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Serotypes of Enterotoxigenic *Escherichia coli* Isolated in the United States

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Strains of enterotoxigenic *Escherichia coli* isolated from humans in the United States were found in 11 of 16 serotypes that previously were documented in the international literature as associated with enterotoxin production. Of 68 strains belonging to these 11 serotypes, 28 (41%) were enterotoxigenic; none of 46 strains belonging to 5 other previously implicated serotypes was enterotoxigenic. Control cultures of various serotypes were selected for comparison and found to contain 0 to 7% enterotoxigenic *E. coli*. *E. coli* belonging to documented enterotoxin-associated serotypes, characterized by both O and H antigens, were selected for toxin testing to determine their prevalence and potential pathogenicity in this country. In this study, a strain possessing any combination of an enterotoxin-associated serotype O antigen and H antigen was more likely to be enterotoxigenic than strains possessing only the specific O antigen or H antigen or neither. Five *E. coli* strains belonging to undocumented enterotoxin-associated serotypes did contain a combination of previously reported enterotoxin-associated serotype O and H antigens and did produce enterotoxin.

Enterotoxigenic *Escherichia coli* (ETEC) have been implicated in sporadic and epidemic outbreaks of diarrhea in both infants and adults in many parts of the world. ETEC produce one or both of two plasmid-mediated (11, 20) enterotoxins: a heat-stable enterotoxin (ST) and a heat-labile enterotoxin (LT). A variety of assays have been developed for detecting the enterotoxins (2, 3, 7, 9, 18), but because all of these assays require some specialized procedure, only a small number of laboratories test for ETEC.

Although one early report (5) suggested the possibility of screening for potential ETEC with biochemical tests, such tests have proved neither specific nor practical. More recent evidence (12, 14-16) suggests that such screening may be better achieved by serotyping. This is based primarily on the observation that a high proportion of enterotoxigenic strains isolated in Asia belonged to a limited number of serotypes. As a result, it was proposed (16) that new groupings of diagnostic antisera be made available to cover those *E. coli* serotypes in which ETEC have most often been found. As part of an ongoing project dealing with the phenotypic and genotypic characterizations of ETEC, strains of *E. coli* belonging to enterotoxin-associated serotypes (EAS) were tested for ST and LT to determine whether these serotypes also contained ETEC in the United States. Control cultures of *E. coli* selected from serotypes containing other combinations of O and H antigens were similarly tested.

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MATERIALS AND METHODS

***E. coli* strains.** All serotyped strains of *E. coli* used in this investigation were originally received and serotyped for O and H antigens in the Enteric Section, Bureau of Laboratories, Center for Disease Control between 1960 and 1978. All serotyped strains were stocked in paraffin-corked blood agar base slants at room temperature. Serotyped strains used in this study were rejuvenated by an initial subculture on Trypticase soy agar (BBL Microbiology Systems, Cockeysville, Md.) plates. They were maintained on blood agar base slants at room temperature. Unserotyped strains were received by the Enteric Section between August 1978 and October 1979 as fresh specimens (isolates on nutrient agar, rectal swabs in transport medium, or both) and were plated on MacConkey agar plates before being assayed for enterotoxin.

A total of 696 strains of *E. coli*, comprising three groups, were tested for ST and LT production. Group 1, containing 204 serotyped strains, consisted of *E. coli* belonging to serotypes that had been documented in the literature on at least two separate occasions before July 1978 to contain ETEC. These serotypes will be referred to as EAS. An initial list of 22 documented EAS was reduced to the 16 for which strains were available (Table 1).

TABLE 1. *EAS[a] of E. coli*

EAS	Test strains available
O6:H16	+
O6:H−[b]	+
O8:H9	+
O8:H?[c]	+
O15:H11	+
O15:H−	+
O20:H−	+
O25:H42	−
O25:H−	+
O27:H20	+
O78:H11	+
O78:H12	+
O109:H21	−
O115:H21	−
O115:H40	−
O128:H7	+
O128:H12	+
O128:H21	+
O148:H28	+
O149:H19	+
O159:H4	?[d]
O159:H20	?[d]

[a] Serotypes reported in the literature on at least two occasions before July 1978 to contain enterotoxigenic strains.

[b] H−, Nonmotile.

[c] H antigens untypable in available antisera (H1 through H49).

