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Escherichia coli O157:H7 and O157:H⁻ Strains That Do Not Produce Shiga Toxin: Phenotypic and Genetic Characterization of Isolates Associated with Diarrhea and Hemolytic-Uremic Syndrome

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We have isolated one sorbitol-nonfermenting (SNF) *Escherichia coli* O157:H7 isolate and five sorbitol-fermenting (SF) *E. coli* O157:H⁻ isolates that do not contain Shiga toxin (Stx) genes (*stx*). Isolates originated from patients with diarrhea ($n = 4$) and hemolytic-uremic syndrome (HUS) ($n = 2$). All isolates harbored a chromosomal *eae* gene encoding gamma-intimin as well as the plasmid genes E-*hly* and *etp*. The *E. coli* O157:H7 isolate was *katP* and *espP* positive. Respective sera obtained from the patient with HUS contained antibodies to the O157 lipopolysaccharide antigen. The *stx*-negative *E. coli* O157:H7 isolate is genetically related to *stx*-positive SNF *E. coli* O157:H7. All *stx*-negative SF *E. coli* O157:H⁻ isolates belong to the same genetic cluster and are closely related to *stx*-positive SF *E. coli* O157:H⁻ isolates. Our data indicate that *stx*-negative *E. coli* O157:H7/H⁻ variants may occur at a low frequency and cannot be recognized by diagnostic methods that target Stx.

Shiga toxin (Stx)-producing *Escherichia coli* (STEC) of various serotypes has been linked to a spectrum of disorders, including watery diarrhea, bloody diarrhea (hemorrhagic colitis), and hemolytic-uremic syndrome (HUS) (37).

STEC has as its cardinal virulence trait the ability to produce one or more Stxs (Stx1, Stx2, or Stx2 variants). Stxs are toxic to cultured human colonic and ileal epithelial cells (25) and endothelial cells (27). Even in the absence of cytotoxicity, Stxs can stimulate the production of vasoactive factors by endothelial cells (5). Thus, the ability to produce an Stx is quite plausibly related to the intestinal and extraintestinal manifestations of human STEC infections.

E. coli strains that express the O157 antigen are the most commonly isolated STEC strains worldwide. Such organisms are easily detected by toxin-independent detection protocols, such as sorbitol-MacConkey (SMAC) agar screening (29), or the immunomagnetic separation (IMS) technique. Unlike approximately 80% of other *E. coli* strains, most O157 STEC isolates do not ferment D-sorbitol after overnight incubation. Therefore, SMAC agar was developed by substituting the carbohydrate sorbitol for lactose in MacConkey agar. SMAC agar has proved to be effective for the isolation of O157 STEC and is the most widely used medium for this purpose.

IMS can isolate sorbitol-fermenting (SF) *E. coli* O157:H⁻ as well as sorbitol-nonfermenting (SNF) *E. coli* O157:H7 (19). However, SF non-O157:H7 STEC can also cause human disease (17, 38), and because of this, Stx detection systems have been used to identify such pathogens in human stools (10, 15, 21, 28).

We have isolated from humans nontoxigenic *E. coli* strains that express the O157 antigen and present data suggesting that Stx may not be obligatorily produced by *E. coli* O157 strains associated with human disease, including HUS.

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MATERIALS AND METHODS

Bacterial strains. The origins and characteristics of the *E. coli* O157 strains used as controls in this study are described in Table 1. The origins of all other strains are described in the text.

Isolation of *E. coli* O157 from stool specimens. A total of 2,785 stool specimens from patients with diarrhea or HUS were screened in 1996 and 1997 for the presence of Stx-producing *E. coli* in the Institute for Hygiene and Microbiology, University of Würzburg, Würzburg, Germany. The majority of the stool samples were obtained from hospitalized children throughout Germany. Detection of *E. coli* O157 was performed as described below.

