Virulence Factors of Lactose-Negative *Escherichia coli* Strains Isolated from Children with Diarrhea in Somalia

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Lactose-negative *Escherichia coli* strains were isolated at high frequency from children with diarrhea in Somalia during a 2-year study on diarrheal diseases. Sixty-four of these strains, considered to be a representative sample, were characterized for virulence factors, plasmid profiles, and antibiotic resistance. Of these strains, 5 were recognized as enteroinvasive *E. coli* (they were serotyped as O135:K-H-), 6 belonged to classical enteropathogenic *E. coli* serotypes, 9 were able to adhere to tissue culture cells (of these, 4 showed a pattern of localized adherence and 1 was an enteropathogenic strain), 18 were both adherent and hemolytic, and 8 were simply hemolytic. None hybridized with ³²P-labeled heat-labile or heat-stable (a and b) enterotoxin gene probes or produced moderate or high-level cytotoxic effects on HeLa cells. Of the 64 strains examined, 24 produced mannose-resistant hemagglutination with human, chicken, and monkey erythrocytes. One of these was serotyped as O4:K:H8, and a rabbit O antiserum raised against this strain allowed us to establish that 23 strains had the same O antigen. The 23 O4 strains were hemolytic and were not enterotoxic for rabbit ileal loops, and intact bacteria were able to destroy tissue culture cell monolayers very rapidly. The uniformity of the antibiotic resistance pattern and of the plasmid DNA content, together with the fact that they were isolated in different years and in different children, suggests that the O4 strains must be epidemiologically relevant in Somalia. A possible diarrheagenic role for the adherent-hemolytic *E. coli* strains is also discussed.

Infectious diarrheal diseases cause considerable morbidity and mortality among infants and children, especially in developing countries (23, 46). The role of *Escherichia coli* in causing diarrhea is well established (13, 16, 29, 41, 45). There are two well-recognized mechanisms by which *E. coli* may cause diarrhea. Enterotoxigenic *E. coli* strains which adhere to epithelial cells colonize the small bowel of humans and animals and cause fluid secretion by elaboration of heat-stable enterotoxin (ST), heat-labile enterotoxin (LT), or both (6, 11, 12). Enteroinvasive *E. coli* (EIEC) strains penetrate and multiply within colonic epithelial cells, damaging the intestinal mucosa and producing symptoms that mimic those seen with shigellosis (18, 26).

Enteropathogenic *E. coli* (EPEC) strains do not invade colonic epithelial cells, and most of them do not produce ST or LT (14). Even though the mechanisms by which EPEC strains cause diarrhea are not fully understood, adherence of the bacteria to the small bowel mucosa must be important in the induction of the disease (2, 30, 36, 40).

Recently, production of a cell-associated cytotoxin similar, if not identical, to Shiga toxin has been reported for some EPEC as well as for non-EPEC strains (14, 39). Enterohemorrhagic *E. coli* strains are the causal agents of hemorrhagic colitis, and *E. coli* serotype O157:H7 (a high-level Shiga-like toxin producer) has been associated with this illness (32).

In January 1983, a surveillance system was set up at the Faculty of Medicine of the Somali National University in Mogadishu to study epidemiological, clinical, and laboratory

characteristics of enteric pathogens associated with diarrheal diseases in children. Between January 1983 and December 1984, 1,667 children with diarrheal diseases who came to the outpatient area of the Pediatric Hospital Banaadir in Mogadishu were studied (M. Casalino, M. W. Yusuf, M. Nicoletti, P. Bazzicalupo, A. Coppo, B. Colonna, C. Cappelli, C. Bianchini, V. Falbo, H. J. Ahmed, K. H. Omar, K. B. Maxamuud, and F. Maimone, submitted for publication). Since non-lactose-fermenting (NLF) *E. coli* strains were isolated at high frequency in the feces of the patients (about 20%, against an estimate of about 9% for a control group of 215 healthy pediatric patients; M. Nicoletti and M. Casalino, unpublished data), we decided to study a representative number of such isolates, screening for virulence factors. In this paper, we describe the virulence traits of 64 NLF *E. coli* strains isolated from stools or rectal swabs or both of infants and children with diarrhea. Bacterial strains were examined for mannose-sensitive and mannose-resistant (MR) hemagglutination (HA), for hemolysin production, for adherence to tissue culture cells, for invasiveness, for production of cytotoxic and enterotoxic factors, for classical EPEC O serotypes, and for iron-sequestering systems. To establish epidemiological relevancy, we also determined antibiotic resistance and plasmid DNA profiles.

