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Colonization Factors Associated with Enterotoxigenic *Escherichia coli* Isolated in Thailand

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Eighty-six percent (72 of 84) of heat-labile and heat-stable, none of 141 heat-labile, and 24% (27 of 111) heat-stable enterotoxigenic *Escherichia coli* isolates from Thailand aggregated in less than 1 M $(NH_4)_2SO_4$, hemagglutinated human group A and bovine erythrocytes in 1% D-mannose, and possessed either colonization factor I or colonization factor II. No other colonization factors were identified by these two methods.

Enterotoxigenic *Escherichia coli* (ETEC) produces heat-labile enterotoxin (LT), heat-stable enterotoxin (ST), or both (2, 15). For diarrhea to result, ETEC must colonize the small intestine (3). Several adhesive fimbriae, or pili, have been shown to be factors that promote attachment to small intestinal epithelial cells of either humans or animals (4, 6, 12). The ability of ETEC to produce mannose-resistant (MR) hemagglutination of human group A and bovine erythrocytes has been used to detect two antigenically distinct adhesive fimbriae, colonization factors I and II (CFA I and CFA II) (4, 7–9). Hemagglutinating fimbriae antigenically distinct from either CFA I or CFA II have also been found on enteropathogenic *E. coli* strains isolated in different geographical locations (1, 17), suggesting that a variety of adhesive fimbriae may exist for ETEC that is enteropathogenic in humans.

Fimbriated *E. coli* strains have a lower electrophoretic mobility than nonfimbriated strains. The lower density of the negative surface charge of fimbriated isolates creates a hydrophobic surface on the bacterial surface. Lindahl et al. (10) have developed a technique to measure the hydrophobicity of bacterial cell surfaces based on aggregation with ammonium sulfate. The more hydrophobic the surface of a bacteria, the lower the salt concentration required to aggregate the cells. For example, CFA I-containing (CFA I^+) LTST ETEC strain H10407 was aggregated by 0.06 M ammonium sulfate, whereas non-CFA I-containing (CFA I^-) strain H10407P required 1.9 M $(NH_4)_2SO_4$ for aggregation. This "salting out" technique has been proposed as a method to detect unknown fimbriae with hydrophobic properties as potential colonization factors for ETEC (10). Both methods were used to identify colonization factors in ETEC isolates from Thailand.

ETEC isolates were stored frozen in skimmed milk at −70°C or lyophilized within 1 month of identification and retested for LT (15) and ST (2) enterotoxin production immediately before being tested for colonization factors by MR hemagglutination (7) and by the salting out technique (10). Isolates that produced MR hemagglutination or aggregated in less than 1 M $(NH_4)_2SO_4$ were tested for agglutination in antisera to CFA I (5), CFA II (4), and *E. coli* 8775A (17). A total of 100 non-ETEC isolates from patients with diarrhea (one isolate per patient) was also tested for colonization factors by the salting out technique (10); they were also tested for MR and mannose-sensitive (MS) hemagglutination of human group A and bovine erythrocytes.

The results showed that 86% (72 of 84) of LTST, none of 141 LT, and 24% (27 of 111) of ST ETEC isolates aggregated in concentrations of less than 0.2 M $(NH_4)_2SO_4$ (Table 1). All ETEC isolates that aggregated in concentrations of less than 0.2 M $(NH_4)_2SO_4$ agglutinated human and bovine or bovine erythrocytes in 1% -mannose and were agglutinated by antisera to CFA I or CFA II. Four LT ETEC isolates and one ST ETEC isolate caused MS and MR agglutination of group A, and one ST ETEC caused MR and MS agglutination of group A and bovine erythrocytes; however, these isolates were not aggregated by less than 2 M $(NH_4)_2SO_4$ and were not agglutinated in antisera to CFA I, CFA II, or *E. coli* 8775A. None of 100 non-ETEC isolates from persons with diarrhea aggregated in low concentrations of salt. Nineteen caused MR and MS hemagglutination of group A, and five caused MR and MS agglutination of bovine erythrocytes, but they were not agglutinated by antisera to specific colonization factors. Sixty-one percent (44 of 72) of LTST ETEC isolates with demonstrable colonization factors were CFA I^+ and 39% (28 of 72) were CFA II^+. All 27 ST ETEC isolates with colonization factors were CFA I^+. None of the 105 hemagglutinating ETEC isolates [of which 99 aggregated in <0.2 M $(NH_4)_2SO_4$] agglutinated in antisera to *E. coli* 8775A.