A total of 10 ml of GN broth Hajna (Difco, Detroit, Mich.) was inoculated with 1 g of the stool sample, and the mixture was incubated for 6 h at 37°C. *E. coli* O157 was sought from 1 ml of this broth by the IMS technique as described previously (19). Fifty microliters of the bacterium-bead suspension was streaked onto SMAC agar and cefixime-tellurite SMAC (CT-SMAC) agar plates. Up to 1,500 colonies from both plates were scraped off with a sterile swab and were suspended in 1 ml of sterile 0.9% NaCl solution. The bacterial cell concentration was determined and was adjusted with the McFarland no. 3 turbidity standard. Fifteen microliters of a 1:25 dilution (10^6 bacterial cells) made from this suspension was subjected to PCR with primer pairs KS7 and KS8, LP43 and LP44, and SK1 and SK2 (34), which are specific for stx_1, stx_2, and *eae*, respectively. The remaining suspension was stored at 4°C until the PCR results were obtained.

PCR-positive samples were processed in the following manner: the bacterial suspension which was stored at 4°C was diluted and restreaked onto three SMAC agar plates to obtain 500 CFU/plate. Colony blot hybridization was then performed with stx_1-, stx_2-, and *eae*-specific probes (33) on these plates to detect and isolate STEC colonies.

Microbiological methods. Detection of the enterohemolytic phenotype was performed on blood agar plates containing washed sheep erythrocytes (34). Fermentation of sorbitol was detected on SMAC agar plates after overnight incubation (29). Resistance to tellurite was determined on CT-SMAC agar containing 2.5 mg of tellurite per liter (40).

PCR techniques. PCR was conducted with 10^6 bacteria. Amplification was performed in a total volume of 50 μl containing each deoxynucleoside triphosphate at 200 μM, 30 pmol of each primer, 5 μl of 10-fold-concentrated DNA polymerase synthesis buffer (Perkin-Elmer, Applied Biosystems, Weiterstadt, Germany), 3 μl of a 25 mM $MgCl_2$ stock solution, and 2.0 U of AmpliTaq DNA polymerase (Perkin-Elmer, Applied Biosystems). For detection of *stx* genes in the isolated strains, primers LP30-LP31 ($stxA_1$), KS7-KS8 ($stxB_1$), and LP43-LP44 ($stxA_2$) were used as described previously (34). JS1 (5′-CAT GAA GAA GAT GTT TAT GGC G-3′) and JS2 (5′-CTC AGT CAT TAT TAA ACT GCA C-3′) were used for the amplification of the entire $stxB_2$ subunit gene by the protocol described previously for GK5 and GK6 (15). For detection of plasmid genes, PCRs for E-*hly* (34), *etp* (34), *katP* (34), and *espP* (9) were performed as described previously. The *eae* genes were amplified with primers SK1-SK2 (32) and with primers LP1-LP2 and LP1-LP3 (31). A 5-μl volume of each PCR sample was analyzed by gel electrophoresis on 1.5% agarose gels. The *fliC* gene was amplified with primers F-FLIC1 and R-FLIC2, and the PCR product was restricted with *Rsa*I as described by Fields et al. (12). Restriction fragments were separated on a 2.5% (wt/vol) agarose gel.

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TABLE 1. *E. coli* strains used as controls in this study[a]

Strain	Serotype	DA	Relevant genetic markers	Fermentation of sorbitol	Reference
EDL933	O157:H7	HC	*stx*$_1$, *stx*$_2$, *eae*	−	26
702/88	O157:H$^-$	HUS	*stx*$_2$, *eae*	+	1
1533/97	O157:H$^-$	HUS	*stx*$_2$, *eae*	+	SC
3817/96	O157:H$^-$	HUS	*stx*$_1$, *stx*$_{2c}$	−	7
E32511	O157:H$^-$	HUS	*stx*$_2$, *stx*$_{2c}$, *eae*	−	35
693/91	O157:H19	WD	*stx*$^-$, *eae*$^-$	+	3
241/88	O157:H43	WD	*stx*$^-$, *eae*$^-$	+	1
1083/87	O157:H45	WD	*stx*$^-$; *eae*,[b] EAF$^+$	+	31
904/90	O157:H45	WD	*stx*$^-$; *eae*,[b] EAF$^-$	+	1

[a] Abbreviations: SC, strain collection (isolation during routine diagnostic work in 1997); HC, hemorrhagic colitis; DA, disease association; WD, watery diarrhea; *stx*$^-$ and *eae*$^-$, negative for *stx* and *eae*, respectively; EAF$^+$, positive for EAF plasmid; EAF$^-$, negative for EAF plasmid.
[b] Enteropathogenic *E. coli eae*.