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MATERIALS AND METHODS

Patients. The 1,667 children surveyed in the 2-year study corresponded approximately to at least 10% of all children with diarrhea who visited the Pediatric Hospital Banaadir in

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Mogadishu. The patients were from newborn to 15 years of age, and the majority (86%) were less than 25 months old. Rectal swabs or stool samples or both were screened for the following enteric pathogens: *Salmonella* spp., *Shigella* spp., enterotoxigenic *E. coli*, EPEC, *Aeromonas hydrophila*, *Plesiomonas shigelloides*, *Vibrio* spp., *Yersinia enterocolitica*, *Campylobacter jejuni*, rotavirus, and intestinal parasites. Of the 64 NLF *E. coli* strains chosen for this study, 35 were isolated in 1983 and 29 were isolated in 1984 from children less than 30 months old.

Bacteriological methods. Well-isolated NLF colonies grown on eosin-methylene blue (Oxoid Ltd., London, England), salmonella-shigella (SS modified; Oxoid), or Hektoen enteric (Oxoid) agar at 37°C for 18 to 24 h were transferred to Kligler iron agar (Oxoid) slants that were incubated overnight at 37°C and then tested for urease and oxidase production by standard methods. Bacterial strains giving reactions typical of *E. coli* were biochemically characterized by the API 20E system (API System S.A., Montalieu-Vercieu, France). *E. coli* isolates were tested with commercially available *Shigella flexneri* polyvalent antiserum (Calbiochem-Behring, La Jolla, Calif.) and then inoculated into nutrient agar stabs. All stabs were incubated overnight at 37°C and then kept at room temperature before they were transported to Italy, where the strains were stored in deep agar at room temperature, as well as in Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) with 15% (wt/vol) glycerol at -70°C.

Media and chemicals. Enriched and minimal growth media were LB medium (31), brain heart infusion broth, Trypticase soy broth, Colonization factor antigen (CFA) medium (15), and M9 medium (31). Congo red-binding activity was examined by growing bacteria in Trypticase soy agar (BBL Microbiology Systems) to which congo red (Sigma Chemical Co., St. Louis, Mo.) was added to a concentration of 0.01%.

Bacterial strains and genetic procedures. ZM46 (a nalidixic acid-resistant mutant of CSH26) and 803 were the *E. coli* K-12 strains used in conjugation and transformation experiments as described previously (8). The wild-type *E. coli* 20-72, a human hemolytic strain isolated in France from a stool culture (28), and *E. coli* O157:H7 E30846, a high-level Shiga-like toxin producer (24), were kindly provided by L. Le Minor and P. J. Sansonetti, respectively.

Serotyping. Boiled bacterial suspensions were screened for EPEC serotypes by slide agglutination with polyvalent and specific antisera (Institut Pasteur Production). The serologic types investigated were: O26, O55, O86, O111, O114, O119, O124, O125, O126, O127, O128, and O142.

Six representative NLF *E. coli* strains were serotyped for both O and H antigens by Ida and Frits Ørskov at the International Escherichia Centre in Copenhagen.

HA tests. The species of blood used for HA tests were human type A, bovine, adult chicken, African Green monkey, and guinea pig. All the HA tests were performed with bacterial cells grown on CFA agar plates (15). Bacteria were mixed with 30 µl of the diluted species of blood with and without 1% (wt/vol) D-mannose on a glass slide at room temperature.