[d] Strains of O159 were not serotyped in the culture collection.

Group 2 consisted of 68 non-EAS strains (Table 2) selected from serotyped stock cultures possessing 1 of 10 O antigens commonly associated with ETEC in humans and 1 (0149) previously reported in swine (10) and in food (19). Additionally, group 2 was limited to strains with H antigens not previously associated with a specific EAS O antigen.

Group 3 consisted of 424 unserotyped strains of *E. coli* obtained by CDC from 18 episodes (that is, sporadic cases and outbreaks) of diarrheal illness. Sources of these strains were limited to (i) suspected outbreaks in infants, (ii) suspected nosocomial outbreaks, (iii) suspected single-source community outbreaks, and (iv) individual cases of protracted diarrhea in which other commonly occurring etiological agents were ruled out. These limitations were specified in a memorandum from the Enteric Section to all state health laboratories and selected hospitals. Telephone confirmations were made before cultures were accepted.

Enterotoxin assays. The production of ST was determined by the infant mouse assay (2, 8), and the production of LT was determined by the Y1 adrenal cell assay (3, 18). As shown in Fig. 1, on day 1 all *E. coli* test strains (both serotyped and unserotyped) were streaked onto fresh blood agar base slants and incubated overnight at 36 ± 1°C. On day 2, a small inoculum from each of these overnight slants was transferred to screw-capped tubes (16 by 100 mm) containing 5 ml of Trypticase soy broth with 0.6% yeast extract and incubated overnight at 37°C on a roller drum (model TC-7, New Brunswick Scientific Co., New Brunswick, N.J.) rotating at 20 rpm. On day 3, the broth cultures were centrifuged at 3,500 × *g* for 30 min, and the supernatants were filtered through 0.45-μm disposable filter units (Millex SLHA0250S). Samples (1 and 3 ml) were taken from each filtrate. Two drops (0.1 ml) of filtered 2% Evans blue dye was added to the tube containing 3 ml of filtrate. The dye-filtrate (0.1-ml amounts) was then injected intragastrically (1-ml tuberculin syringe with 25-gauge needle) into each of four or five randomly selected mice, 2 to 4 days of age. After incubation for 4 h at room temperature (13), the animals were sacrificed with chloroform, the entire intestinal tract was removed and weighed, and the ratio of intestinal weight to remaining carcass weight was used as the measure of ST-mediated enterotoxigenicity. A ratio of ≥0.83 was considered positive. From the second tube containing 1

TABLE 2. *Non-EAS selected by EAS O antigen (group 2)*

O antigen	H antigens
6	1, 6, 7, 31, 49
8	−,[a] 4, 5, 10
15	1, 10, 16, 18
20	4, 5, 9, 11, 26, 27, 34
25	1, 2, 12, 28
27	7, 12, 18, 30, 45
78	−, 2, 9, 33
128	−, 2, 8, 47
148	−, 2, 30, 40
149	−, 7, 16

[a] −, Nonmotile.

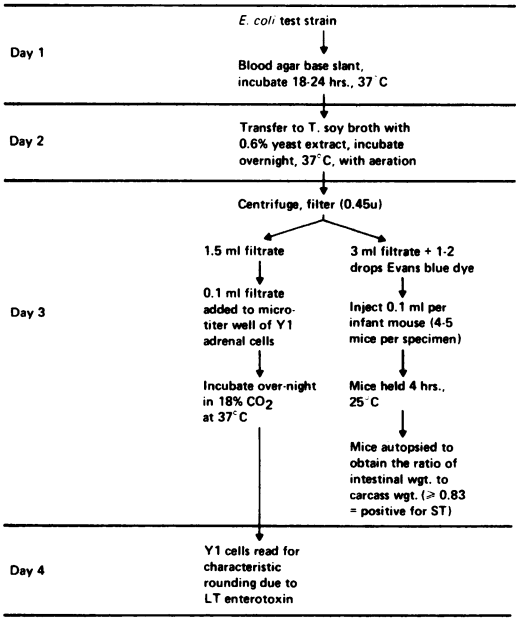

FIG. 1. *Flow diagram of the assays for ST and LT production in E. coli.*

ml of filtrate, 2 drops (0.1 ml) were added to a microtiter well of nearly confluent Y1 adrenal cells. The cells were then incubated overnight in 18% CO_2 at 36 ± 1°C. On day 4, the Y1 cells were read for the characteristic rounding caused by LT (3).