Analysis of genomic DNA of *E. coli* O157 strains was performed by random amplification of polymorphic DNA (RAPD) PCR fingerprinting with a single primer, primer 1247 (5′-AAG AGC CCG T-3′) (16). Internal standards (PCR products with known sizes) were run in each lane for standardization of gels for analysis. Gels were stained with ethidium bromide and were digitized for computer-aided analysis. The GelCompar software package (Applied Maths, Kortrijk, Belgium) was used for analysis. Calculation of the similarity matrix was performed with the Jacquard algorithm after defining each single band. The hierarchic clustering was achieved by the unweighted pair group method with arithmetic averages clustering algorithm (36).

Standard DNA techniques. Restriction endonuclease digestion (New England Biolabs) was performed according to the supplier's instructions. Restriction fragments were separated electrophoretically in 0.6 to 0.9% agarose gels in 0.5-fold TBE (Tris-borate-EDTA) buffer (30), stained in ethidium bromide, and analyzed. For Southern blot hybridization, DNA was prepared by the method of Heuvelink et al. (16): 100 ng of DNA was separated electrophoretically and was transferred from agarose gels to Zeta-probe nylon membranes (Bio-Rad, Munich, Germany) by standard methods (30). For hybridization assays, the nonradioactive DNA Labeling and Detection Kit (Boehringer GmbH, Mannheim, Germany) was used according to the manufacturer's instructions. The specific washing step was performed twice (5 min each time) in SSC buffer (sodium chloride, sodium citrate) and 0.1% (wt/vol) sodium dodecyl sulfate. The concentration of SSC and the temperature of the washings were calculated as described by Meinkoth and Wahl (24) to effect different stringencies. Probes were labeled with digoxigenin-11-dUTP by random hexamer labeling. The probes used included fragments of *stx*$_1$ (amplified from EDL933 by PCR with primers KS7 and KS8), *stx*$_2$ (amplified from EDL933 by PCR with primers GK3 and GK4), and *eae* (amplified from EDL933 with primers SK1 and SK2 [32]).

Vero cell assay. The Vero cell toxicities of bacterial culture supernatants and fecal filtrates were determined as described previously (14, 20, 34). Ten, 100, and 1,000 50% cytotoxic doses of Stx2 per ml were added to filtrates to confirm that no inhibitors of cytotoxicity were present.

Serological and immunological techniques. Isolation of lipopolysaccharide (LPS) and electroblotting were performed as described previously (4). For detection of immunoglobulin M (IgM) antibodies against O157 LPS by immunoblotting, the techniques of Bitzan et al. (4) were used. The *E. coli* O and H antigens were determined with the Wellcolex *E. coli* O157:H7 Rapid Latex Test Kit (Murex Diagnostica/Abbott Laboratories, Wiesbaden, Germany) according to the manufacturer's instructions. *E. coli* serotypes were confirmed by the method described by Bockemühl et al. (6).

An enzyme immunoassay (Premier EHEC EIA; Meridian Diagnostics Inc., Cincinnati, Ohio) was used to detect the expression of Stxs from isolated organisms according to the manufacturer's instructions, with minor modifications. Briefly, 4 ml of tryptic soy broth was supplemented with 0.05 μg of mitomycin C per ml and was inoculated with the test strain, and the mixture was incubated overnight at 37°C with shaking. A total of 50 μl of the overnight culture was mixed with 200 μl of sample dilution buffer, and 100 μl of this mixture was transferred to the well of a microtiter plate coated with polyclonal anti-Stx IgG antibodies. After 1 h of incubation at room temperature, the well was washed five times with washing buffer. Detection of bound antigen was performed as described in the manual of the Premier EHEC EIA. Stx-positive strain *E. coli* O157:H7 EDL933 and *stx*-negative strain *E. coli* O157:H19 693/91 were used as controls.

