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Laboratory Investigation of Diarrhea in Travelers to Mexico: Evaluation of Methods for Detecting Enterotoxigenic *Echerichia coli*

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A laboratory investigation was conducted on cultures collected from travelers before, during, and after a trip to Mexico to characterize the etiology of traveler's diarrhea. Four laboratory methods for detecting enterotoxigenicity of *Escherichia coli* were evaluated: the infant mouse assay, the Chinese hamster ovary (CHO) cell assay, the Y1 adrenal cell assay, and the rabbit ileal loop. Although a number of common enteric pathogens were identified as a cause of traveler's diarrhea, including six serotypes of *Salmonella*, two serotypes of *Shigella*, *Vibrio parahaemolyticus*, *Giardia lamblia*, and *Entamoeba histolytica*, enterotoxigenic *Escherichia coli* was most commonly isolated. Strains were identified that produced only heat-labile enterotoxin (LT), only heat-stable enterotoxin (ST), or both LT and ST. The infant mouse assay yielded results falling into two distinct groups, providing a clear separation of positive and negative cultures. The CHO assay also formed two groups, with positive cultures producing 11% or more of the elongated cells. There was good agreement between the CHO and the Y1 adrenal cell assays for detection of LT. The adrenal cell system for detection of LT was more suitable than the CHO assay for processing large numbers of specimens because of the miniculture modification of this method utilized in this study. The infant mouse method was a simple and reliable method for detecting ST.

Diarrheal disease occurs frequently in travelers and is commonly referred to as traveler's diarrhea. The disease is usually mild and self-limiting, but in severe cases it may incapacitate the otherwise active traveler.

Although numerous studies have been conducted, the etiology of the disease has not been clearly defined (1, 5, 12, 13, 15, 17, 24, 29). The importance of *Escherichia coli* as a cause of traveler's diarrhea was first shown in a report of an outbreak of diarrhea in British soldiers caused by *E. coli* 0148, a strain that does not belong to one of the classical enteropathogenic serotypes (18). Subsequently, Shore et al. (24) reported that enterotoxigenic *E. coli* was partly responsible for traveler's diarrhea. Using the rabbit skin permeability assay, Gorbach et al. (9) recently found that 72% of the American students who developed traveler's diarrhea in Mexico had enterotoxigenic *E. coli* in their stool.

Recent studies have shown that *E. coli* causes disease by several mechanisms. Some strains cause a disease resembling shigellosis by invading the epithelium of the large bowel (4, 14). These invasive strains are identified in the laboratory by the Sereny test, in which positive strains cause keratoconjunctivitis in the eye of the guinea pig (23). Other strains cause disease by elaborating a heat-labile (LT) and/or a heat-stable (ST) enterotoxin. LT is a large protein molecule, which activates adenyl cyclase, is destroyed by heat (60 C), and is immunologically related to the enterotoxin of *Vibrio cholerae* (27). Production of LT is commonly measured by the rabbit ileal loop assay (20), the Y1 adrenal tumor cell culture assay (3, 21), the Chinese hamster ovary (CHO) cell culture assay (10), and the skin vascular permeability factor assay (7). ST is a small molecule, which is relatively stable to boiling and is not thought to be immunogenic. Production of ST is measured in the rabbit ileal loop assay (20) and the infant mouse assay (2).

In this paper we describe a study involving the laboratory investigation of traveler's diar-

rhea in a group of physicians and their families, primarily from the United States, who attended the World Congress on Gastroenterology in Mexico City in October 1974. In this investigation we evaluated four laboratory methods for detecting the enterotoxigenicity of *E. coli*: the infant mouse assay, the CHO cell assay, the Y1 adrenal cell assay, and the rabbit ileal loop. The epidemiological and clinical data from this study as well as the serological and viral studies are published elsewhere (16, 20).

