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Clinical Cholera Caused by Enterotoxigenic *Escherichia coli*

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A woman returning from Mexico was hospitalized as an emergency patient with hypovolemic shock due to diarrheal disease of less than 1-day duration. Her clinical course was similar to that of severe cholera—she excreted greater than 60 liters of stool and urine in a 4-day period. The etiological agent was a non-enteropathogenic serotype but enterotoxigenic strain of *Escherichia coli* (063:NM). The patient responded with both agglutinating and antitoxic antibodies against this strain and its enterotoxin. An "enteropathogenic serotype," 0111:B4, was also isolated but this finding had no etiological significance.

On Sunday, 13 April 1975, a 36-year-old, 52.6-kg female (P.T.), who had just returned from a cruise to Mexico, was taken from Dallas/Ft. Worth Regional Airport to Parkland Memorial Hospital, Dallas, Tex., as an emergency patient in hypovolemic shock due to diarrheal disease of less than 1-day duration. With adequate intravenous and oral fluid replacement therapy her subsequent clinical course was uneventful, although from the period between 14 April and 17 April she had discharged a total of 60.7 liters of stool (51.2 liters) and urine (9.5 liters). She was asymptomatic on 18 April and was released from the hospital at that time.

Anecdotal information indicated that more than 100 of the 500 passengers on the ship had manifested some forms of gastrointestinal disturbances although none were as severely affected as P.T. However, only 12 passengers were recorded in the ship's log as having been seen by the ship's physician for diarrheal disease. Routine stool culture on admission failed to reveal enteric pathogens (Salmonella or Shigella). On Tuesday, 15 April, the diagnosis of cholera (which had occurred in a single, isolated case in Texas in 1973 [9]) was considered.

A sample of typical "rice-water" stool was submitted to the Department of Microbiology for examination and culture. Immediate microscopic examination of a hanging-drop preparation revealed many rod-shaped bacteria but no motile organisms, thus reducing the likelihood of *Vibrio cholerae*. There was no evidence of pus cells, ova, or parasites. The stool specimen was streaked on meat-extract agar (3), TCBS agar (BBL), and MacConkey agar (Difco). Examination of the meat-extract agar plates at 6 h and subsequently with a stereoscope and transmitted oblique illumination failed to reveal colonies characteristic of cholera vibrios and there was no growth on TCBS agar held for 48 h. Lactose-fermenting colonies typical of *Escherichia coli* predominated on MacConkey agar. Ten of these were picked for further study and were confirmed as *E. coli* by fermentation reactions. Five isolates were agglutinated in enteropathogenic serotype 0111:B4 antiserum (Difco) in slide-agglutination tests. Five isolates were nontypable with the antisera available to us. A single lactose-negative colony produced an alkaline slant and an acid-plus-gas butt in triple sugar iron agar but was not further identified, since convalescent serum (taken 6 May) from the patient was found subsequently to agglutinate four of the five nontypable *E. coli* isolates with a titer of 1:1,280 in tube-agglutination tests. A representative subculture was subsequently found to belong to O group 063 by Frits and Ida Ørskov of the Collaborative Centre for Reference and Research on Escherichia (W.H.O.), Statens Seruminstitut, Copenhagen, Denmark. (They indicated [personal communication] that a single toxinogenic motile 063 strain was found previously in their extensive collection [F. Ørskov et al., submitted for publication].) Acute phase serum was unreactive with these strains and no rises in titer were observed with the lactose-negative strain or with the 0111:B4 strains. Each of the 11 isolates was cultivated in 50 ml of dialyzed Trypticase soy broth (BBL) (Finkelstein et al., J. Infect. Dis., in press) with shaking at 37 C and the 24-h sterile culture filtrates were concentrated approximately 25-fold by ultrafiltration on PM-10 membranes (Amicon Corporation). These concentrates were

