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Relationship Among Enterotoxigenic Phenotypes, Serotypes, and Sources of Strains in Enterotoxigenic *Escherichia coli*

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The relationship among O groups, O:H serotypes and enterotoxigenic phenotypes was examined in 76 *Escherichia coli* strains isolated in Brazil from different sources. Of the 17 heat-labile and -stable enterotoxin (LT/ST)-producing strains whose O antigens were identified, 15 belonged to serotypes O6:H16 (7 strains), O63:H$^-$ (5 strains), and O139:H28 (3 strains). All 11 ST strains were in group O128ac, which was represented by four O:H serotypes. The 23 LT strains with the O antigen identified were distributed among serotypes of 14 O groups. Colonization factor CFA/I was not found in any of the LT strains, but it was found in six LT/ST and three ST strains. On the whole, each *E. coli* O:H serotype had a particular fermentation pattern. LT/ST as well as ST strains were all isolated from patients with diarrhea, whereas LT strains were isolated from patients with diarrhea, normal children, food, and river water.

Enterotoxigenic *Escherichia coli* are now recognized as an important cause of intestinal infections in both children and adults in different parts of the world (5, 6, 11, 14). Some strains produce heat-labile enterotoxin (LT), others produce heat-stable enterotoxin (ST), and still others produce both LT and ST (7, 13, 17, 18). Until recently, it was thought that any *E. coli* serotype might contain enterotoxin-controlling plasmids and therefore be enterotoxigenic (13). However, accumulating evidence from the last 2 years suggests that enterotoxigenic *E. coli* strains isolated from patients with diarrhea commonly belong to a small number of serogroups or serotypes (9) and that some of these may produce primarily both toxins or only one of them (8). On the other hand, it has been shown that enterotoxigenic O:H types correspond on the whole to a typical fermentation pattern (8, 10, 15). Data obtained in this study of 76 enterotoxigenic *E. coli* strains isolated from different sources in Brazil support the evidence of a relationship between toxin production and serogroup or serotype and suggest that the enterotoxigenic phenotype may be also related to the sources of the strains.

MATERIALS AND METHODS

Strains. A total of 76 enterotoxigenic *E. coli* strains isolated in Brazil were studied. Seven were isolated by R. L. Guerrant in Florianopolis (Santa Catarina), two were isolated by A. F. Pestana de Castro in Campinas (São Paulo), and the remainder were isolated in our laboratory during the last 3 years. The sources of the strains were as follows: 51 were isolated from patients with diarrhea, 11 were isolated from individuals without diarrhea, 9 were isolated from food of animal origin, and 5 were isolated from river water. A total of 21 strains produced both LT and ST, 11 produced only ST, and 44 produced only LT. Of the 11 strains of *E. coli* serogroup O128ac included in this study, 6 were previously described as ST producers (12). The infant mouse test of Dean et al. (1) was used for detection of ST, whereas the Y1 adrenal cell test (2) was used to detect LT.

Serological identification and biochemical examination. O and H antigens determinations were performed as described by Edwards and Ewing (3). The strains which were not identified in our laboratory were investigated by M.R.F.T. when she was visiting the Center for Disease Control, Atlanta, Ga.; for this she used O sera O1 through O157 and H sera H1 through H50. Biochemical examination for biotype determination was done by standard procedures, using all tests recommended by Edwards and Ewing for identification of the *Enterobacteriaceae* (3). The following sugars were included: lactose, mannitol, sorbitol, arabinose, rhamnose, xylose, trehalose, maltose, inositol, adonitol, sucrose, salicin, dulcitol, raffinose, and cellobiose.

Detection of CFA/I. We used a hemagglutination test that was previously described (4), except that the strains were grown in blood agar base (Difco Laboratories) and a 5% suspension of washed group A human erythrocytes with or without 1% mannose was employed. Results were recorded as positive and negative only. Bacterial agglutination with anti-CFA serum was done by using a rabbit anti-CFA/I serum prepared with strain H10407, which was kindly supplied by D. J. Evans, Jr. Slide agglutination tests at room temperature were used, and the bacterial suspensions for this test contained approximately 1.2×10^{10} cells per ml in phosphate-buffered saline.

RESULTS

The relationships between O serogroups and enterotoxigenic phenotypes of the 76 strains are shown in Table 1. LT/ST strains were found to belong to 5 O serogroups, ST strains belonged to only one serogroup, and LT strains belonged to 14 serogroups. A total of 18 strains (2 LT/ST and 16 LT) were not agglutinated by sera O1 through O157, and 7 strains (2 LT/ST and 5 LT) were rough. Of the 21 LT/ST strains, 15 belonged to groups O6, O63, and O139. Table 2 shows the relationships between the enterotoxigenic phenotypes and O:H types. Strains producing both LT and ST enterotoxin were of the same serotype in groups O6, O63, and O139. In group O128ac, the only enterotoxin ST producer, there were four different serotypes.