MATERIALS AND METHODS

Specimen collection. Specimens were analyzed from 107 study participants before they left for Mexico, while they were in Mexico City, and after they returned from Mexico. Within 3 days prior to departure to Mexico, each participant was directed to defecate in a stool cup, insert three cotton swabs into the stool, rotate the swabs to coat them with feces, and put them into a tube of Cary-Blair transport medium. They then put the tube containing the swabs in a mailing kit and mailed it (Air Mail-Special Delivery) to the Center for Disease Control (CDC). The average time in transit was 2 days. On arrival at CDC the tubes were immediately refrigerated until they were inoculated to appropriate media. All specimens were inoculated on the day of arrival. Within 3 days after returning to the United States, each participant followed the same procedure to collect and ship three more swabs. In Mexico City, stool specimens were collected from each participant whether or not he or she became ill. Each participant was instructed to defecate in a stool cup and deliver the stools to CDC personnel stationed in Mexico City within 3 h after collection. Messenger service was provided between the laboratory and pick-up stations located at the meeting center and various hotels. All stools were inoculated to appropriate media as soon as they were received in the laboratory. (The average interval from collection of stool by participant to time of inoculation to laboratory media was 4.7 h.)

Bacteriology. The pre- and post-Mexico fecal swabs were inoculated as follows. One swab was inoculated directly to MacConkey agar and xylose-lysine-deoxycholate (XLD) agar. The second swab was inoculated to bismuth sulfite agar and then inserted into a tube of tetrathionate-brilliant green (TBG) broth. The TBG broth was incubated for 48 h at 37 C and streaked to brilliant green agar. The third swab was inoculated to thiosulfate citrate bile salts sucrose (TCBS) agar. All agar plates were incubated for 24 h at 37 C except bismuth sulfite agar, which was incubated for 48 h. For those specimens collected in Mexico, three swabs were inserted into the stool and rotated to coat the swab with feces. They were inoculated to plating media and enrichment broth by the same methods used for the pre- and post-Mexico swabs. In addition, approximately 10 g of stool was divided and inoculated into two preservatives, 5% polyvinyl alcohol and 10% formalin, to be examined for *Giardia lamblia* and *Entamoeba histolytica*.

The media were examined for pathogens as follows. (i) For *Salmonella* spp., three colonies characteristic of *Salmonella* were selected from the bismuth sulfite agar and brilliant green agar plates. Colonies were subcultured to triple sugar iron (TSI) and lysine iron agar, and cultures typical of *Salmonella* were identified and serotyped by previously described methods (6). (ii) For *Shigella* spp., three colonies characteristic of *Shigella* were selected from the MacConkey agar and XLD agar plates. Colonies were subcultured to TSI agar, and cultures typical of *Shigella* were identified and serotyped (6). (iii) For *Vibrio* spp., three sucrose-negative and three sucrose-positive colonies, if present, were selected from TCBS agar and subcultured to TSI agar. Colonies typical of *V. parahaemolyticus* or *V. cholerae* were biochemically and serologically examined (8). (iv) For *E. coli* and other gram-negative bacteria, five colonies (four lactose positive and one lactose negative) were selected from MacConkey agar and subcultured to heart infusion agar slants. If a predominance of non-*E. coli*-like colonies occurred on a plate, four additional colonies were selected. A total of 1,916 cultures of *E. coli* and other gram-negative bacteria were isolated. They were identified using the API Enteric 20 system (Analytab Products, Inc.), which has been demonstrated to have an accuracy of over 96% (28). Each of the *E. coli* and other gram-negative isolates was inoculated to heart infusion agar plates and incubated for 24 h; the growth was harvested with a cotton swab and suspended in 4 ml of medium consisting of 20% glycerol in Trypticase soy broth. Four 1-ml aliquots were dispensed in vials and stored at −70 C for use in the pathogenicity tests described below. Frozen stock cultures were used for all tests except the infant mouse test, for which stock cultures stored on agar slants were used.

Pathogenicity tests. (i) Infant mouse test. All isolates of *E. coli* and other gram-negative bacteria were tested for ST production using the infant mouse method of Dean et al. (2). Studies have shown this test to be specific for ST (11). Intragastric, percutaneous injections of culture filtrate were performed using at least four mice for each isolate. Positive strains produced intraintestinal fluid accumulation as indicated by a mean ratio (intestine weight/remaining body weight) of 0.083 or greater. One isolate from each culture-positive participant was retested after heating the culture filtrate at 100 C for 15 min.

(ii) CHO test. Forty-five *E. coli* cultures found positive by the adrenal cell test, isolates from all other ill individuals (303 isolates), and 263 randomly selected cultures found negative by the adrenal cell test and isolated from healthy individuals were tested for LT production using the CHO cell culture assay described by Guerrant et al. (10). Tests yielding more than 10% elongated cells were considered positive. When the results in the initial test fell in the range 8 to 13% they were retested and the results were averaged.