Carbohydrate fermentation. Fermentation medium was prepared by the methods of Edwards and Ewing (4) except that Andrade indicator was prepared with 0.2 g of acid fuchsine rather than 0.5 g/100 ml of water. Adonitol (ribitol), dulcitol, D-sorbitol, D-raffinose, sucrose, D-xylose, L-rhamnose, maltose, salicin, inositol, lactose, and L-sorbose fermentation media, in screw-capped or cotton-plugged tubes (13 by 100 mm), were inoculated with the test strains, incubated at 36 ± 1°C, and read after 2, 5, and 30 days.

RESULTS

Enterotoxin assays. A search through *E. coli* stock cultures yielded 204 EAS strains that belonged to group 1 (Table 1). Of these strains, 55 were enterotoxigenic (Table 3). Of these 55, 16 produced both ST and LT and belonged to five serotypes; the remaining 39 produced only ST or LT and belonged to eight serotypes.

Excluding duplicate patient isolates, multiple isolates from outbreaks, and known foreign and animal isolates, there were 111 human strains from the United States. Although a few of these strains may not have been contracted in the United States, all resulting cases of diarrheal illness were seen by U.S. physicians, and all strains were initially isolated in this country. Of these 111 strains, 28 were enterotoxigenic and belonged to 11 different EAS; none of 46 strains from the 5 remaining EAS were enterotoxigenic (Table 4). As Table 5 shows, the 28 human ETEC were isolated from geographically diverse areas throughout the continental United States. The enterotoxin(s) produced and the date of original isolation apparently were not associated. Between 1969 and 1977, CDC received an average of three EAS strains per year. The number of ETEC falling into any one EAS ranged from one to five (Fig. 2). Of 68 non-EAS strains in group 2, 5 (7.4%) proved to be enterotoxigenic (Table 6). In all five strains, both the O and H antigens were previously implicated in EAS; however, the O-H combination in these five strains was new, that is, undocumented in the literature. All 424 strains of unserotyped *E. coli*

TABLE 3. *ETEC[a] listed by serotype and enterotoxin production*

Group of test strains	Serotype	ST	LT	No. of enterotoxigenic strains
1[b] (*n* = 204)	O15:H−[c]	+	+	3
	O15:H11	+	+	7
	O20:H−	+	+	1
	O78:H12	+	+	1
	O148:H28	+	+	4
	O6:H16	+	−	4
	O15:H11	+	−	1
	O27:H20	+	−	7
	O78:H12	+	−	2
	O128:H7	+	−	9
	O128:H21	+	−	5
	O15:H−	−	+	2
	O15:H11	−	+	3
	O25:H−	−	+	1
	O78:H11	−	+	5
2[d] (*n* = 68)	O25:H28	−	+	1
	O27:H7	+	−	1
	O27:H45	+	−	1
	O78:H−	−	+	1
	O149:H7	+	−	1

[a] Total ETEC found, including duplicate strains and foreign and animal isolates.
[b] ETEC belonging to EAS.
[c] H−, Nonmotile.
[d] ETEC possessing O antigen 6, 8, 15, 20, 25, 27, 78, 128, 148, or 149, but not belonging to an EAS.

TABLE 4. *Results of ST and LT assays covering 111 human E. coli isolates from the United States belonging to EAS[a] of E. coli*

Serotype	ST	LT	No. of enterotoxigenic strains	No. of strains assayed
O6:H16	+	+	1	4
O15:H−[b]	+	+	2	18
O15:H11	+	+	4	7
O78:H12	+	+	1	5
O148:H28	+	+	3	3
O6:H16	+	−	2	
O27:H20	+	−	4	8
O78:H12	+	−	1	
O128:H7	+	−	1	1
O128:H21	+	−	3	7
O15:H−	−	+	2	
O15:H11	−	+	1	
O20:H−	−	+	1	5
O25:H−	−	+	1	6
O28:H11	−	+	1	1
O6:H−	−	−	0	22
O8:H9	−	−	0	9
O8:H?[c]	−	−	0	3
O128:H12	−	−	0	9
O149:H19	−	−	0	3

[a] Serotypes reported in the literature on at least two separate occasions before July 1978 to contain enterotoxigenic strains.
[b] H−, Nonmotile.
[c] H antigens untypable in available antisera (H1 through H49).