RESULTS

Identification of *stx*-negative *E. coli* O157 strains. During routine diagnostic work in 1996 and 1997, tests for the detection of STEC were performed with 2,875 stool specimens. From 145 of these stool specimens, 145 STEC O157 (118 sorbitol-negative and 27 sorbitol-positive strains) could be isolated in our laboratory. Eighty-five strains were from patients with HUS and 60 were from patients with diarrhea without HUS. In addition, five stool specimens from five different patients (Table 2) did not show toxic activity for Vero cells, and PCR screening with *stx*$_1$ and *stx*$_2$-specific primers (*stx* PCRs) after enrichment by the IMS technique was negative. However, the PCR of the five stool specimens with *eae*-specific primers (*eae* PCR) was positive. Stool samples from diarrheal patients were taken 1 to 2 days after the onset of diarrhea and from

TABLE 2. Characteristics of *stx*-negative *E. coli* O157 isolates[a]

Strain designation	Serotype	DA or origin	Age (yr)[b]	Presence of free fecal Verotoxin	Fermentation of sorbitol	Growth on CT-SMAC	Presence of the following:				Anti-O157 LPS immunoblotting result
							E-*hly*[c]	*etp*	*katP*	*espP*	
19685	O157:H7	WD	4	−	−	+	+	+	+	+	ND
2937	O157:H$^-$	WD	3	−	+	−	+	+	−	−	ND
6790	O157:H$^-$	HUS	6	−	+	−	+	+	−	−	+
431	O157:H$^-$	WD	3	−	+	−	+	+	−	−	ND
659	O157:H$^-$	WD	3	−	+	−	+	+	−	−	ND
2576	O157:H$^-$	HUS[d]	1	+	+	−	+	+	−	−	+

[a] Abbreviations: DA, disease association; WD, watery diarrhea; ND, not determined.
[b] Age of patients.
[c] All isolates were also enterohemolytic on blood agar plates.
[d] STEC O103:H2 was found to be coisolate.

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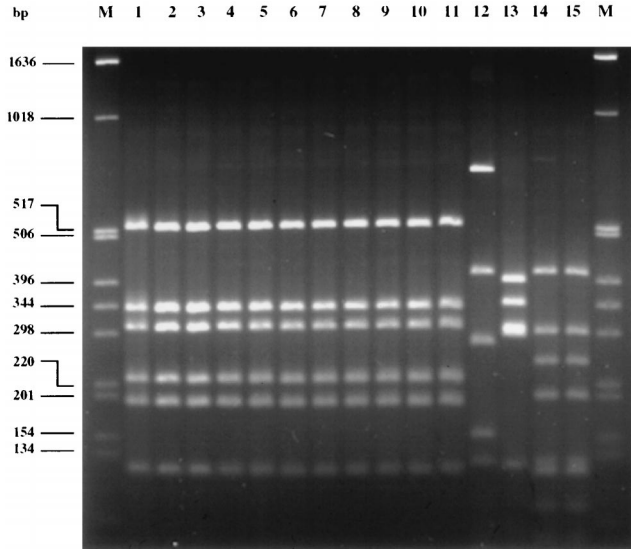