CFA/I was found in 9 of the 76 strains by both the mannose-resistant hemagglutination test and agglutination in anti-CFA/I serum. Six strains were LT/ST, and three were ST only. Of the six LT/ST strains, four belonged to serotype O63:H$^-$, one belonged to serotype O62:H$^-$, and one was rough. The three ST strains belonged to serotype O128ac:H12. All CFA/I-bearing strains were isolated from patients with diarrhea.

Table 3 shows the fermentation patterns of the strains of the 10 serotypes which included two or more strains. It is evident that with few exceptions each serotype had a constant fermentation pattern with the sugars used. Lactose, mannitol, arabinose, trehalose, sorbitol, and maltose were fermented by all strains, whereas none of them fermented inositol. Reactions with the other biochemical tests were the usual ones for *E. coli*.

Table 4 shows the relationships between enterotoxigenic phenotypes and sources of all strains. Both LT/ST strains and ST strains were found only in patients with diarrhea, whereas LT strains were found in patients with diarrhea, in individuals without diarrhea, in food, and in river water.

TABLE 1. *O groups and enterotoxigenic phenotypes of 76 E. coli strains isolated in Brazil*

Group	No. of strains	No. of strains with enterotoxigenic phenotype:		
		LT/ST	ST	LT
O6	7	7		
O8	4			4
O9	1			1
O25	2	1		1
O36	1			1
O60	2			2
O62	1	1		
O63	5	5		
O78	2			2
O112	1			1
O114	1			1
O115	1			1
O128	11		11	
O132	1			1
O139	5	3		2
O149	4			4
O151	1			1
OX1	1			1
Negative O1-O157	18	2		16
Rough	7	2		5

TABLE 2. *O:H serotypes and enterotoxigenic phenotypes of 76 E. coli strains isolated in Brazil*

Serotype	No. of strains	No. of strains with enterotoxigenic phenotype:		
		LT/ST	ST	LT
O6:H16	7	7		
O8:H4	1			1
O8:H9	1			1
O8:H5	1			1
O8:H8	1			1
O9:H21	1			1
O25:H42	1	1		
O25:H$^-$	1			1
O36:H5	1			1
O60:H21	2			2
O62:H$^-$	1	1		
O63:H$^-$	5	5		
O78:H10	2			2
O112:H21	1			1
O114:H21	1			1
O115:H6	1			1
O128ac:H21	4		4	
O128ac:H12	3		3	
O128ac:H27	2		2	
O128ac:H$^-$	2		2	
O132:H$^-$	1			1
O139:H28	5	3		2
O149:H10	4			4
O151:H8	1			1
OX1:H4	1			1
Negative O1-O157, with different H antigens	18	2		16
Rough, with different H antigens	7	2		5

DISCUSSION

Ørskov et al. (9) were the first to suggest a relationship between enterotoxin production and *E. coli* serotypes. Among 106 enterotoxigenic *E. coli* strains received from different parts of the world, serotypes O6:H16, O8:H9, O15:H11, O55:H42, O78:H11, and O78:H12 were found with particularly high frequencies and only rarely among 20,000 *E. coli* strains collected over many years from several locations and sources. However, these authors did not try to establish

TABLE 3. *Fermentation patterns of enterotoxigenic E. coli serotypes isolated in Brazil*

Substrate	Fermentation patterns of the following serotypes:[a]									
	O128ac:H21 ($n = 4$)	O128ac:H12 ($n = 3$)	O128ac:H27 ($n = 2$)	O128ac:H$^-$ ($n = 2$)	O6:H16 ($n = 7$)	O63:H$^-$ ($n = 5$)	O139:H28 ($n = 5$)	O149:H10 ($n = 4$)	O60:H21 ($n = 2$)	O78:H10 ($n = 2$)
Sucrose	+	−	+	+	−	+	+	−	+	+
Salicin	+	−	+	+	+	−	+	−	+	(+)[b]
Dulcitol	+	+	−	+	−	+	+	−	+	−
Adonitol	−	−	−	−	+	−	−	+	−	−
Raffinose	+	−	+	+	D	+	+	−	+	+
Rhamnose	+	+	+	+	D	+	+	+	+	+
Xylose	+	+	+	+	+	−	+	+	+	+
Cellobiose	−	−	−	−	−	−	−	−	D	D

[a] +, Positive reaction within 1 or 2 days; −, negative reaction within 30 days; D, different reactions with different strains; (+), positive reaction after 3 days.