TABLE 5. *Twenty-eight human ETEC from the United States (1960 through 1977) belonging to 11 EAS*

CDC no.	Serotype	Origin	Source	Yr	ST	LT
5203-70	O128:H21	N.Mex.	Stool	1970	+	−
2372-77	O128:H7	N.Dak.	Stool	1977	+	−
2375-77	O128:H7	N.Dak.	Stool	1977	+	−
2523-77	O128:H7	La.	Stool	1977	+	−
1782-77	O148:H28	Cruise ship	Stool	1977	+	+
0423-77	O148:H28	U.S. Army	Stool	1970	+	+
5448-72	O148:H28	U.S. Army	?	1972	+	+
1108-76	O15:H−[a]	Ariz.	Stool	1976	+	+
2521-77	O15:H−	La.	Stool	1977	+	+
1519-70	O15:H−	Mass.	?	1970	−	+
3805-71	O15:H−	Calif.	?	1971	−	+
2417-77	O15:H11	Mass.	Stool	1977	+	+
3502-77	O15:H11	Md.	Stool	1977	+	+
2707-69	O15:H11	Md.	?	1969	+	+
5474-72	O15:H11	U.S. Army	?	1972	+	+
1252-60	O15:H11	Pa.	Stool	1960	−	+
0115-75	O20:H−	D.C.	?	1975	−	+
1203-79	O25:H−	Cruise ship	Stool	1976	−	+
0563-73	O27:H20	Mass.	?	1973	+	−
1694-71	O27:H20	Traveler	Stool	1971	+	−
1789-72	O27:H20	Traveler	Stool	1972	+	−
1943-72	O27:H20	Traveler	Stool	1972	+	−
2513-72	O6:H16	Mass.	Stool	1972	+	−
5460-72	O6:H16	U.S. Army	?	1972	+	−
1916-79	O6:H16	Oreg.	Stool	1975	+	+
1116-73	O78:H11	Calif.	?	1973	−	+
0100-76	O78:H12	Tex.	Stool	1976	+	−
1119-73	O78:H12	Calif.	?	1973	+	+

[a] H−, Nonmotile.

in group 3, isolated from cases of diarrheal illness, were found to be negative for ST or LT or both.

Carbohydrate fermentation. Fermentation patterns from 202 strains of *E. coli* belonging to 16 serotypes are shown in Table 7. A comparison between ETEC and non-ETEC belonging to the same EAS is shown in Table 8. A total of 16 strains of *E. coli* were adonitol positive ($Adon^+$) and dulcitol negative (Dul^-); 13 (81%) of these 16 were enterotoxigenic (Table 9). Of five strains belonging to serotype O78:H11, all were D-xylose negative and enterotoxigenic (Table 9). The inability to ferment sucrose was not associated with enterotoxigenicity. Of the 12 carbohydrates tested, only adonitol fermentation in conjunction with a lack of dulcitol fermentation and a lack of xylose fermentation were found to be possible phenotypic markers for strains of ETEC.

DISCUSSION

Most of the ETEC serotype and enterotoxin data presently available are based on studies of Asian and some Mexican strains (6, 12, 14, 17). To facilitate the development and evaluation of serotyping as a diagnostic screening procedure for ETEC diarrhea, additional information is needed on both the EAS present in different geographical areas and on O- and H-antigen specificities.

The results of our enterotoxin assays show that, between 1960 and 1977, ETEC were present in the United States and that at least 11 of the serotypes defined in other parts of the world as EAS also contained ETEC in the United States. The four EAS for which no strains were available (Table 1) may be quite rare in this country; as such, these serotypes may be of little importance as sources of ETEC in the United States. In these conclusions and in this investigation, it is necessary to consider that the enterotoxin genes are usually plasmid mediated and are often susceptible to spontaneous curing (6). The latter may have occurred during storage.