Hemolysin production. Hemolysin production was determined on sheep blood agar plates. Hemolytic titers were evaluated essentially as described by Bhakdi et al. (4) and expressed in arbitrary hemolytic units, defined as the last dilution giving >90% hemolysis.

Tests for antibiotic resistance. Antimicrobial agent susceptibility was determined by a disk diffusion method (3).

Plasmid extraction. Plasmid extractions were performed

by the method of Kado and Liu (25) and analyzed by electrophoresis through 0.7% agarose gels.

Hybridization with enterotoxin gene probes. *E. coli* isolates were analyzed for the presence of DNA sequences homologous to probes for LT, STa, and STb by in situ colony hybridization (31). The LT probe was derived from plasmid pWD299 (11), the STa probe was from plasmid pRIT10036 (36), and the STb probe was from plasmid pSLM004 (37) as previously described (8). Plasmids pWD299, pRIT10036, and pSLM004 were kindly provided by S. Falkow.

Detection of hydroxamate-type compounds. Hydroxamates were assayed by the ferric perchlorate assay of Atkin et al. (1) or by the more sensitive Csáky test (9) as previously described (8).

Adhesiveness and invasiveness. *E. coli* strains were assayed for adhesiveness and invasiveness essentially in HeLa or HEp-2 cells or both by using the tissue culture method of LaBrec et al. (27). Cell monolayers were prepared in 12-well Costar tissue culture dishes. When needed, the medium was replaced 3 h before infection with complete medium containing 0.5% D-mannose. Bacterial cultures were harvested in the exponential phase and suspended in Eagle minimal essential medium with or without 0.5% D-mannose. Cell monolayers were infected with 1 ml of the suspended bacteria and incubated at 37°C for different times.

When adhesiveness was to be tested, infection was carried out for 20 min at 37°C. Then the cell monolayers were washed five times with Earle balanced salt solution, fresh medium was added, and the plates were incubated for an additional 3 h. A strain was considered to be adherent if it was observed to adhere to more than 40% of the cells. For the adherent strains, it was noted whether the MR-adherence patterns were diffuse (DA) or localized (LA) (43).

For invasiveness assays, infection was carried out for 3 h, after which the cell monolayers were washed three times with Earle balanced salt solution. Fresh medium with 50 µg of gentamicin (Sigma) per ml was added to each well (all the 64 *E. coli* strains studied were susceptible to this antibiotic), and incubation was continued for 3 h more to allow intracellular multiplication. At the end of both the adhesiveness and invasiveness experiments, the monolayers were washed five times with Earle balanced salt solution, fixed in methanol, and stained with Giemsa stain.

Sereny test. Sereny tests (44) were performed with adult albino guinea pigs. Strains which elicited keratoconjunctivitis within 96 h were considered invasive.

Tests for cytotoxicity. Cytotoxicity tests were performed by a previously described method (39). Shiga-like toxin production was assayed with bacteria grown under aeration in 50 ml of iron-depleted syncase broth (39). Concentrated bacteria were disrupted by sonication. Serial twofold dilutions of neutralized and filter-sterilized supernatants were assayed for cytotoxicity on HeLa or HEp-2 cells or both. The O157:H7 *E. coli* E30846 strain (24) was used as a positive control.

Filter-sterilized supernatants and sonicated extracts of bacteria grown simply in Trypticase soy broth were also tested for cytotoxic activities as well as for the presence of the cytotoxic necrotizing factor (a protein toxin present in *E. coli* strains isolated from children with diarrhea in Italy, which causes necrosis in rabbit skin and multinucleation and lethal activity in several types of tissue cultures) (7).

Rabbit ileal loop assay. The rabbit ileal loop assay was performed in duplicate by a previously described method (19). Ileal loops were inoculated with 1-ml volumes containing approximately 10^9 cells of the test organism.

