype</u>	<u>Antibiotic resistance</u>
1. PCV with travelers' diarrhea	8	LTST	<u>08:K25:H9</u>	Tc
	1	LTST	<u>08:K25:H9</u>	Ap Cm Km Nm Sm Su Tc
	1	LTST	<u>020:K?:H-</u>	Ap Cm Km Nm Sm Su Tc
2. PCV with travelers' diarrhea*	10	LTST	<u>025:K?:H42</u>	MS
	1	LTST	<u>020:K?:H-</u>	Ap Cm Sm Su Tc
	1	LTST	<u>039:K?:H9</u>	Ap Cm Sm Su Tc
3. Child with diarrhea in Bangkok	7	LTST	<u>078:H12</u>	Cm Sm Su Tc
	1	LTST	<u>0127:H46</u>	Ap Cm Km Nm Sm Su Tc
4. Hostess without diarrhea in the Philippines	1	LT	<u>08:K25:H9</u>	Sm Su
	2	LT	<u>075:K?:H?</u>	Sm Su Tc

* cultured on two successive days

"special serotypes" underlined

PCV = American Peace Corps volunteer

LT = *E. coli* heat labile enterotoxin

ST = *E. coli* heat stable enterotoxin

Ap, ampicillin; Cm, chloramphenicol; Km, kanamycin; Nm, neomycin; Sm, streptomycin;

Su, sulfonamides; Tc, tetracycline

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