The screening procedure for EAS employs polyvalent and monovalent O antisera in slide agglutination tests, confirmed by tube agglutination with monovalent antisera and heated cell suspensions (B. Rowe, personal communication). In the United States, such a procedure based on O antigens alone may not prove suffi-

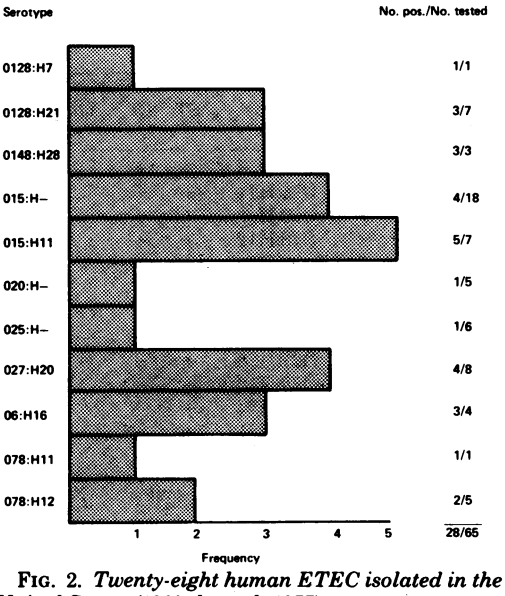

FIG. 2. *Twenty-eight human ETEC isolated in the United States (1960 through 1977).*

TABLE 6. *Serotypes of ETEC previously unreported to produce enterotoxin*

CDC no.	Serotype	Origin	Source	Yr	ST	LT
3505-77	O25:H28	?	Stool	1977	−	+
0635-79-1	O27:H7	Cruise ship	Stool	1975	+	−
0635-79-2	O27:H45	Cruise ship	Stool	1975	+	−
4549-63	O78:H−[a]	Ga.	Stool	1963	−	+
2694-73	O149:H7	Ohio	Urine	1973	+	−

[a] H−, Nonmotile.

TABLE 7. *Carbohydrate fermentation patterns for 202 E. coli strains belonging to serotypes associated with enterotoxin production*

Carbohydrate	Fermentation pattern with following serotype:															
	O128:H12 (n = 13)	O128:H21 (n = 5)	O128:H7 (n = 15)	O148:H28 (n = 4)	O15:H−[a] (n = 31)	O15:H11 (n = 17)	O20:H− (n = 7)	O25:H− (n = 14)	O27:H20 (n = 17)	O6:H− (n = 27)	O6:H16 (n = 7)	O78:H− (n = 11)	O78:H11 (n = 5)	O78:H12 (n = 6)	O8:H?[b] (n = 5)	O8:H9 (n = 18)
Adonitol	2	−[c]	−	−	4	−	−	−	−	−	2 (1)[d]	−	+[e]	−	−	−
Dulcitol	11	3 (2)	12 (3)	+	26	15	+	6 (1)	−	24	2	10	−	4 (2)	+	+
Sorbitol	+	+	+	+	+	+	+	12	+	+	+	+	+	+	+	+
Raffinose	7	+	+	+	28	2	3	−	−	+	−	+	−	+	2	+
Sucrose	6	+	+	+	28	2	4	−	−	+	−	+	−	+	2	14 (3)
Xylose	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+
Rhamnose	11 (2)	+	13 (2)	+	+	+	+	+	+	25 (2)	+	+	3	+	+	+
Maltose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salicin	6 (1)	4 (1)	11 (4)	+	22 (5)	7 (4)	3	10 (3)	+	14 (12)	5	2 (6)	+	5	3 (1)	8 (6)
Inositol	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Lactose	+	+	+	+	27 (1)	17	+	+	+	+	+	+	+	+	+	+
Sorbose	−	1	1	−	2	2 (2)	−	3	−	+	−	8 (1)	−	3	2	+

[a] H−, Nonmotile.
[b] H antigens untypable in available antisera (H1 through H49).
[c] −, Negative after 30 days.
[d] 2 (1), Two strains positive within 48 h and one strain positive between 2 and 7 days.
[e] +, All strains positive within 48 h.