RESULTS

Enterotoxins. When the 64 NLF *E. coli* strains were hybridized with ³²P-labeled enterotoxin gene probes for LT, STa, and STb, we could not show the presence of DNA sequences homologous to the probes.

HA typing. To define the nature of the pili, we tested the 64 strains for HA and grouped them into different HA types as described by Evans et al. (17). The results are reported in Table 1. Of the 64 strains studied, 24 (37%) were isolated from stools of patients in which one or more enteric pathogens were identified (the isolation rate was 61% for the 1,667 children with diarrhea in the 2 years of surveillance). The distribution of these 24 strains with respect to the HA type was: 1 HA type III, 5 HA type V, 7 HA type VI, 10 HA negative, and 1 untypable strain.

Hemolysin production. Hemolysin production was detected in 26 of the 64 NLF *E. coli* strains tested, and titers of culture supernatants were in the range of 1/4 to 1/16 hemolytic units. Remarkably, 24 of the 26 hemolytic strains belonged to HA type VI (Table 1).

EPEC strains. Of the 64 lactose-negative *E. coli* strains, 6 belonged to the traditional EPEC serogroup. Two strains were O111, two were O114, and two were O142. The two O114 strains were HA type V, while the O111 and O142 strains were HA negative (Table 1).

Adhesiveness and cytotoxicity of *E. coli* strains on tissue culture cells. MR adherence was shown for 27 (42%) of the 64 *E. coli* strains studied (Table 1). The behavior of the 24 HA type VI strains was remarkable: all were highly cytotoxic when tissue culture cells and bacteria (ratio, 1:100) were incubated together. A similar cytotoxic effect was observed for *E. coli* HN9 (HA type V) and HN184 (HA untypable) and for control strain 20-72, and this may be explained because they all produced hemolysin. Of the 24 HA type VI *E. coli* strains, 18 showed MR adherence (Table 1), while HN9 and HN184 were not adherent and *E. coli* 20-72 showed mannose-sensitive adherence. The 18 adherent-hemolytic HA type VI *E. coli* strains detached the monolayers more rapidly (about 50% detachment after 30 min of incubation) than the hemolytic strains (about 50% detachment after 3 h of incubation).

MR adherence to HeLa cells was shown also for the HA type II, the HA type III, two HA type V, and five HA-negative *E. coli* strains, and of these, four showed an LA pattern and five showed a DA pattern (Table 1). Of the six EPEC strains, only HN45 (HA negative) was able to adhere to HeLa cells, and it showed an LA pattern.

Bacterial cell extracts and culture supernatants of 30 strains among the 64 NLF *E. coli* strains which had been grown in iron-depleted medium were tested for cytotoxin production on HeLa cells as described in Materials and Methods. The strains tested were the HA type II and III adherent strains; one adherent, one hemolytic, and one nonadherent-nonhemolytic HA type V; six adherent-hemolytic and two hemolytic HA type VI; four invasive, four adherent, and two nonadherent-noninvasive HA negative; one hemolytic untypable; and the six EPEC strains. All the bacterial cell extracts and culture supernatants tested produced only low amounts of cell-associated cytotoxin, while the control strain E30846 produced high-level cytotoxic effect.

A low-level cytotoxic effect was also obtained when these strains were grown simply in Trypticase soy broth, and moreover, we did not observe multinucleation of HEp-2 cells, which is characteristic of cytotoxic necrotizing factor-producing *E. coli* strains (7).

HA type VI *E. coli* strains. Of the 64 *E. coli* strains studied, 24 had the HA type VI pattern (Table 1). All produced hemolysin, and 18 showed MR adherence to tissue culture cells. A rabbit O antiserum raised against *E. coli* HN2, chosen as the prototype of the HA type VI strains, allowed us to establish that 23 of the 64 *E. coli* strains had the same O antigen and that all were HA type VI. *E. coli* HN2 has been serotyped at the International Escherichia Centre in Copenhagen as O4:K:H8.