FIG. 1. Agarose gel electrophoresis of *fliC* PCR products of *E. coli* O157 strains restricted with *Rsa*I. Lanes M, molecular size marker (1-kb ladder; Gibco GmbH); lane 1, *E. coli* O157:H7 EDL933; lane 2, *E. coli* O157:H7 19685; lane 3, SF *E. coli* O157:H⁻ 702/88; lane 4, SF *E. coli* O157:H⁻ 1533/97; lane 5, SNF *E. coli* O157:H⁻ 3817/96; lane 6, SNF *E. coli* O157:H⁻ E32511; lane 7, SF *E. coli* O157:H⁻ 2937; lane 8, SF *E. coli* O157:H⁻ 6790; lane 9, SF *E. coli* O157:H⁻ 431; lane 10, SF *E. coli* O157:H⁻ 659; lane 11, SF *E. coli* O157:H⁻ 2576; lane 12, *E. coli* O157:H19 693/91; lane 13, *E. coli* O157:H43 241/88; lane 14, *E. coli* O157:H45 1083/87; and lane 15, *E. coli* O157:H45 904/90.

both HUS patients during the acute phase of disease 7 days after the onset of diarrhea.

By colony blot hybridization, five *E. coli* O157 strains were isolated (Table 2). These isolates were also positive by the universal *eae* PCR but negative by the *stx* PCRs. Filtrates of stools from five of the six patients from which these bacteria were obtained were also negative by the Vero cell assay. To ensure that no inhibitors of Stx were present in the stools, the supernatant of an Stx2-positive culture was added at different concentrations to samples of all stools that were primarily negative by the Vero cell assay. All samples supplemented with Stx2 were cytotoxic by the Vero cell assay, indicating that no inhibitors were present in the stools.

A sixth, Stx-positive stool which contained an Stx-producing *E. coli* O103:H2 isolate was noticed. However, serum from the same patient reacted with *E. coli* O157 LPS. Therefore, the presence of *E. coli* O157 was likely. An enrichment culture of the stool sample was streaked onto blood agar plates, and the enterohemolytic phenotypes were investigated. Beside strongly hemolytic colonies, which were later shown to be *E. coli* O103:H2, colonies surrounded by the typical enterohemolytic phenotype were detected. Analysis with the Wellcolex *E. coli* O157:H7 Rapid Latex Test Kit revealed that these colonies were serotype O157:H⁻.

Sera from the two children with HUS were obtained during the acute phase of HUS 8 days after the onset of diarrhea and were tested by immunoblot analysis with O157 LPS. Both sera were positive by the IgM immunoblot assay.

Strain characteristics. One of the Stx-negative isolates was of serotype O157:H7, and the other five were of serotype O157:H⁻. All isolates were from patients with primary cases of infection, and no temporal or geographic clustering could be observed. The origins of these isolates are described in Table 2. PCR was performed to characterize the *fliC* gene of the *E. coli* O157:H7 isolates and selected controls (see Fig. 1). Restriction fragment length polymorphism (RFLP) analysis of the PCR products after digestion with *Rsa*I demonstrated a pattern identical to that obtained by the *fliC* PCR with strains of the H7 or H⁻ type as described by Fields et al. (12). The results of *fliC* PCR and RFLP analysis of all tested strains are depicted in Fig. 1. *E. coli* O157:H7 strains EDL933 and 19685 and *E. coli* O157:H⁻ control strains 702/88, 1533/97, 3817/96, and E32511 as well as *stx*-negative SF O157:H⁻ isolates 2937, 6790, 431, 659, and 2576 demonstrated identical RFLP patterns after digestion with *Rsa*I. This pattern is identical to that described by Fields et al. (12) and confirmed the presence of an H7/H⁻ antigen in the respective isolates. *E. coli* control strains 693/91 (O157:H19), 241/88 (O157:H43), 1083/87 (O157:H45), and 904/90 (O157:H45) showed different patterns after digestion with *Rsa*I, and these patterns were distinguishable from that of *E. coli* O157:H7/H⁻.