TABLE 8. *Carbohydrate fermentation patterns of ETEC and non-ETEC belonging to the same serotypes*

Carbohydrate	Fermentation pattern with following serotype:															
	O128:H7		O15:H−[a]		O15:H11		O20:H−		O25:H−		O27:H20		O6:H16		O78:H12	
	ETEC (n = 8)	Non-ETEC (n = 7)	ETEC (n = 5)	Non-ETEC (n = 26)	ETEC (n = 11)	Non-ETEC (n = 6)	ETEC (n = 2)	Non-ETEC (n = 5)	ETEC (n = 3)	Non-ETEC (n = 11)	ETEC (n = 7)	Non-ETEC (n = 10)	ETEC (n = 4)	Non-ETEC (n = 3)	ETEC (n = 3)	Non-ETEC (n = 3)
Adonitol	−[b]	−	4	−	−	−	−	−	−	−	−	−	1 (1)[c]	1	−	−
Dulcitol	4 (1)	+[d]	1	25	+	4	+	+	+	3 (1)	−	−	−	2	+	+
Sorbitol	+	+	+	+	+	+	+	+	+	9	+	+	+	+	+	+
Raffinose	+	+	2	−	2	−	−	3	−	−	−	−	−	(1)	+	+
Sucrose	+	+	1	2	2	−	1 (1)	3	−	−	−	−	−	−	+	+
Xylose	+	+	+	+	+	+	+	+	+	+	+	+	3	2	+	+
Rhamnose	+	5 (2)	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salicin	+	+	2	19 (5)	7 (3)	(1)	−	3	+	7 (3)	+	+	+	1	+	2
Lactose	+	+	+	22 (1)	+	+	+	+	+	+	+	+	+	+	+	+
Sorbose	−	1	−	2	1 (1)	1 (1)	1 (1)	−	−	−	−	−	−	−	1	2

[a] H−, Nonmotile.
[b] −, Negative after 30 days.
[c] 1 (1), One strain positive within 48 h and one strain positive between 2 and 7 days.
[d] +, All strains positive within 48 h.

TABLE 9. *Adon⁺ Dul⁻ strains of E. coli*

CDC no.	Serotype	Xylose fermented	ST	LT
1109-76	Orough:H−[a]	+	+	+
1783-72	O128:H12	+	−	−
1785-72	O128:H12	+	−	−
3805-71	O15:H−	+	−	+
1086-76	O15:H−	+	+	+
1108-76	O15:H−	+	+	+
2521-77	O15:H−	+	+	+
1947-72	O27:H12	+	−	−
5460-72	O6:H16	+	+	−
1916-79-1	O6:H16	−	+	+
1916-79-2	O6:H16	−	+	−
5726-70	O78:H11	−	−	+
5727-70	O78:H11	−	−	+
2426-75	O78:H11	−	−	+
1116-73	O78:H11	−	−	+
1117-73	O78:H11	−	−	+

[a] H−, Nonmotile.

ciently specific. For example, O antigen 6, a documented EAS O antigen, is also the most frequently occurring O group reported in urine, blood, and normal stools (21). Our group 2 was selected to evaluate screening by O antigen alone.

Several investigators (12, 14) have suggested that the particular combination of O and H antigens making up an ETEC serotype is necessary for enterotoxigenicity. To test this supposition, we randomly selected group 2 (68 strains possessing EAS O antigens, but lacking the H antigens commonly associated with these EAS O antigens) from stock cultures and tested it for ST and LT. The fact that only 5 (7.4%) of 68 strains proved to be enterotoxigenic supports the proposal that enterotoxigenicity occurs more frequently in strains possessing a specific H antigen in conjunction with a specific O antigen. In addition, among ETEC, a relatively small number of H antigens (Table 10) have repeatedly been associated with a relatively small number of O antigens. The fact that a limited number of O and H antigens are most commonly encountered suggests specificity and a possible role for both in predisposing a strain to acceptance or maintenance or both of an enterotoxin plasmid. As evidence of this, each of the five enterotoxigenic strains (Table 6) from group 2 contained O and H antigens that previously had been associated separately with a number of EAS. None of the serotypes (O-H combinations) exhibited by these five strains, however, had previously been reported to contain ETEC.

We found that ETEC producing both ST and LT belonged to a smaller number of serotypes ($n = 6$) than did those producing ST or LT alone ($n = 13$). These data agree with a recent report by Merson et al. (12), who suggested that the larger ST-LT plasmid may be more readily accepted and retained by strains of *E. coli* with specific surface characteristics that include the O antigen. We would also include H antigen or, perhaps more importantly, a lack of H antigen (Table 10). It may be noteworthy that, of the five ETEC not belonging to a previously reported EAS, all produced only ST or LT alone. However, in recent studies (manuscripts in preparation), we have found evidence suggesting that plasmid incompatibilities may account to some extent for the presence of one or both enterotoxin plasmids.

