Severe Protracted Diarrhea due to Multiresistant Adherent *Escherichia coli*

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• We studied 15 cases of severe protracted diarrhea due to a strain of *Escherichia coli* serotype 0111:K58:H2. The clinical features of these patients were compared with those of 18 patients infected with other enteropathogenic serotypes of *E coli*. More patients infected with 0111:K58:H2 strains had been treated with ampicillin (six of 15 *v* one of 18), and more were dehydrated (nine of 15 *v* two of 18) and in a toxic condition (nine of 15 *v* three of 18). The number of stools per day (14.1 *v* 6.8), the total duration of the diarrhea (24.7 *v* 7.0 days), and the frequency of relapses (six of 15 *v* none of 18) were also greater in these patients. A large number had a serum sodium level below 135 mEq/L (ten of 15 *v* none of 18) and a serum protein level below 11 mEq/L (six of 15 *v* none of 18). At least two infants infected with *E coli* 0111:K58:H2 manifested a urinary tract infection. All 15 strains of *E coli* 0111:K58:H2 studied adhered to HeLa cell lines, whereas none of the strains isolated from the second group were adherent. None of the 0111:K58:H2 strains produced enterotoxins, and they were negative in the Serény test. All adherent strains were resistant to many antibiotics.

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In 1945, Bray demonstrated that *Escherichia coli* could cause diarrhea.[1] In the decades that followed this discovery, it was shown that *E coli* could cause gastrointestinal disease by two mechanisms: invasion of the intestinal epithelium and production of several enterotoxins.[2] Strains of a third group, called the enteropathogenic serogroup, were also recognized as primary intestinal pathogens; however, the pathogenesis of the illness caused by these strains remained largely an enigma.[3] Certain strains of this last group were shown to secrete enterotoxinlike substances.[4] Since 1977, massive adherence to and histologic changes of the epithelium of the intestinal tract by *E coli* has emerged as a new mechanism of intestinal disease in animals and people.[5-8]

We report a series of cases of severe protracted diarrhea due to a strain of adherent *E coli* (0111:K58:H2). The purpose of this article is to present evidence that adherent enteropathogenic *E coli* may be associated with severe diarrhea, and to describe the clinical setting and findings in these patients.

PATIENTS AND METHODS

Study Patients

During an 11-month period beginning in the summer of 1980, all patients hospitalized on the same ward for a bout of gastroenteritis associated with an enteropathogenic serotype of *E coli* were studied. A total of 15 patients were infected with *E coli* serotype 0111:K58:H2, resistant to multiple antibiotics, whereas six patients had *E coli* serotype 0111:K58 that was sensitive to antibiotics (four strains were H21, one strain was H12, and one strain was nonmotile), and 12 patients grew other enteropathogenic serotypes of *E coli* sensitive to antibiotics (0128:K67, four strains; 0125:K70 and 026:K60, two strains each; 0119:K69, 0126:K71, 0124:K72, and 086:K61, one strain each). Clinical data collected were previous treatment with antibiotics, hydration status, toxic appearance, temperature, vomiting, duration of diarrhea, maximum number of stools per day, macroscopic blood in stools, and rash. Diarrhea was defined as the observation of more than three liquid stools per day. Relapses were defined as the recurrence of diarrhea after normal stools were defecated. Laboratory data collected included blood cell counts, serum electrolyte levels, and protein levels.

Stool Cultures

Stool specimens were inoculated onto sheep blood agar, MacConkey agar, *Salmonella-Shigella* agar, and bismuth sulfite agars, all of which were incubated at 35 °C, and on a selective medium for *Campylobacter*,[9] which was incubated in a 5% oxygen atmosphere at 42 °C. A cross-smear of the growth on the blood agar plate was screened by slide agglutination with commercial antiserum pools for the presence of enteropathogenic serotypes of *E coli*. Following a positive screening test, individual strains were isolated, identified biochemically, and typed with monospecific antiserum by slide agglutination. All strains identified as enteropathogenic serotypes of *E coli* were sent to the Quebec Laboratory of Public Health, Montreal, for confirmation and complete seroidentification by tube agglutination. Serotyping was done according to standard techniques.[10] Strains were stab inoculated into semisolid medium containing 0.8% nutrient broth and 0.75% agar and kept at room temperature. Enteric viruses were looked for by electron microscopy in 16 instances, and parasites by optic microscopy on stool specimens in 12 instances.