[b] With this combination of substrate and serotype, there were different reactions with the same strain.

TABLE 4. *Sources of enterotoxigenic E. coli phenotypes isolated in Brazil*

Enterotoxigenic phenotype	Serotype[a]	No. of strains	No. of strains from the following sources:			
			Human		Food[b]	Water[c]
			Diarrhea	No diarrhea		
LT/ST	O6:H16	7	7			
	O63:H$^-$	5	5			
	O139:H28	3	3			
	O25:H42	1	1			
	O62:H$^-$	1	1			
	NT:H4	1	1			
	NT:H$^-$	1	1			
	R:H$^-$	2	2			
ST	O128ac:H21	4	4			
	O128ac:H12	3	3			
	O128ac:H27	2	2			
	O128ac:H$^-$	2	2			
LT	O149:H10	4			4	
	O139:H28	2	1	1		
	O60:H21	2	1	1		
	O78:H10	2	2			
	O8:H4, O8:H9, O8:H5, O8:H8, O25:H$^-$, O112:H21, O114:H21, O132:H$^-$, O9:H21, O35:H5, O115:H6, O151:H8, OX1:H4	13 (one of each)	6	3	1	3
	Negative O1-O157, with different H antigens	16	6	4	4	2
	Rough, with different H antigens	5	3	2		

[a] H$^-$, Nonmotile; NT, negative with O sera used; R, rough.

[b] From animals.

[c] From rivers of São Paulo.

a relationship between the enterotoxigenic phenotypes and the serotypes of the strains. Scotland et al. (15) observed that among several serotypes of *E. coli* groups O6 and O148 only serotypes O6:H16 and O148:H28 were enterotoxigenic; the former produced enterotoxins LT and ST, and the latter produced enterotoxin ST only. More recently, Merson et al. (8) reported that among 109 enterotoxigenic *E. coli* strains isolated from patients with diarrhea in Bangladesh, 90% of the LT/ST strains belonged to serogroups O6, O8, O78, and O115, whereas the ST strains were included in 15 O serogroups. The six LT strains encountered belonged to six serogroups. With the exception of serogroup O6, which included only strains of serotype O6:H16, more than one serotype were found in groups O8, O78, and O115. However, in group O8 all of the LT/ST strains were of serotype O8:H9. In the present study it was found that of the 17 LT/ST strains whose O antigens were identified, 15 were in groups O6, O63, and O139. The 11 ST strains all belonged to serogroup O128ac, and the 23 LT strains with identified O antigens were distributed in 14 O serogroups (Table 1). Although only one serotype was found in the LT/

ST serogroups, four serotypes were found in serogroup O128ac. These results support the finding of Merson et al. (8) concerning the relationship between serogroups or serotypes and enterotoxigenic *E. coli* LT/ST phenotypes. However, in this study, the major serotypes were O6:H16, O63:H$^-$, and O139:H28 (Table 2), whereas in the study of Merson et al., most frequently found serotypes were O6:H16, O8:H9, O78:H11, O78:H12, O115:(H51), and O115:H40. It should be noticed that the two LT O78 strains found in this study belonged to serotype O78:H10, whereas the LT/ST O78 serotypes reported by Merson et al. (8) belonged to O78:H11 and O78:H12. Drawing conclusions concerning the relationship between LT or ST strains and serotypes is more difficult because these strains may be derived from the LT/ST strains (4). However, a relationship between O128 serotypes and production of enterotoxin ST seems to be highly probable. In this study all ST only strains belonged to serogroup O128ac, and a similar relationship has been also observed by Merson et al. (8), Reis et al. (12), and Serafim et al. (16).

The results of the biochemical tests confirm the findings of other authors (8, 10, 15); i.e., a specific fermentation pattern is associated with a given serotype. This may be a helpful characteristic for the identification of these bacteria.

Table 4 shows a clear correlation between the enterotoxigenic phenotypes and sources of the strains. Although all LT/ST and ST strains were found only in patients with diarrhea, the LT strains were found throughout the four sources (i.e., patients with diarrhea, normal individuals, food, and river water). The finding of LT/ST and ST strains only in patients with diarrhea and the presence of CFA/I in some of them suggest that these strains were the etiological agents of the diarrhea. On the other hand, the distribution of LT strains and the absence of CFA/I in all of them seem to be indications that production of LT is a more general property of *E. coli*. Although these strains may have CFA/II or even an unknown colonization factor, we think that these findings point to the need for more studies on the association of *E. coli* LT only strains and infantile diarrhea. In our laboratory, although the frequency of LT only strains has been the same in children with endemic diarrhea and normal children, LT/ST and ST only strains have been found significantly more frequently in children with diarrhea (Reis and Trabulsi, Abstr. Joint U.S. - Japan Conf. Cholera 15th, p. 76–77, 1979). Similar results have been reported by others (11).