It appears that a given strain of *E. coli* has the greatest chance of being enterotoxigenic if it possesses a specific EAS O antigen in conjunction with a specific EAS H antigen. However, a strain possessing a combination of any EAS O antigen and EAS H antigen may still have a better chance of being enterotoxigenic than do strains possessing an EAS O antigen alone, an EAS H antigen alone, or neither. If this proves to be correct and the presence or lack of individual H antigens is as important as O antigens are to ETEC, then any range of diagnostic antisera that is developed to screen most effectively for ETEC must include the capability of detecting specific H antigens and specific O antigens.

To our knowledge, the enterotoxigenic O149 strain (Table 6) is the first reported from a human patient; its isolation from urine is even more unusual. Of 20 strains known to have originated from urine specimens and tested for ST and LT, only this strain (2694-73, O149:H7) proved to be enterotoxigenic (ST+). These data agree with previous observations that ETEC do not occur regularly at extraintestinal sites (21). It is not known whether it can produce diarrheal disease in humans.

In an attempt to determine the prevalence of ETEC in the United States, 424 fresh, unserotyped isolates of *E. coli* from patients with diarrhea were sent to CDC between August 1978

TABLE 10. *H antigens most commonly expressed by ETEC belonging to the 21 EAS*[a]

H antigen	O antigen(s)
−[b]	6, 15, 20, 25, 78
7	27, 128
11	15, 78
12	20, 78, 128
16	6
19	8, 149
20	27, 159
21	109, 115, 128
28	148

[a] Serotypes reported in the literature on at least two occasions before July 1978 to contain enterotoxigenic strains.

[b] H−, Nonmotile.

and October 1979 specifically for ST and LT assays. None of these strains were enterotoxigenic. These data, plus the small number of ETEC received by CDC over the last 18 years, indicate that few, if any, enterotoxin assays will prove cost-effective without some screening procedure such as serotyping first being used. In another study (1), ETEC accounted for only 2.3% of diarrhea cases in any population, at any season, in midwestern Canada. Data from that study and ours lead to the conclusion that, although it may be useful for a reference laboratory to screen diarrheal outbreaks for ETEC, routine screening of *E. coli* isolates by clinical microbiology laboratories in the United States and Canada would be neither cost-effective nor medically justified at the present time.

In general, the serotypes of *E. coli* that were biotyped in this study possessed patterns of fermentation matching those reported in earlier studies (12, 15). The data in Table 7 agree with these earlier reports that EAS have predictable fermentation patterns; however, this does not seem sufficiently specific for screening ETEC. Non-ETEC in a particular serotype usually possess the same biotype as ETEC belonging to the same serotype (Table 8). In the past, a majority of ETEC from Bangladesh were found to be negative for sucrose fermentation (5), and it was proposed that such a phenotypic characteristic might serve to identify ETEC. As shown in Tables 7 and 8, a given strain of *E. coli* does or does not use sucrose depending on the serotype of the strain, not the enterotoxigenicity. Sucrose fermentation was of no value as a screen for ETEC among the 202 *E. coli* strains tested.

Most EAS were Adon$^-$ Dul$^+$, a few EAS (O25:H−, O27:H20) were mostly Adon$^-$ Dul$^-$, and only one EAS (O78:H11) was Adon$^+$ Dul$^-$ (Table 7). Since 13 (24%) of 55 ETEC were Adon$^+$ Dul$^-$, whereas only 3 (2%) of 147 non-ETEC were Adon$^+$ Dul$^-$ ($\chi^2 > 25$, $P < 0.001$), any Adon$^+$ Dul$^-$ strain of *E. coli* has a good chance of being enterotoxigenic. Only 7 (3.2%) of 202 *E. coli* strains were xylose negative; however, all 7 of these were both enterotoxigenic and Adon$^+$ Dul$^-$. Although the number of available strains was small, any strain of *E. coli* failing to ferment xylose should also be suspected of being enterotoxigenic. These data agree with those of other authors where there are comparable data (12, 15).

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