LTST and ST ETEC isolates with colonization factors were found in a similar proportion of persons with diarrhea, asymptomatic contacts, or environmental sources within the home (Table 2). Among the 274 ETEC isolates that were not epidemiologically associated, 82% (54 of 60) of LTST and 21% (20 of 93) of ST ETEC isolates had colonization factors. In this group, 54% (29 of 54) of LTST and 100% (20 of 20) of ST ETEC isolates were CFA I^+, and 46% (25 of 54) of LTST ETEC isolates were CFA II^+. The serovars of ETEC isolates with colonization factors that had been serotyped previously (11) are shown in Table 3.

CFA I and CFA II have been associated with a higher proportion of ETEC isolates in Thailand than has been reported for other locations (14). Of 83 LTST isolates 9 (11%) lost their ability to produce CFA I or CFA II after passage on colonization factor agar. Since plasmids coding for both toxigenicity and colonization factors can be lost (3, 6, 13, 16), it is important to determine the toxigenicity of the

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TABLE 1. Ammonium sulfate aggregation and MR hemagglutination of 336 ETEC isolates[a]

ETEC isolate	No. of isolates	Aggregation in $(NH_4)_2SO_4$[b]	MRHA	MRHB	MRHA and MRHB	Colonization factor
LIST	84	72	12 (12[c])	28 (28+)	32 (32[c])	44 CFA I^+, 28 CFA II^+
LT	141	0	5 (0)	0	0	
ST	111	27	5 (3[c])	0	24 (24[c])	27 CFA I^+
Non-ETEC	100	0	19 (0)	4 (0)	0	

[a] MRHA, MR hemagglutination of human group A erythrocytes; MRHB, MR hemagglutination of bovine erythrocytes.
[b] Aggregation in <0.2 M $(NH_4)_2SO_4$.
[c] Number of CFA I^+ ETEC isolates.
[d] Number of CFA II^+ ETEC isolates.

TABLE 2. Prevalence of colonization factors associated with 336 ETEC isolates from different sources in Thailand

ETEC isolate	Diarrhea patients				Asymptomatic contacts				Environment			
	No. of isolates tested	CFA I^+[a]	CFA II^+[b]	ND[c]	No. of isolates tested	CFA I^+	CFA II^+	ND	No. of isolates tested	CFA I^+	CFA II^+	ND
LTST	48	26	15	7	23	9	11	3	13	9	2	2
LT	62	0	0	62	62	0	0	61	18	0	0	18
ST	81	18	0	63	19	5	0	14	11	4	0	7

[a] Number of isolates with CFA I.
[b] Number of isolates with CFA II.
[c] Number of isolates with no detectable colonization factor.

TABLE 3. Serotypes of ETEC isolates with colonization factors

Colonization factor and serotype	No. of isolates	No. of toxin-producing isolates
CFA I		
O78:H12	11	3 LTST, 8 ST
O78:H^-	1	1 ST
O128:H12	3	3 LTST
O126:H^-	1	1 LTST
O126:H12	1	1 ST
O159:H^-	1	1 ST
O7:H18	1	1 LTST
CFA II		
O6:H16	5	5 LTST
O6:H^-	2	2 LTST

ETEC isolate immediately before testing for colonization factors to determine the actual prevalence of colonization factors associated with it. As 86% (72 of 84) of LTST and 24% (27 of 111) of ST ETEC isolates were either CFA I^+ or CFA II^+, active or passive immunological interventions directed against these colonization factors might help prevent the majority of infections caused by LTST isolates and one-quarter of ST isolates causing ETEC infections in Thailand.

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