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used in tests for the presence of heat-labile *E. coli* enterotoxin (LT) in Ouchterlony-type double diffusion precipitation with antisera against isolated LT preparations (R. A. Finkelstein, et al., J. Infect. Dis., in press) in rabbit skin (1, 2), in Chinese hamster ovary (CHO) cell cultures (5), and in adult rabbit ileal loops. The results are summarized in Table 1. Concentrates of nine *E. coli* culture supernatants revealed some precipitin activity with one or both antisera against *E. coli* LT (Finkelstein et al., J. Infect. Dis., in press) but, of these, only four were highly active in rabbit skin and CHO cells in which they caused typical elongation when diluted ≥1:625. These results were available on 18 April. These four positive filtrates were from the nontypable strains with which the patient's serum subsequently showed diagnostic rises in agglutinin titer. Two of these strains produced positive rabbit ileal loops with fluid/length ratios ranging from 0.58 to 2.01 ml/cm. Two of the 0111:B4 strains had traces of activity at dilutions up to 1:25 in CHO cells and two (one CHO cell and antigen negative and one CHO cell and antigen positive) produced positive responses (0.67 to 0.94 ml/cm) in rabbit ileal loops. As the latter tests were performed only in duplicate on a single occasion, the possibility of nonspecific reactions cannot be excluded. The unidentified lactose-negative strain exhibited a slight precipitin reaction but was unreactive in CHO cells and was not tested in ileal loops.

Because of the demonstrated presence of enterotoxigenic *E. coli* in the patient's stool, the presumptive diagnosis of diarrhea due to nontypable, nonmotile enterotoxigenic *E. coli* was entertained on 18 April and this was confirmed by the subsequent demonstration of the patient's specific antibody rise to these organisms. Subsequently, LT was isolated from fermentor-grown cultures of one of the isolates (Finkelstein et al., J. Infect. Dis., in press) and the patient's serum was found to exhibit a rise in titer of neutralizing antibody against *E. coli* LT in rabbit skin tests.

The case is illustrative of several points. (i) It indicates the potential of *E. coli* strains to produce severe secretory diarrhea resembling severe cholera in some individuals. (ii) It raises the question of why some individuals are more responsive than others to infection with enterotoxigenic bacteria. None of the other cruise members was as severely affected. Although toxinogenic *E. coli* strains are increasingly being implicated in diarrheal disease of adults, e.g., (4), and children (7), the diarrhea is generally much milder than that of patient P.T. (iii) It points out quite clearly that belonging to an enteropathogenic serotype does not necessarily mean that a strain is pathogenic. In fact, the term "enteropathogenic serotype" ought to be rendered inoperative until more meaningful criteria are established. In this instance, the 0111:B4 isolates were most likely adventitious. (iv) As the capability of elaborating *E. coli* enterotoxin is genetically determined by means of transmissible extrachromosomal elements, or plasmids (6, 8), it appears that any strain of *E. coli*, and probably other genera as well, may acquire the capability of producing enterotoxin

TABLE 1. *Identification of enterotoxigenic E. coli (ETEC) from patient P.T.*

Colony	Serotype	Motility	Precipitin reaction[a]	Skin test	CHO cells	Ileal loop	Rise in agglutinin titer
1	0111:B4	−	+	−	−	ND[b]	−
2	0111:B4	−	+	−	± (1:25)	ND	ND
3	0111:B4	−	−	−	−	±	ND
4	0111:B4	−	±	−	−	−	ND
5	0111:B4	−	+	±	± (1:25)	±	ND
6	NT[c]	+	+	−	−	ND	−
7	NT	−	+	++++	++++ (≥1:625)	++	1:1,280
8	NT	−	+	++++	++++ (≥1:625)	±	1:1,280
9	NT	−	+	++++	++++ (≥1:625)	ND	1:1,280
10	NT	−	+	++++	++++ (≥1:625)	ND	1:1,280

[a] With anti-*E. coli* enterotoxin in Ouchterlony tests with concentrated culture supernatants.
[b] ND, Not done.
[c] NT, Nontypable—strain 7 was subsequently identified as 063 (see text).

and may become an enteropathogen if it can also colonize the small bowel. It is possible that other strains isolated in the present study may have acquired a transmissible *ent* plasmid, as evidenced by their precipitin reactions, but were either unable to produce a fully effective enterotoxin or to localize in the small bowel. It is also possible that the precipitating antisera used are not completely specific for *E. coli* enterotoxin. Further study will be needed to resolve these issues. (v) The study also points out that new methodology must become available to diagnostic laboratories if they are to establish the etiology of diarrheal disease cases. (vi) The classic approach of confirming etiology by means of simple and old-fashioned agglutination tests with patients' acute and convalescent sera should not be overlooked.

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