(iii) Adrenal cell test. All isolates were tested for LT production by a miniculture modification (21) of the adrenal tumor cell culture assay described by Donta et al. (3).

(iv) Rabbit ileal loop test. Strains found positive in any of the above assays and 28 of the negative isolates were tested in the rabbit ileal loop model (20). Bacteria were grown in glucose syncase medium (21) (10 ml in a 50-ml Erlenmeyer flask) on a rotary shaker at 37 C (200 rpm). A 1-ml sample of the sterile culture filtrate was injected into 5-cm ileal loops, and rabbits were sacrificed after 6 or 18 h. Both heated (100 C for 15 min) and unheated filtrates were tested in at least three rabbits, and a mean volume/length (milliliter/centimeter) ratio was determined. Only results from rabbits with appropriate positive and negative control loops were accepted.

(v) Sereny test. All isolates from at least one specimen collected during illness of each participant with traveler's diarrhea were tested for invasiveness. Heart infusion agar cultures were harvested and suspended in physiological saline. One drop of a suspension containing approximately 10^9 cells per ml was inoculated directly into the guinea pig conjunctival sac in a manner similar to that described by Sereny (23). The animals were observed for 72 h for development of keratoconjunctivitis.

Serotyping. Enterotoxin-producing and invasive *E. coli* were serotyped using 138 *E. coli* O antisera in 21 pools (6). When isolates from two or more participants had the same antigen, the H antigens were determined. Antisera for serotyping were obtained from the Biological Products Division, CDC.

RESULTS

All pre-Mexico specimens were negative for pathogens. However, common enteric pathogens accounted for 19 of 48 (40%) of the pathogens isolated from specimens collected in Mexico and after the trip. *Salmonella* was isolated from nine ill travelers and four well excretors. Six serotypes were represented among these 13 isolates. *Shigella* organisms of two different serotypes were isolated from two ill individuals, parasites were isolated from three, and *V. parahaemolyticus* was isolated from one (Table 1).

TABLE 1. *Common enteric pathogens isolated from 107 participants*

Pathogen	Ill	Well	Total
Salmonella (all serotypes)	9	4	13
S. saint-paul[a]	2	1	3
S. blockley	1	0	1
S. senftenberg	1	0	1
S. anatum	3	1	4
S. infantis	2	1	3
S. newport	0	1	1
Shigella flexneri	1	0	1
Shigella sonnei[a]	1	0	1
Giardia lamblia	1	1	2
Entamoeba histolytica	0	1	1
Vibrio parahaemolyticus	1	0	1

[a] One patient had a multiple infection of *S. saint-paul* and *S. sonnei*.

Other gram-negative bacteria isolated are shown in Table 2. *E. coli* accounted for 87.1% of the isolates collected in pre-Mexico specimens, 94.9% of isolates from specimens collected in Mexico, and 91.6% from post-Mexico specimens. Seven different bacterial species were isolated from specimens collected in Mexico, whereas pre-Mexico and post-Mexico specimens yielded 10 and 14 species, respectively. No significant difference was noted in the frequency of the various species in ill and well participants (Table 3).

Values obtained on 1,916 cultures in the infant mouse assay expressed in ratios of intestinal weight/remaining body weight are plotted in Fig. 1. The most frequent ratio was 0.060, and the negative isolates formed a symmetrical bilateral peak around this point. There were two distinct populations among these cultures clearly separating the positive and negative groups; i.e., 1,873 negative cultures had a ratio ≤ 0.076, and 43 positive cultures a ratio ≥ 0.083. All cultures found positive in the infant mouse assay were positive in the 6-h rabbit ileal loop assay, and their activity was stable in both assays after filtrates were boiled for 15 min. Cultures yielding values of 0.074 to 0.076 were negative in the rabbit ileal loop.

The percentage of elongated cells in the CHO test for 611 *E. coli* cultures was determined, and the number of cultures was plotted for each value. The values ranged from 0 to 38% (Fig. 2). Two populations were demonstrated of positive and negative cultures, with very few cultures falling in the intermediate zone. Forty-seven cultures were positive, and 564 were negative.