Antibiotic Susceptibility Testing

A standard agar dilution technique was used to determine the susceptibility of the microorganisms to ampicillin, ticarcillin disodium, cephalothin sodium, chloramphenicol, kanamycin sulfate, gentamicin sulfate, and trimethoprim-sulfamethoxazole.[11] Resistance was defined as a minimum inhibitory concentration greater than 64 mg/L for ampicillin, 128 mg/L for ticarcillin disodium, 32 mg/L for cephalothin sodium, 16 mg/L for chloramphenicol, 32 mg/L for kanamycin sulfate, 12 mg/L for gentamicin sulfate, and 5 mg/L of trimethoprim and 100 mg/L of sulfamethoxazole for the combination. Microorganisms were considered sensitive if inhibited by less than or equal to 4 mg/L of ampicillin, 16 mg/L of ticarcillin disodium, 4 mg/L of cephalothin sodium, 4 mg/L of chloramphenicol, 4 mg/L of kanamycin sulfate,

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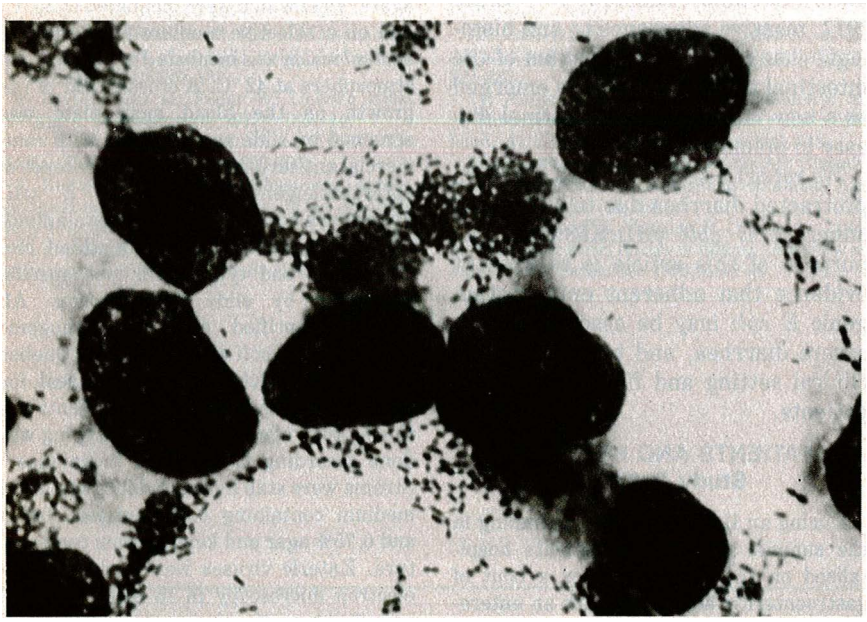
Clinical Findings

	Enteropathogenic *Escherichia coli*		
	0111:K58:H2 (15 Patients)	Other Strains (18 Patients)	P
Dehydration >5% of body weight	60%	11%	<.01
Temperature >38.5 °C	60%	33%	NS
Vomiting	60%	33%	NS
Toxic appearance	60%	16%	.045
Maximum number of stools/day (mean ± SD)	14.1 ± 5.7	6.8 ± 3.7	<.001
Duration of diarrhea, days (mean ± SD)	24.7 ± 15.3	7.0 ± 6.2	<.001
Macroscopic blood in stool	6%	16%	NS
Recurrence of diarrhea	60%	0%	.009



Escherichia coli adherence to HeLa cell lines. Large bacterial clumps are obvious (Giemsa stain, ×1,000).

4 mg/L of gentamicin sulfate, and 1 mg/L of trimethoprim and 20 mg/L of sulfamethoxazole for the combination. No strains were of intermediate sensitivity in our study.

Adherence Assay

In vitro adherence of the strains was determined by the tissue culture assay of Clausen and Christie[7] using HeLa instead of HEp-2 cells. Five positive and two negative strains were tested with both cell lines and gave identical results. The slides were interpreted by an observer unaware of the origin and serotype of the strains.

Enterotoxin Production and Invasiveness

Tests for enterotoxin production included the VERO cell assay for cytotoxin,[12] the Chinese hamster ovary cell assay[13] for heat-labile enterotoxin, and the suckling mouse assay[14] for heat-stable enterotoxin. Invasiveness was looked for with the Serény test.[15] These procedures are done on a regular basis at the Quebec Laboratory of Public Health, and are submitted to a quality control program, including the use of control strains.

Statistical analysis was done by the χ^2 test with Yates' correction, the two-tailed Fisher exact probability test, and by the Student's *t* test.

RESULTS
Clinical Features

Fifteen patients with adherent *E coli* in their stool were included in this study. Every adherent strain was serotyped 0111:K58:H2. Seven patients were North American Indians who lived on reservations in northern Quebec. We saw five secondary cases in hospital and foster home contacts of these children. Three sporadic cases from the Montreal region were also seen.