Virulence determinants of the *stx*-negative *E. coli* O157 strains. None of the *E. coli* O157 strains gave a PCR product with primers LP30-LP31 and LP43-LP44, which are complementary to the A-subunit genes of stx_1 and stx_2, respectively. To test the hypothesis that A-subunit genes different from $stxA_1$ and $stxA_2$ were present, *stx* PCR was repeated with primers KS7-KS8 and JS1-JS2, which are complementary to the B-subunit genes of stx_1 and stx_2, respectively, but these PCRs were also negative. Moreover, the chromosomal DNAs of all isolates were hybridized with probes complementary to stx_1 and stx_2 sequences under low-stringency conditions, but no signal could be demonstrated (Fig. 2B and C). Control hybridization with an *eae*-specific probe showed a signal in all cases (Fig. 2A). None of the strains reacted in the Premier EHEC EIA.

The *E. coli* O157:H7 strain was E-*hly*, *etpP*, *katP*, and *espP* positive by PCR. The five *E. coli* O157:H⁻ isolates, however, contained only E-*hly* and *etp* and lacked *espP* and *katP*.

By PCR with primers LP1 and LP3 it could be shown that *eae* encoding gamma-intimin, which is typically found in Stx-producing *E. coli* O157, is present in all strains. The *E. coli* O157:H7 strain showed the typical enterohemolytic phenotype on blood agar plates after 18 to 24 h of incubation. However, of the *E. coli* O157:H⁻ isolates, only 30% of the colonies were enterohemolytic when they were streaked for isolation onto blood agar plates.

Clonal analysis. We compared the genetic relatedness of these strains by RAPD analysis. Figure 3 depicts a dendrogram constructed with GelCompar software. The dendrogram shows the genetic relatedness of the *stx*-negative *E. coli* O157 isolates and *stx*-positive *E. coli* O157:H7 and O157 isolates with other H antigens.

RAPD analysis demonstrated that the *stx*-negative isolate *E. coli* O157:H7 19685 is identical to the *stx*-positive reference strain *E. coli* O157:H7 EDL933. The SNF O157:H7 strains are more closely related to the SNF *stx*-positive *E. coli* O157:H⁻ strains than to the SF O157:H⁻ isolates. Moreover, as expected, the SF *stx*-negative *E. coli* O157:H⁻ strains are clonal and are most closely related to the *stx*-positive *E. coli* O157:H⁻ strains. *E. coli* O157 strains of the H⁻ and H7 types are more closely related to each other than to *E. coli* strains of other H types.

DISCUSSION

Our finding of clinical *E. coli* O157:H7/H⁻ isolates that lack *stx* genes was surprising. Such *stx*-negative *E. coli* O157 strains have rarely been identified, presumably because in strains of this serotype, unlike other toxigenic *E. coli* strains that produce Stx, the toxin genes are retained after multiple replications. It

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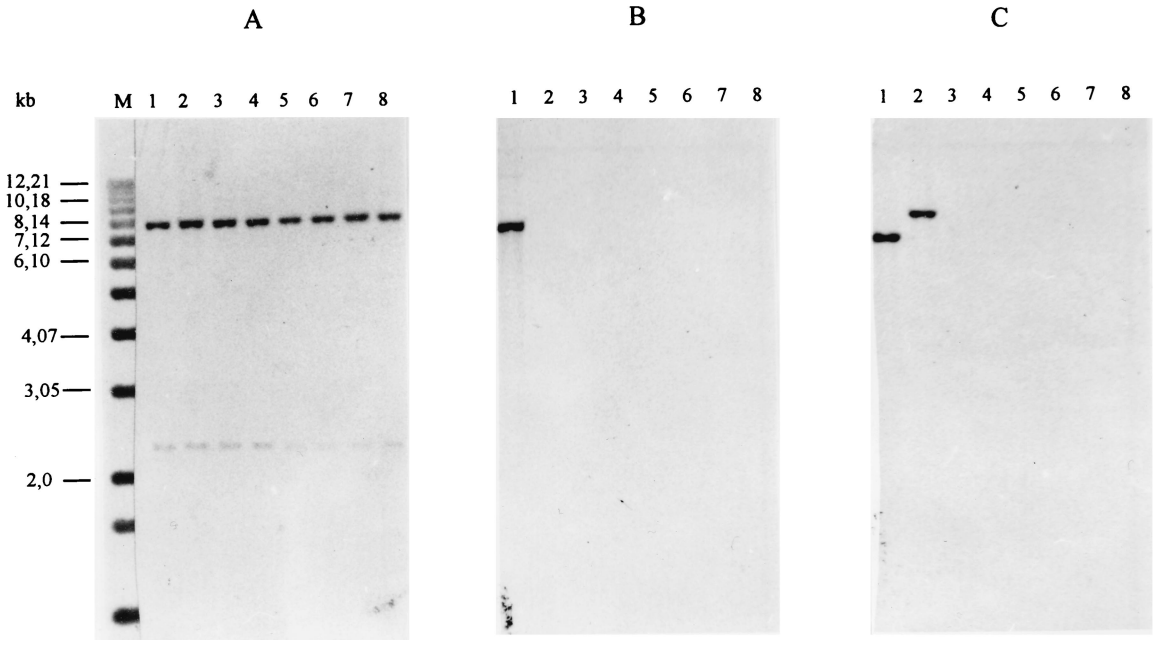