A comparison of the CHO assay and the adrenal cell assay with the 611 cultures is shown in Table 4. Identical results were obtained, except in two instances in which the CHO test was positive and the adrenal test was negative. These two isolates yielded strongly positive values in the CHO test, one producing 18% and the other producing 23% elongated cells, but these isolates were negative in the rabbit ileal loop at 18 h. Cultures yielding values of 9 and 10% were also negative in the rabbit ileal loop.

The results of all laboratory tests used to determine pathogenic strains are shown in Table 5. Those tests considered indicators of LT production were CHO cell tests, the adrenal cell test, and the 18-h rabbit ileal loop assay. Those tests considered indicators of ST production were the infant mouse test and the heated culture filtrate in the 6-h rabbit ileal loop assay. The Sereny test was used to detect invasive strains. Only two cultures were positive in the Sereny test. Of the 1,916 gram-negative bacteria tested, only *E. coli* strains were found to be

TABLE 2. *Frequency of isolation of gram-negative bacteria from pre-, post-, and in-Mexico specimens*[a]

Identity	Frequency of isolation							
	Pre-Mexico		In Mexico		Post-Mexico		Total	
	No.	%	No.	%	No.	%	No.	%
Escherichia coli	467	87.13	599	94.92	686	91.59	1,752	91.44
Klebsiella pneumoniae	22	4.10	9	1.43	26	3.74	57	2.97
Citrobacter freundii	22	4.10	11	1.74	7	0.93	40	2.09
Enterobacter cloacae	10	1.87	5	0.79	7	0.93	22	1.15
Enterobacter hafniae	5	0.93	5	0.79	1	0.13	11	0.57
Klebsiella pneumoniae oxytoca	3	0.56			8	1.07	11	0.57
Proteus morganii					6	0.80	6	0.03
Aeromonas hydrophila	1	0.19	1	0.16	2	0.27	4	0.02
Enterobacter agglomerans	4	0.74					4	0.02
Proteus mirabilis			1	0.16	1	0.13	2	0.01
Pseudomonas sp.	1	0.19			1	0.13	2	0.01
Serratia marcescens	1	0.19			1	0.13	2	0.01
Aeromonas sp.					1	0.13	1	<0.01
Citrobacter sp.					1	0.13	1	<0.01
Providencia alcalifaciens					1	0.13	1	<0.01
Total	536		631		749		1,916	

[a] Colonies were selected from MacConkey agar for pathogenicity tests. Isolates were not randomly selected (see Materials and Methods). Isolates were identified by the API enteric 20 system.

TABLE 3. *Frequency of isolation of gram-negative bacteria in specimens from ill and healthy subjects*

Identity	Frequency of isolation			
	Ill		Well	
	No.	% Total	No.	% Total
E. coli	398	90.05	1,354	91.86
K. pneumoniae	12	2.71	45	3.05
C. freundii	8	1.81	32	2.17
E. cloacae	11	2.49	11	0.75
E. hafniae	4	0.90	7	0.47
K. pneumoniae oxytoca			11	0.75
P. morganii	3	0.68	3	0.20
A. hydrophila	2	0.45	2	0.14
E. agglomerans			4	0.27
P. mirabilis	1	0.23	1	0.07
Pseudomonas sp.	1	0.23	1	0.07
S. marcesens			2	0.14
Aeromonas sp.	1	0.23		
Citrobacter sp.			1	0.06
P. alcalifaciens	1	0.23		
Total	442		1,474	

enterotoxigenic or invasive by the above methods. The methods by which they were determined to be pathogenic were: (i) production of both LT and ST, (ii) production of LT only (positive in both the CHO and adrenal cell test), (iii) CHO positive reaction only, (iv) production of ST only, and (v) invasiveness.

In this study only one pathogenic strain was of a serotype normally considered to be enteropathogenic in infants (serotype O128, ST only). In fact, one would not have been able to detect pathogenic strains in these individuals by serotyping the strains. All strains producing both LT and ST, however, were *E. coli* serotype 06:H16, whereas multiple serotypes were noted in all other categories of pathogenic *E. coli* (Table 5). The strains producing both LT and ST also had multiply sensitive antibiotic resistance patterns, whereas strains producing only LT and only ST had a variety of resistance patterns (16).