Apart from HN132, the HA type VI strain not recognized by the O antiserum, the 23 O4 *E. coli* strains produced an identical API numerical profile (5144512). They showed multiple antibiotic resistance patterns (18 of the 23 O4 strains were resistant to at least six drugs), but mating experiments performed with six strains seemed to rule out the possibility that the antibiotic resistance genes reside on conjugative plasmids. Transformation experiments failed to give transformants for any of the antibiotic resistance markers considered.

The 23 O4 HA type VI NLF *E. coli* strains were examined for their plasmid content. Twenty strains were very uniform in terms of plasmid profiles: a large plasmid of about 100 megadaltons (MDa) (in two strains the large plasmid was about 70 to 80 MDa), a smaller one in the range of 20 to 30 MDa, and small plasmids ranging between 2 and 5 MDa. Figure 1 shows the plasmid DNA content of six HA type VI NLF *E. coli* strains.

Serial passages of the HA type VI *E. coli* strains revealed that 12 strains generated stable lactose-positive colonies at

TABLE 1. HA profiles of the 64 NLF *E. coli* strains isolated from children with diarrhea in Somalia and distribution of hemolytic, adherent, EIEC, and EPEC strains according to HA type

HA type ^a	HA with ^b :					No. of strains	Hemolytic strains	Adherent strains ^c	EIEC strains	EPEC strains
	Hu	Bv	Ck	Mk	Gp					
II	—	R	R	—	—	1	0	1 (LA)	0	0
III	—	—	S	S	S	1	0	1 (LA)	0	0
V	R	—	—	—	—	5	0	1 (DA)	0	2
	—	—	R	—	—	2	1	1 (LA)	0	0
VI	R	—	R	R	—	24	24	18 (ND)	0	0
Negative	—	—	—	—	—	29	0	5 (1,LA;4,DA)	5	4
Untypable	—	—	S	—	S	2	1	0	0	0

^a HA types are according to Evans et al. (17).

^b Erythrocytes tested were: Hu, human group A; Bv, bovine; Ck, chicken; Mk, African Green monkey; Gp, guinea pig. R, MR HA; S, mannose-sensitive HA; —, negative HA.

^c Number of strains showing HeLa cell adhesivity. In parentheses are the number of strains showing LA or DA pattern; ND, not determined.

FIG. 1. Agarose gel electrophoresis of plasmid DNA obtained from six independent HA type VI NLF *E. coli* strains isolated from children with diarrhea in Somalia. Lanes: A, strain HN2; B, strain HN33; C, strain HN107; D, strain HN18; E, strain HN168, F, strain HN119. Chr, Chromosomal DNA. See text for details.

low frequency on salmonella-shigella and/or MacConkey agar plates. The lactose-positive colonies behaved in exactly the same way in terms of HA type, hemolysin production, O-antigen relatedness, cytotoxicity, HeLa cell adhesivity, antibiogram, total plasmid DNA pattern, and hydroxamate-type compound production, a result indicating that all were derived from parental strains.

Of the 23 *E. coli* strains (the same O4 strains that we tested for cytotoxin production), 8 were also assayed in the rabbit ileal loop test and they did not produce any enterotoxin effect under our experimental conditions.

EIEC *E. coli* strains. When the 64 NLF *E. coli* strains were tested with polyvalent anti-*S. flexneri* antiserum, only 5 strains produced strong agglutinations. These were HN11, HN13, HN19, HN280, and HN300. The five strains were serotyped as O135:K:H- at the International Escherichia Centre in Copenhagen.