FIG. 2. Southern blot hybridization of *Eco*RI-restricted chromosomal DNAs of control strains *E. coli* O157:H7 EDL933 (lanes 1) and *E. coli* O157:H$^-$ E32511 (lanes 2) and the O157 isolates 19685 (lanes 3), 2937 (lanes 4), 6790 (lanes 5), 431 (lanes 6), 659 (lanes 7), and 2576 (lanes 8) with probes specific for *eae* (A), stx_1 (B), and stx_2 (C) under low-stringency conditions. M, molecular size marker.

is probable that a progenitor of the organisms that we detected contained *stx* genes that were subsequently lost. The emergence of pathogenic *E. coli* O157:H7 proposed by Feng et al. (11) does not accommodate the existence of a nontoxigenic *E. coli* strain that expresses the O157 LPS antigen.

We are unable to state with certainty that Stx had no role in the diseases observed in the patients who we report on here. Specifically, we cannot prove that we did not inadvertently overlook the true toxigenic pathogens in the isolation process, and we cannot affirm that the organisms that were initially shed did not contain toxin genes that were lost during isolation or subculture, a phenomenon that has been seen among STEC strains belonging to serotypes O2:H5, O26:H11, O73:H34, and O100:H32 (18).

Several lines of evidence suggest that these explanations do not apply. First, had we neglected to recover toxin-producing *E. coli* O157:H7/H$^-$ strains that were actually present or if *stx* genes were lost on subculture, the probability of identification of toxin in the stool by Vero cell assay should have been high, especially as the stools from early in the course of the illness were analyzed. Second, our isolation protocol contains no obvious bias against the isolation of toxin-producing organisms.