The association of *E. coli* found positive by any of the four tests with illness is summarized in Table 6. Specimens from eight ill individuals were positive for organisms producing both LT and ST. Specimens from 14 individuals yielded isolates which produced only LT. Eleven of these were from ill individuals, and three were from well participants. One of the ill individuals also yielded an isolate of *Salmonella anatum* and one yielded an isolate of *V. parahaemolyticus*; another ill individual (Table 5, no. 327) had two serotypes of LT-producing *E. coli* in his stool. Isolates from five individuals, all of whom were ill, produced only ST. One of these individuals was also positive for *G. lamblia*, and one was positive for invasive *E. coli*. Finally, specimens from two ill individuals yielded invasive strains of *E. coli*; one person was also positive for *Salmonella senftenberg*. In all participants the enterotoxigenic and invasive *E. coli* were isolated from the specimen obtained earliest in their illness. No enterotoxigenic or invasive *E. coli* strains were isolated from pre-Mexico specimens.

Many isolates were tested on multiple occasions. One of two invasive, one of five ST-only,

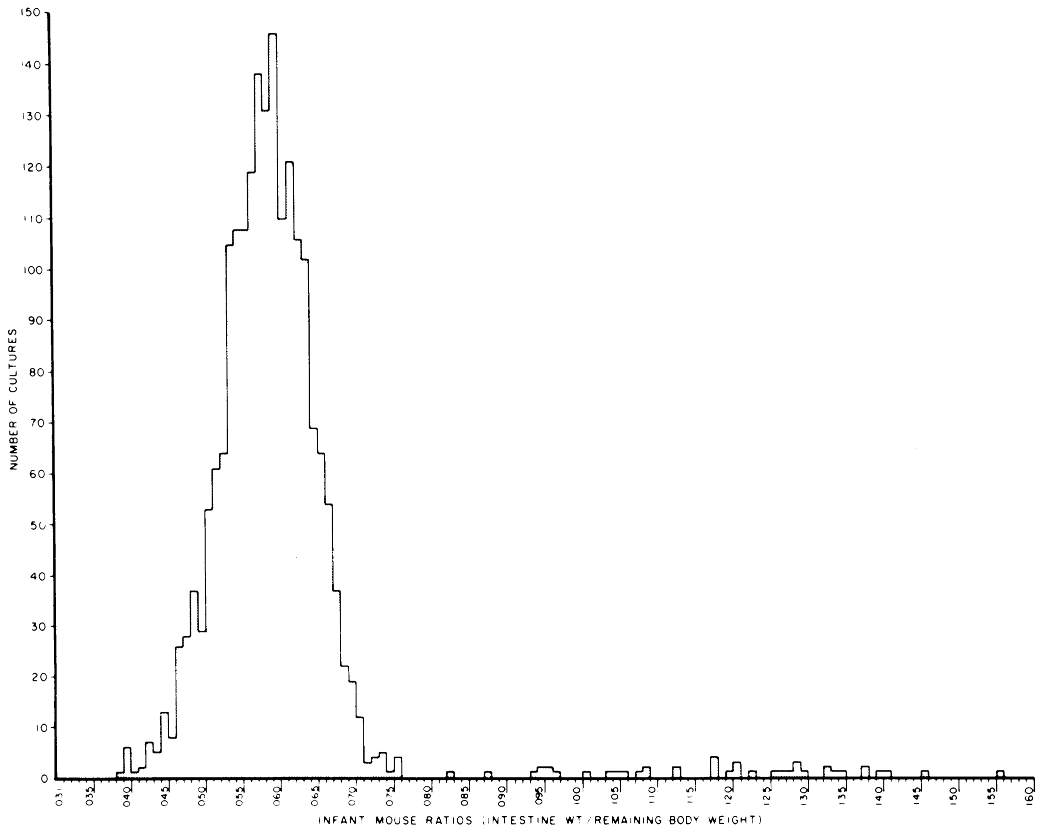

FIG. 1. *Infant mouse test for detection of toxigenic E. coli.*

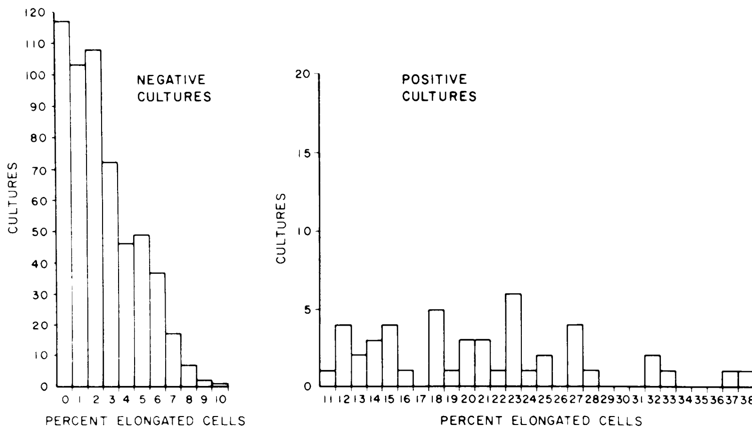

FIG. 2. *CHO cell culture for detection of LT.*

and one of two CHO-detectable LT-only strains lost the pathogenic property after being held on laboratory media for 3 months. Otherwise, in cultures producing LT only or LT and ST, there was no evidence of permanent loss of enterotoxigenicity, although some cultures gave variable results from test to test.

Five isolates were examined from each specimen for detection of enterotoxigenic *E. coli*. The results of the efficiency of examining multiple isolates are shown in Table 7. Fifty-four percent of those individuals positive for ST *E. coli* and 52% of those positive for LT *E. coli* would have been detected by examination of

TABLE 4. *Comparison of CHO cell assay and adrenal cell assay*

CHO cell assay	Adrenal cell assay	
	Positive[a]	Negative
Positive	45	2
Negative	0	564

[a] Values indicate the number of cultures positive by each test.

only one isolate; three isolates would have detected 85 and 76%, respectively.

DISCUSSION

A recent study indicated that LT-producing enterotoxigenic *E. coli* plays a predominant role in traveler's diarrhea in Mexico (9). In this study we also found that enterotoxigenic *E. coli* played a significant etiological role, accounting in part for 45% of cases (16, 20). However, we found that the more commonly recognized enteric pathogens also were an important cause of diarrhea. *Salmonella* was isolated from thirteen (12%) of participants, nine of whom were symptomatic, *Shigella* was isolated from two ill individuals, and *V. parahaemolyticus* was isolated from one ill person. The parasite *G. lamblia* or *E. histolytica* was found in one ill and two well participants. This may be an underestimate of the etiologic significance of these parasites, since the incubation period for giardiasis and amebiasis is usually 1 week or more, and in this study whole stools were not collected after the participants left Mexico. We also isolated invasive *E. coli* from two ill participants.