Only HN13, HN19, HN280, and HN300 were able to invade HeLa cell monolayers. The four strains produced keratoconjunctivitis in the eyes of guinea pigs, were enterotoxic in the rabbit ileal loop assay, were able to bind congo red dye, and had the 140-MDa invasive plasmid (Fig. 2), while *E. coli* HN11 did not. Plasmid DNA analysis revealed that HN11 lacked the 140-MDa invasive plasmid (Fig. 2, lane C). All five strains were HA negative and lysine decarboxylase negative and had homogeneous plasmid DNA profiles (Fig. 2).

HN11, HN280, and HN300 were susceptible to all antibiotics used, while HN13 was resistant to chloramphenicol, mercuric ion, streptomycin, sulfonamide, and tetracycline, and HN19 had the same pattern but was not resistant to tetracycline. HN13 and HN19 had all the antibiotic resistance markers localized on conjugative plasmids pHN13 and pHN19, respectively (Fig. 2).

HA-negative *E. coli* strains. Of the 64 NLF *E. coli* strains studied, 29 (45%) were HA negative (Table 1). Of these, five were EIEC, five were adherent to tissue culture cells, four belonged to classical EPEC serotypes (see above), three were able to produce stable lactose-positive colonies at low frequency (one strain was adherent and generated lactose-

positive colonies), and none produced hemolysin. The 29 strains were quite different in their antibiotic resistance patterns and total plasmid DNA profiles. Twelve strains were resistant to at least four drugs, and nine strains were susceptible. Analysis of the agarose gel electrophoretic plasmid DNA profiles revealed that although some strains had similar electropherotypes (the EIEC strains, two EPEC strains, three adherent strains, three noninvasive-nonadherent strains), the HA-negative strains were a heterogeneous class.

Hydroxamate production. Culture supernatants of 28 strains were positive for hydroxamate production by both assays used. Positive strains were 14 of the 24 HA type VI, 8 of the 9 adherent strains (including the EPEC strain HN45 that was adherent), 4 of the 5 nonadherent EPEC, and 2 of 6 chosen among the strains without relevant phenotypes. The addition of iron to the medium (50 μ M) strongly repressed hydroxamate production. All five O135:K:H- EIEC strains failed to produce hydroxamate-type compounds.

Antibiotic susceptibility. The 64 NLF *E. coli* isolates were susceptible to gentamicin, rifampin, and nalidixic acid. A total of 35 (55%) were resistant to ampicillin, 32 (50%) to chloramphenicol, 34 (53%) to mercuric ion, 27 (42%) to kanamycin, 38 (59%) to streptomycin, 29 (45%) to spectinomycin, 40 (62%) to sulfamide, 49 (76%) to tetracycline, and 14 (22%) to trimethoprim. Nine (14%) were susceptible to all the antibiotics tested.

DISCUSSION

One of the characteristic biochemical reactions of *E. coli* is the fermentation of lactose with the production of acid and gas within 24 to 48 h, even if there are some strains which ferment it slowly or not at all (33). In all recent studies (5, 20, 21, 38) reported thus far regarding the characterization of enteric pathogens associated with childhood diarrhea in developing countries, pathogenic *E. coli* (enterotoxigenic *E.*

FIG. 2. Agarose gel electrophoresis of plasmid DNA obtained from five independent NLF EIEC strains isolated from children with diarrhea in Somalia. Lanes: A, strain HN280, invasive; B, strain HN300, invasive; C, strain HN11, noninvasive; D, strain HN13, invasive; E, strain HN19, invasive. Arrows indicate the conjugative plasmids pHN13 and pHN19, carrying the antibiotic resistance markers and the 140-MDa invasive plasmid (see text for details). Chr, Chromosomal DNA.

coli, EPEC, etc.) have been searched for only among *E. coli* strains which formed typical lactose-positive *E. coli* colonies when stool specimens were plated on primary standard enteric media. NLF *E. coli* strains can be isolated, albeit not frequently, from stools of patients with diarrhea. In the past, such strains have caused difficulty in the diagnosis of enteric infections, being often mistaken for members of the genera *Salmonella* or *Shigella* (33). To our knowledge, recent extensive studies on NLF *E. coli* strains isolated from cases of diarrhea in children in developing countries have not been reported.