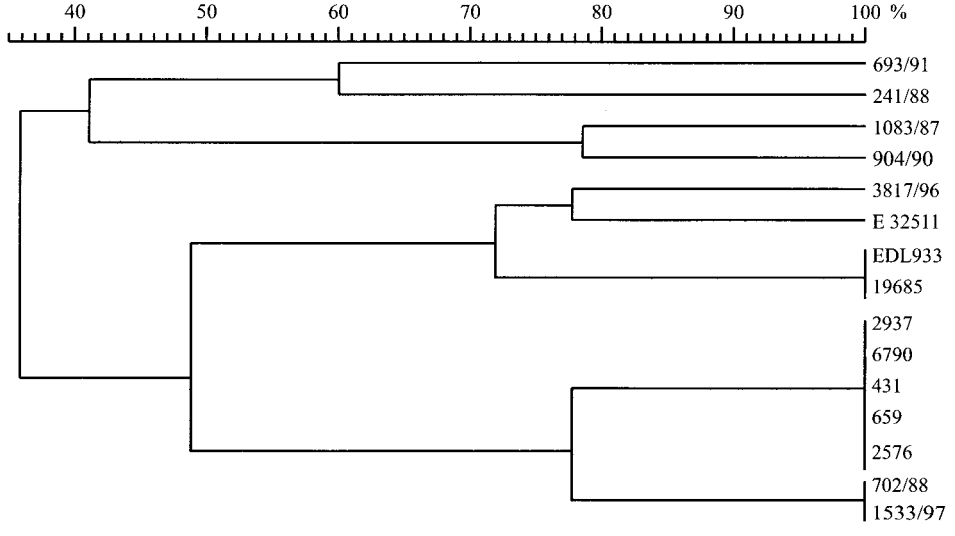

FIG. 3. Dendrogram of *E. coli* O157 strains used in this study based on RAPD analysis-PCR pattern similarity. The percentages represent band pattern similarities as calculated with the Jacquard algorithm.

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In addition, *stx*-negative *E. coli* O157:H7/H⁻ presumably contains many of the virulence traits contained by nontoxigenic, enteropathogenic *E. coli* O55:H7 (11), an organism closely related to *E. coli* O157:H7/H⁻.

E. coli strains of this serotype have been recovered from children with nonbloody diarrhea in North America (8), and it is plausible that nontoxigenic *E. coli* O157:H7 is similarly pathogenic.

Our data have a variety of implications for the diagnosis of *E. coli* O157:H7/H⁻ infections and for our understanding of the pathogenesis of diseases caused by this organism. First, screening on SMAC agar is inadequate for the detection of SF non-O157:H7 STEC strains, including stx_2-positive SF *E. coli* O157:H⁻. To identify these organisms, it is necessary to detect the Stx phenotype by cytotoxicity assay or antigen detection or to detect *stx* genes. However, the presumptively pathogenic strains that we reported on above would be overlooked by protocols that rely on toxin detection. Thus, we strongly urge the performance of parallel and complementary tests that address a variety of nontoxin phenotypes and loci possessed by such organisms when performing thorough enteric microbiologic evaluations.

Second, our data support the role of Stx as a cause of bloody diarrhea in humans, in contrast to its questionable role as a cause of nonbloody diarrhea. None of the patients described in this report developed bloody diarrhea, suggesting that toxin production is necessary for hemorrhagic colitis in most patients.

Third, Stx does not appear to be necessary for all manifestations of the diseases associated with *E. coli* O157:H7. It is interesting that *Shigella dysenteriae* serotype 1, which does not have the ability to produce Stx, causes nonbloody diarrhea in monkeys (13) and that Stx production is not needed for diarrhea in gnotobiotic piglets challenged with STEC (39). Also, Li et al. (23) demonstrated that *E. coli* O157:H7 strains deficient in the ability to produce Stxs were able to induce abnormalities of colonic structure and ion transport in New Zealand White rabbits.

Fourth, HUS might result from non-Stx factors produced by *E. coli* O157:H7/H⁻. Other bacteria that do not produce Stx, such as *Streptococcus pneumoniae* and *Neisseria meningitidis*, have occasionally been associated with HUS (2, 22). Our data raise the possibility that other properties of *E. coli* O157:H7 might also be sufficient to produce HUS in susceptible hosts. However, we observed HUS caused by an Stx-negative *E. coli* O157 strain without evidence of the presence of other pathogenic *E. coli* strains in only a single patient, and we urge clinicians to use caution before attributing HUS to nontoxigenic *E. coli*. Certainly, additional reports of the isolation of such potential pathogens from children with HUS, without evidence of the presence of fecal Stx, would lend support to our speculation.

In summary, we have identified *stx*-deficient *E. coli* O157 strains from infected humans with diarrhea and HUS using *stx*-independent recovery techniques. Moreover, these patients had no evidence of fecal Stx. The infecting isolates had other characteristics of *E. coli* O157:H7/H⁻ and were closely related to pathogenic toxigenic *E. coli* O157. Our findings suggest that Stx- and *stx* gene-based detection systems should be complemented by additional methods for the identification of stx-negative *E. coli* O157 in microbiologic evaluations and that the diseases in humans caused by *E. coli* O157:H7/H⁻ do not uniformly require the production of Stx by these pathogens.

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