The variety of pathogenic mechanisms demonstrated in vitro by the *E. coli* strains was of great interest. Strains were identified with three types of enterotoxigenic mechanisms: (i) production of LT and ST, (ii) production of LT only, and (iii) production of ST only. The isolation of strains producing only LT and only ST confirmed the importance of LT as a cause of human diarrhea and demonstrated clearly for the first time the pathogenic role of ST (20). Excellent correlation was found in the adrenal and CHO cell assays for measurement of LT. The meaning of the positive results on two strains in the CHO cells, but not in the adrenal cell test, is unclear, especially since these strains were negative in the rabbit ileal loop model. A wide variety of gram-negative bacteria was isolated (Table 2), but only *E. coli* were found to be pathogenic by the laboratory tests used in this study.

No enterotoxigenic or invasive *E. coli* was isolated from pre-Mexico specimens. This is consistent with previous studies showing that enterotoxigenic *E. coli* strains are rarely encountered in stools from nondiarrheal adult patients in the United States (9, 30). Rudoy and Nelson (19) found 41% of stools from nondiarrheal children positive for enterotoxigenic *E. coli*; however, these strains did not produce LT and their criterion for positivity in the infant mouse assay was 0.071, a ratio less than that considered positive in this study.

It appears from these studies that one should examine *E. coli* cultures for pathogenicity by at least three tests: one to detect LT production, another to detect ST production, and a third to detect invasiveness. We preferred the adrenal cell assay for the detection of LT production because it does not require the quantitative cell count needed in the CHO system, and when adapted to the microculture method as used in this study it was more rapid and economical. However, recent adaptations of the CHO assay to the microculture technique may facilitate use of this assay (K. I. Steinberg, J. C. McLaughlin, S. H. Richardson and R. L. Guerrant, personal communication). We found the infant mouse assay reliable for detection of ST and found it correlated well with the 6-h rabbit ileal loop, which is a more expensive and technically more difficult method. Analyzing only one colony from a plate detected 52 to 54% as many positive patients as examining five colonies, whereas selecting three colonies was 76 to 85% as effective as examining five. It should be pointed out that in defining a case as an individual with at least one out of five cultures positive for enterotoxigenic *E. coli*, a small number of true cases were missed, i.e., those in which the first five colonies were negative, but the sixth or subsequent colony, if selected, would have been positive.