The clinical characteristics of these 15 patients were compared with those of 18 patients seen during the same period who were infected with other enteropathogenic serotypes of *E coli*. Patients with 0111:K58:H2 strains were younger (6.5 *v* 13.2 months), had previously been treated with ampicillin more often (six of 15 *v* one of 18; $P = .045$), and had more severe illness (Table). Dehydration (nine of 15 *v* two of 18), toxic appearance (nine of 15 *v* three of 18), and relapses (six of 15 *v* none of 18) were noted more frequently. The maximum number of stools per day as well as the duration of diarrhea were greater in the first group. However, the frequency of fever (nine of 15 *v* six of 18), vomiting (nine of 15 *v* six of 18), and macroscopic blood in stool (one of 15 *v* three of 18) were similar. No acrodermatitislike rash was observed. The duration of diarrhea among patients with adherent strains was greater when they had previously been treated with ampicillin (28.8 *v* 20.1 days).

All patients were given 5% dextrose and 0.45% sodium chloride solutes or intravenous (IV) hyperalimentation. Serum sodium levels below 135 mEq/L were noted in ten of 15 infants infected with *E coli* 0111:K58:H2, but in none of the children in the second group ($P < .001$). Moderate to severe hyponatremia (serum sodium level <130 mEq/L) developed in five patients and stool sodium concentration reached a level of 115 mEq/L in one infant. A serum protein level below 11 mEq/L was found in six of 15 infants of the first group, but in none of the second ($P < .01$). No patient manifested metabolic alkalosis. Finally, all 33 patients recovered completely.

Urine cultures were done on all patients. Of the 18 patients with nonadherent enteropathogenic *E coli* in their stool, only one had a positive urine culture. Among the 15 patients infected with adherent *E coli* 0111:K58:H2, we found seven with at

least one positive urine culture, but this result must be interpreted cautiously since most urine cultures were collected by bag.

A urinary tract infection was proved by suprapubic tap in two cases. The first patient, a 10-month-old French-Canadian boy, was admitted after 18 days of severe diarrhea. Twenty-four hours prior to admission, he became febrile and vomited. On admission, the physical examination showed 10% dehydration and failure to thrive. Oral administration of clear liquids was stopped, and IV hyperalimentation was begun. The diarrhea persisted, however, but with adequate hydration the patient became afebrile. Stool cultures disclosed adherent *E coli* 0111:K58:H2. Assays for toxin production and invasiveness were negative on this strain. The serum protein level was 10.3 mEq/L. The sodium level was 122 mEq/L in the serum, 72 mEq/L in the stool, and 34 mEq/L in the urine. Five days after admission, fever recurred. Two bladder taps done on consecutive days showed more than 100,000 *E coli* 0111:K58. This strain was not tested for H2 antigen. Both the strains from the stool and the urine showed the same antibiotic resistance pattern. Oral trimethoprim-sulfamethoxazole was administered, and two days later the fever disappeared. The urine became rapidly sterile and the diarrhea ended 33 days later, with the infant recovering completely. An IV pyelogram that was done two months later was normal.

The second case was an 8-month-old Cree Indian. He was admitted after 19 days of severe diarrhea. Ten days earlier, he had appeared with high fever and was treated with IV ampicillin. On admission, the baby was dehydrated (10%), febrile (40.5 °C), and in a toxic condition. *Escherichia coli* 0111:K58:H2 was isolated from the stool; the strain was adherent but nontoxicogenic and noninvasive. Urine and blood cultures at admission were negative. The serum protein level was 10.7 mEq/L, the serum sodium level was 128 mEq/L, and the stool sodium concentration was 115 mEq/L. Treatment with IV hyperalimentation was initiated. The diarrhea persisted (up to 300 mL/kg/day), but the fever soon disappeared. One month after admission, diarrhea still persisted and fever recurred. A bladder tap disclosed more than 100,000 *E coli* 0111:K58 (the H antigen was not looked for). The strains isolated from both the stool and the urine exhibited the same resistance pattern to antibiotics. With the stool culture still positive for adherent *E coli* 0111:K58:H2, oral nitrofurantoin was given. The child's condition improved and urine cultures became negative. An IV pyelogram was normal.

Bacteriology

The 15 strains of *E coli* 0111:K58:H2 were found to be resistant to chloramphenicol, ampicillin, kanamycin, cephalothin, and ticarcillin. Resistance to trimethoprim-sulfamethoxazole was present in all but two strains. One strain was resistant to gentamicin. Of the 18 other strains, only one was resistant to ampicillin and ticarcillin.

All 15 strains of *E coli* 0111:K58:H2 were strongly adherent to HeLa cell lines, as shown in the Figure: adherence in these strains consisted of aggregation of large bacterial clumps ("microcolonies" as described by Clausen and Christie[7]) onto most or all epithelial cells.