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LITERATURE CITED

1. **Dean, A. G., Y. C. Ching, R. G. Williams, and L. B. Harden.** 1972. Test for *Escherichia coli* enterotoxin using infant mice: application in a study of diarrhea in children in Honolulu. J. Infect. Dis. **125**:407–411.
2. **Donta, S. T., H. W. Moon, and S. C. Whipp.** 1974. Detection of heat-labile *Escherichia coli* enterotoxin with the use of adrenal cells in tissue culture. Science **183**:334–336.
3. **Edwards, P. R., and W. H. Ewing.** 1972. Identification of the *Enterobacteriaceae*, 3rd ed. Burgess Publishing Co., Minneapolis.
4. **Evans, D. G., and D. J. Evans, Jr.** 1978. New surface-associated heat-labile colonization factor antigen (CFA/II) produced by enterotoxigenic *Escherichia coli* of serogroups O6 and O8. Infect. Immun. **21**:638–647.
5. **Giugliano, L. G., M. A. M. Meneguetti, and L. R. Trabulsi.** 1978. LT enterotoxin production by *Escherichia coli*. Rev. Microbiol. **9**:11–17.
6. **Gorbach, S. L., and C. M. Khurana.** 1972. Toxigenic *Escherichia coli*: a cause of infantile diarrhea in Chicago. N. Engl. J. Med. **293**:567–573.
7. **Gyles, C. L.** 1971. Discussion: heat-labile and heat-stable forms of the enterotoxin from *E. coli* strains enteropathogenic for pigs. Ann. N.Y. Acad. Sci. **176**:314–322.
8. **Merson, M. H., F. Ørskov, I. Ørskov, R. B. Sack, I. Hug, and F. T. Koster.** 1979. Relationship between enterotoxin production and serotype in enterotoxigenic *Escherichia coli*. Infect. Immun. **23**:325–329.
9. **Ørskov, F., I. Ørskov, D. J. Evans, Jr., R. B. Sack, D. A. Sack, and T. Wadström.** 1976. Special *Escherichia coli* serotypes among enterotoxigenic strains from diarrhoea in adults and children. Med. Microbiol. Immunol. **162**:73–80.
10. **Ørskov, I., and F. Ørskov.** 1977. Special O:K:H serotypes among enterotoxigenic *E. coli* strains from diarrhea in adults and children. Occurrence of the CF (colonization factor) antigen and of hemagglutinating abilities. Med. Microbiol. Immunol. **163**:99–110.
11. **Pickering, L. K., D. J. Evans, Jr., O. Munoz, H. L. Du Pont, P. Coello-Ramirez, J. J. Vollet, R. H. Conklin, J. Olarte, and S. Kohl.** 1978. Prospective study of enteropathogens in children with diarrhea in Houston and Mexico. J. Pediatr. **93**:383–388.
12. **Reis, M. H. L., A. F. P. Castro, M. R. F. Toledo, and L. R. Trabulsi.** 1979. Production of heat-stable enterotoxin by the O128 serogroup of *Escherichia coli*. Infect. Immun. **24**:289–290.
13. **Sack, R. B.** 1975. Human diarrheal disease caused by enterotoxigenic *Escherichia coli*. Annu. Rev. Microbiol. **29**:333–353.
14. **Sack, R. B., S. L. Gorbach, J. G. Banwell, B. Jacobs, B. D. Chatterjee, and R. C. Mitra.** 1971. Enterotoxigenic *Escherichia coli* isolated from patients with cholera-like disease. J. Infect. Dis. **123**:378–385.
15. **Scotland, S. M., R. J. Gross, and B. Rowe.** 1977. Serotype-related enterotoxigenicity in *Escherichia coli* O6:H16 and O148:H28. J. Hyg. **79**:395–403.
16. **Serafim, M. B., A. F. P. Castro, H. A. Rangel, and L. P. Neto.** 1977. Isolation of heat-stable enterotoxin-producing strains of *Escherichia coli* from cases of diarrhea in Campinas, S.P., Brazil. Rev. Inst. Med. Trop. São Paulo **19**:144–147.
17. **Wachsmuth, K., J. Wells, P. Shipley, and R. Ryder.** 1979. Heat-labile enterotoxin production in isolates from a shipboard outbreak of human diarrheal illness. Infect. Immun. **24**:793–797.
18. **Wachsmuth, I. K., S. Falkow, and R. W. Ryder.** 1976. Plasmid-mediated properties of a heat-stable enterotoxin-producing *Escherichia coli* associated with infantile diarrhea. Infect. Immun. **14**:403–407.