There is concern that enterotoxigenic strains may quickly become non-enterotoxigenic after isolation (R. L. Guerrant and M. D. Dickins, Prog. Abstr. Intersci. Conf. Antimicrob. Agents Chemother., 14th, San Francisco, Calif., abstr. 130, 1974), since enterotoxin production in *E. coli* isolated from humans has previously been shown to be associated with transmissable plasmids (25, 26). Loss of pathogenicity was observed in one of two invasive strains, in one of five strains producing only ST, and in one of 2 CHO-detectable LT strains. Otherwise, there was no permanent loss of enterotoxigenicity in cultures producing LT only or LT and ST, although some isolates yielded different results from test to test. Nevertheless, all cultures should be tested promptly after isolation.

TABLE 5. *Results of laboratory tests on pathogenic strains of E. coli*

Specimen no.	Ill	Isolate no.	Serotype	Results of pathogenicity test: Sereny test	Infant mouse[a]	Rabbit ileum[b] 6-h Unheated	6-h Heated[c]	18-h Unheated	18-h Heated	CHO cell[d]	Adrenal cell	Interpretation
293	Yes	C5	R	+	0.065					4	–	Invasive
213	Yes	C1	NT[e]	+	0.052					0	–	Invasive
213	Yes	C2	O128	–	0.121	0.61	0.46	0.09	0.20	5	–	ST only
		C3	O128	–	0.113	0.76	0.48	0.37	0.30	5	–	ST only
		C4	O128	–	0.140	0.79	0.56	0.39	0.35	3	–	ST only
		C5	O128	–	0.130	0.91	0.50	0.35	0.29	0	–	ST only
214	Yes	C1	NT	–	0.135	0.46	0.34	0.26	0.29	0	–	ST only
		C2	NT	–	0.108	0.66	0.67	0.14	0.20	0	–	ST only
		C3	NT	–	0.118	0.62	0.46	0.59	0.44	0	–	ST only
		C4	NT	–	0.123	0.45	0.67	0	0.19	6	–	ST only
		C5	NT	–	0.120	0.56	0.42	0.76	0.70	0	–	ST only
471	Yes	C2	O8	–	0.134	0.81	0.58	0.49	0.78	2	–	ST only
514	Yes	C1	NT	–	0.118	0.54	0.49	0.2	0.2	6	–	ST only
		C2	NT	–	0.104	0.85	0.60	0.6	0	2	–	ST only
		C3	NT	–	0.088	0.40	0.52	0.23	0.8	5	–	ST only
		C4	NT	–	0.083	0.70	0.69	0		6	–	ST only
		C5	NT	–	0.101	0.57	0.68	0		7	–	ST only
617	Yes	C5	O50	–	0.096	0.56	0.34	0	0	0	–	ST only
219	Yes	C1	O6:H16	–	0.141			1.07		19	+	LT-ST
		C2	O6:H16	–	0.121					12	+	LT-ST
		C3	O6:H16	–	0.128					15	+	LT-ST
		C4	O6:H16	–	0.105					18	+	LT-ST
		C5	O6:H16	–	0.095					14	+	LT-ST
260	Yes	C1	O6:H16	–	0.127			1.65		33	+	LT-ST
		C2	O6:H16	–	0.117					27	+	LT-ST
322	Yes	C5	O6:H16	–	0.118			2.16		25	+	LT-ST
325	Yes	C1	O6:H16	–	0.138			1.13		18	+	LT-ST
		C2	O6:H16	–	0.126					14	+	LT-ST
		C3	O6:H16	–	0.118					15	+	LT-ST
		C4	O6:H16	–	0.129					23	+	LT-ST
		C5	O6:H16	–	0.097					12	+	LT-ST
405	Yes	C3	O6:H16	–	0.146			1.57		37	+	LT-ST
412	Yes	C1	O6:H16	–	0.138			1.26		32	+	LT-ST