Fifteen of the 18 strains of other enteropathogenic serotypes (three strains were inadvertently discarded) were also tested for adherence. Thirteen of these strains showed no adherence; only two showed moderate adherence consisting of the attachment of an occasional clump, smaller than those noted in adherent microorganisms, with most epithelial cells devoid of bacteria. Ten strains isolated from normal stool flora were negative on the adherence test.

Eight adherent strains associated with the most severe cases of diarrhea (including two strains from patients with laboratory evidence of a secretory diarrhea) were tested for toxin production by the Chinese hamster ovary cell assay, the suckling mouse assay, and the VERO cell assay; no evidence of toxin production could be found. They were also tested for invasiveness by Serény test; all were negative. No other enteric pathogen was found in the stools of the study patients.

COMMENT

We have reported that a strain of *E coli* 0111:K58:H2, resistant to multiple antibiotics, and strongly adherent in vitro to human epithelial cell lines, can cause severe diarrhea in infants. Six of the 15 patients had previously been treated with antibiotics. All patients passed at least five stools per day and many of them more than 15 per day. The diarrhea was protracted, secretory in type, and persisted for more than 15 days in nine of 15 cases. Despite IV administration of large amounts of sodium, serum sodium levels lower than 130 mEq/L were recorded while the patients received treatment in the hospital in five of 15 cases, with the lowest level at 113 mEq/L. One patient who was eight months old passed more than 1 L of stool per day with a stool sodium concentration of 115 mEq/L. The exact mechanism of this diarrhea is unclear. No toxin production was shown in our adherent strains. Adherent *E coli* may be an important cause of intractable diarrhea of infancy.[6-8] Our findings suggest that North American Indians may be especially vulnerable to this disease.

Patients with severe and prolonged gastroenteritis caused by enteropathogenic adherent *E coli* could be at risk for urinary tract infection. Seven patients had a positive culture and a urinary tract infection was clearly demonstrated in two cases. In each instance it was a late event since the diarrhea had begun three to four weeks earlier. Adherence of *E coli* to urinary epithelial cells is a well-known phenomenon,[16] and adherence of the same *E coli* strain to different epithelia has been described by Schaeffer et al,[17] who have shown a correlation between adherence to vaginal and buccal epithelial cells. Our data suggest that enteropathogenic adherent *E coli* could also adhere to urinary epithelium; this remains to be demonstrated.

In our study, we were able to show massive in vitro adherence of the particular strain of *E coli* 0111:K58:H2 found in our patients, but we did not have the opportunity to test for in vivo

adherence due to the lack of biopsy material. The biologic characteristics of the disease caused by adherent *E coli* described to date are the following: massive adherence of bacteria in vivo to the epithelium of the jejunum and rectum,[5-8] histologic changes consisting of blunting of the villi, flattening, and disorganization of the epithelium,[5-8] and degeneration of the microvilli by electron microscopy.[6,8,18] Studies of some strains have shown that they adhere massively in vitro to epithelial cell lines.[7] No evidence of toxin production or of invasiveness has been found.[5-8]

A particular characteristic of the outbreak was the resistance to several antibiotics including ampicillin. Of interest is the fact that human adherent *E coli* 0111:K58:H- isolated by Clausen and Christie[7] was resistant to ampicillin, carbenicillin, cephalothin, kanamycin, and sulfonamides while the strains of Rothbaum et al[8] (serotype 0119:B14) were resistant to ampicillin and carbenicillin. This suggests that adherent strains may be subjected to important selective pressure in their environment, possibly from antibiotic usage. Previous treatment with ampicillin may have rendered ten of the 15 infants from the series of Rothbaum et al[8] and six of the 15 infants from our series susceptible to adherent and ampicillin-resistant *E coli*. The physician who finds in a severe case of gastroenteritis a strain of enteropathogenic *E coli* resistant to multiple antibiotics should ponder the possibility that it may be adherent.

All our adherent *E coli* were of 0111:K58:H2 antigenic composition. Other serotypes have been found in adherent strains: 0125:H21,[6] 0111:K58:H-,[7] and 0119:B14.[8] It is possible that adherent *E coli* belong to a restricted group of serotypes; however, more studies are needed to confirm this point. At this time, serotyping alone seems insufficient to predict adherence in a strain of *E coli*. One of our strains lost its adherence property spontaneously; this strain remained 0111:K58:H2. Therefore, it appears that adherence is not mediated by one of these antigens.

In conclusion, it now seems established that adherent *E coli* strains can cause severe and protracted intestinal disease in infants. Usually, these infants are young, in a toxic condition, cachectic, dehydrated, and febrile. Often they have been treated unsuccessfully with antibiotics (especially ampicillin), and the enteropathogenic strain of *E coli* is found to be resistant to a number of antibiotics. When seen, this clinical setting must alert the physician to the possibility of an adherent *E coli* infection. Urine cultures ought to be taken.

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