Experiments conducted to assess the ability of the 64 independent NLF *E. coli* isolates that we chose in this study to cause HA of different species of erythrocytes revealed that the majority (83%) belonged to only two different HA types.

The 24 HA type VI strains represented a very homogeneous and epidemiologically interesting group. All were hemolytic and multiple antibiotic resistant, and the fact that we found identical plasmid profiles in strains isolated in different years (Fig. 1) suggests that those strains are epidemiologically relevant (as far as Somalia is concerned) and raises the hypothesis of a possible clonal origin. Nevertheless, 18 O4 strains showed MR adherence to HeLa cells. Because these adherent-hemolytic strains were highly cytotoxic for tissue culture cells, we could not ascertain whether they presented DA or LA patterns. Recently, it has been found that enteroadherent *E. coli* strains may represent another group of diarrheagenic *E. coli* (30, 34, 36, 40). Enteroadherent *E. coli* strains are defined as strains that exhibit MR adherence to HeLa and/or HEP-2 tissue culture cells, do not produce conventional enterotoxins, and are not enteroinvasive; they may be EPEC as well as non-EPEC strains (35). The elaboration of surface components that mediate adhesion to both erythrocytes and culture epithelial cells reflects the presence of bacterial structures that could promote adhesion of these strains to host intestinal epithelial cells. Adherence to mucosal surfaces of the intestine is an important early event in colonization and in the development of diarrheal disease.

E. coli strains of the serogroup O4 have been included in the family of the facultative enteropathogenic *E. coli* strains, i.e., *E. coli* strains which belong to certain serogroups which have been associated with sporadic cases of diarrhea, as well as to extraintestinal infections such as meningitis, urinary tract infections, and bacteremia (10, 17). Specific biological tests must be done to assess the possible diarrheagenic role of the HA type VI strains. However, the fact that they all produce hemolysin, an extracellular protein toxin which disrupts the membranes of erythrocytes and other differentiated eucaryotic cells (22), allows us to hypothesize that the HA type VI strains induce diarrhea by adhering to enterocytes and causing mucosal injury.

In this report, we also describe five O135:K:-H- NLF EIEC strains. Even though O135 EIEC strains have been isolated in the past, albeit not frequently (I. Ørskov and F. Ørskov, personal communication), this is the first time that isolates are described and characterized.

The presence of the 140-MDa invasive plasmid in four of the five EIEC strains examined seems to be correlated with fluid accumulation in ligated rabbit ileal loops. In fact, bacterial cell extracts and culture supernatants of the five strains grown on iron-depleted medium were similarly and scarcely cytotoxic in the HeLa tissue culture cell assay. Recently, Sansonetti et al. (42) have used Hfr chromosomal gene transfer techniques to transfer *S. flexneri* virulence

traits to a strain of *E. coli* K-12 in which the 140-MDa Tn5-tagged invasive plasmid of *S. flexneri* 1b had been mobilized. Analysis of the resulting hybrid *E. coli* strains allowed them to establish that the *Shigella mtl*⁺ and *rha* loci were associated with fluid accumulation. Evidence accumulated in recent years indicates that EIEC strains appear to mimic *Shigella* species in their virulence characteristics and moreover that they also encode the same chromosomal and plasmid virulence factors as *Shigella* species (26). Our results suggest that, for the EIEC strains we isolated, a 140-MDa invasive plasmid-encoded function(s) is also necessary for the provocation of fluid accumulation in the ileal loop.

In conclusion, the results obtained in the present study assess the occurrence of relevant virulence factors in lactose-negative *E. coli* strains isolated from children with diarrhea in Somalia and lead to the suggestion that this class of strains should also be monitored, where possible, in epidemiological studies on diarrhea-inducing *E. coli* in developing countries.

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