		C2	O6:H16	–	0.129				24	+	LT-ST
		C3	O6:H16	–	0.129				21	+	LT-ST
		C4	O6:H16	–	0.121				27	+	LT-ST
451	Yes	C2	O6:H16	–	0.096			1.03	32	+	LT-ST
462	Yes	C1	O6:H16	–	0.106	0.95	0.40	0.56	25	+	LT-ST
		C2	O6:H16	–	0.094				22	+	LT-ST
		C3	O6:H16	–	0.095				14	+	LT-ST
206	Yes	C2	R[f]	–	0.060	1.04	0	1.67	23	+	LT only
		C3	NT	–	0.059				28	+	LT only
218	No	C1	O40	ND[g]	0.059	1.18	0.15	1.79	23	+	LT only
		C2	O40	ND	0.053				15	+	LT only
224	Yes	C3	NT	ND	0.055	0.88	0	0.71	13	+	LT only
		C4	NT	ND	0.061				38	+	LT only
		C5	NT	ND	0.057				20	+	LT only
225	Yes	C4	O75	–	0.055			1.44	18	+	LT only
286	No	C2	O8:NT	ND	0.060	1.4	0	2.08	27	+	LT only
289	No	C1	O8:NT	–	0.058	0.29	0	0.97	23	+	LT only
		C2	O8:NT	–	0.058				27	+	LT only
		C3	O8:NT	–	0.057				16	+	LT only
308	Yes	C4	NT	–	0.058	0.50	0	1.58	13	+	LT only
327	Yes	C1	O25:NM[h]	–	0.059	0.70	0.10	1.29	21	+	LT only
		C2	O5	–	0.062				20	+	LT only
		C4	O25:NM	–	0.060				21	+	LT only
427	Yes	C1	O25:NM	–	0.058				15	+	LT only
		C2	O25:NM	–	0.052	0.44	0	1.08	18	+	LT only
		C4	O25:NM	–	0.048				12	+	LT only
484	Yes	C7	NT	–	0.055	0.86	0	0.78	11	+	LT only
490	Yes	C1	NT	–	0.060	0.55	0	0.9	12	+	LT only
504	Yes	C1	O25:NM	–	0.054	0.68	0	2.2	20	+	LT only
		C2	O25:NM	–	0.057				23	+	LT only
290	Yes	C5	O1	–	0.060	0	0	0	23	–	CHO only
294	Yes	C4	NT	–	0.055	0	0	0	18	–	CHO only

[a] Intestine weight/remaining body weight.
[b] Volume (milliliter)/length (centimeter).
[c] Cell-free filtrates heated at 100 C for 15 min.
[d] Percentage of elongated cells.
[e] NT, Nontypable.
[f] R, Rough strains.
[g] ND, Not done.
[h] NM, Nonmotile strains.

TABLE 6. *Summary of enterotoxigenic and invasive E. coli*

Mechanism of pathogenicity	Ill	Well	Total	Other pathogens isolated
LT and ST	8	0	8	
LT only	11[a]	3	14	*S. anatum* (1)[b] *V. parahaemolyticus* (1)[b]
ST only	5	0	5	*G. lamblia* (1) Invasive *E. coli* (1)
Invasive	2	0	2	*S. senftenberg* (1) ST *E. coli* (1)
Total	26	3	29	

[a] Included two cultures positive for CHO-detectable enterotoxin only.
[b] Isolated from ill participants.

TABLE 7. *Efficiency of testing multiple fecal isolates for diagnosing patients with enterotoxigenic E. coli diarrhea*

Colony no.[a]	Patients with enterotoxigenic *E. coli*			
	ST		LT	
	No.	% of total	No.	% of total
1	7	54	11	52
2	10	77	14	67
3	11	85	16	76
4	11	85	19	90
5	13	100	21	100

[a] Numbers indicate order in which colonies were selected from the plates. Values on the first line indicate the number of positive participants that would have been detected if only one colony had been tested, the second line gives results for two colonies, etc.

The serotype O6:H16 was commonly found, and travelers with this organism may have been exposed to a common source. Of the 29 people yielding positive strains of *E. coli* by one or more tests for pathogenicity, only one patient yielded an isolate with a serotype commonly referred to as "enteropathogenic *E. coli*." This supports previous findings (22) and the concept that pathogenicity in adults is not a function of these serotypes but rather may be related to other serotypes or other virulence factors such as plasmid-acquired ability to produce enterotoxin.

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