JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 1995, p. 3174–3178 Vol. 33, No. 12
0095-1137/95/$04.00+0

Clonal Relatedness of Shiga-Like Toxin-Producing *Escherichia coli* O101 Strains of Human and Porcine Origin

SYLVIA FRANKE,[1] DAG HARMSEN,[1] ALFREDO CAPRIOLI,[2] DENIS PIERARD,[3] LOTHAR H. WIELER,[4] AND HELGE KARCH[1]*

Institut für Hygiene und Mikrobiologie der Universität Würzburg, 97080 Würzburg,[1] and Institut für Hygiene und Infektionskrankheiten der Tiere der Universität Gießen, 35392 Gießen,[4] Germany; Laboratorio di Ultrastrutture, Istituto Superiore di Sanità, 00161 Rome, Italy[2]; and Department of Microbiology, Academisch Ziekenhuis, Free University of Brussels, 1090 Brussels, Belgium[3]

Received 16 June 1995/Returned for modification 22 August 1995/Accepted 18 September 1995

Shiga-like toxin (SLT)-producing *Escherichia coli* (SLTEC) O101 has recently been associated with hemorrhagic colitis and hemolytic-uremic syndrome in humans. In this study, SLTEC O101 strains from humans and pigs were characterized for clonal relatedness by nucleotide sequence analysis of their *slt* genes, DNA fingerprinting of genomic DNA, and determination of virulence factors. The *slt* genes of five *E. coli* O101 strains were cloned and sequenced. For all strains, the deduced amino acid sequences of the B subunits were identical to those of the SLT-IIe present in the classical SLTEC O139 strains that cause edema disease in pigs. The A subunit revealed more than 99% homology to that of SLT-IIe. DNA fingerprinting revealed a high degree of genetic relatedness between the human and porcine O101 isolates. None of the O101 strains investigated had virulence factors frequently found in porcine (F107 fimbriae or heat-stable or heat-labile enterotoxins) or human SLTEC strains (*eaeA* or enterohemorrhagic *E. coli* hemolysin). The absence of virulence factors typical of SLT-I- and SLT-II-producing *E. coli* together with the presence of SLT-IIe, a toxin previously seen only in porcine *E. coli*, suggests a new pathogenic mechanism for *E. coli* O101 infection of humans. For diagnostic purposes, we recommend the use of PCR primers and DNA probes complementary to *slt*-IIe to correctly identify such strains and to further evaluate their role in human diseases.

Escherichia coli strains associated with hemorrhagic colitis and hemolytic-uremic syndrome (HUS) typically produce Shiga-like toxins (SLT), also called verocytotoxins. Cattle appear to be the major natural reservoir for SLT-producing *E. coli* (SLTEC) associated with human disease, and many outbreaks traced to contaminated cattle products have been reported (8, 13). SLTEC from humans and cattle generally produce SLT-I, which is homologous to the Shiga toxin of *Shigella dysenteriae* type 1 (16, 22), and either SLT-II (16) or a variant of SLT-II termed SLT-IIc (29). Genes encoding other alleles of the SLT-II cytotoxin family, termed the edema disease principle (7), SLT-IIv (34), or SLT-IIe (21), have been recently cloned and sequenced from porcine *E. coli* (15, 34).

The *E. coli* strains which produce SLT-IIe usually belong to serogroups O138, O139, and O141 and cause edema disease in pigs (11). These strains are generally considered to be pathogenic only to pigs and nonpathogenic to humans (11). However, SLTEC strains harboring *slt* genes which reacted in PCRs with *slt*-IIe-specific primers have been recently isolated from a patient with diarrhea (25) and from a patient with a case of HUS (31). Interestingly, both patients yielded strains of serogroup O101 that have never been reported among those associated with edema disease in pigs. However, the porcine origin of the O101 strains isolated from humans seems to be supported by the isolation of SLTEC O101 strains from the intestinal contents of 4 of 242 healthy pigs in Italy (6).

Since the precise structure of the *slt* genes present in *E. coli* O101 strains is unknown, we determined the complete sequence of the *slt* structural genes of five *E. coli* O101 strains of human and porcine origin and further assessed their genetic relatedness by DNA fingerprinting and analysis of virulence factors.

* Corresponding author. Mailing address: Institut für Hygiene und Mikrobiologie der Universität Würzburg, Josef-Schneider-Str. 2, 97080 Würzburg, Germany. Phone: 0931/201-5162. Fax: 0931/201-3445. Electronic mail address: hkarch@hygiene.uni-wuerzburg.de.

MATERIALS AND METHODS

Bacterial strains. *E. coli* O101:H9 strain VUB-EH60 was isolated from a patient with diarrhea in Belgium (25). Strains E-D42, E-D43, E-D53, and E-D68 were isolated in Italy from the intestinal contents of four different slaughtered pigs from three different farms (6). Strains E-D43, E-D53, and E-D68 were of serotype O101:H$^-$, strain E-D42 was of serotype O101:H14. Additional strains used for genetic analyses were *E. coli* lysogens C600 (H19J) and C600 (933W) and wild-type strains E57 (O138), E32511 (O157), EDL933 (O157), 76/5 (O143), HUS2/86 (O111), 4503/87 (O26), and WF96 (O7), which have been previously described (4, 20). In addition, *E. coli* O139:H1 strain 2458 from a pig with edema disease was used in Southern hybridization experiments.

PCR. Oligonucleotides and PCR conditions for amplification of *slt*-IB (28), *slt*-IIB (14), and *slt*-IIeB (10) have been published previously. Primers FK5 (5′-ATG AAG AAG ATA TTT GTA GCG-3′) and FK6 (5′-TTA ATT AAA CTG CAC TTC AGC-3′) were designed to amplify the B-subunit genes of SLT-IIOX3 and SLT-IIO111 (24), and oligonucleotides FK7 (5′-ATG AAG AAG ATG ATT ATT GCA-3′) and FK8 (5′-TCA GTT AAA CTT CAC CTG GGC-3′) were used for amplification of *slt*-IIvaB (12). *slt*-IIeA was amplified with primers FK9 (5′-CCC GGA TCC ATG AAG TGT ATA TTG TTA-3′) and FK10 (5′-CCC GAA TTC AGC ACA ATC CGC CGC CAT-3′).

Bacterial DNA was prepared by incubating 10 μl of a bacterial suspension (10^4 bacteria) for 10 min at 95°C. Amplifications were performed in a total volume of 50 μl containing 200 μM deoxynucleoside triphosphates (dATP, dCTP, dGTP, and dTTP), 30 pmol of each primer, 5 μl of 10-fold-concentrated polymerase synthesis buffer, and 2.5 U of *Taq* DNA polymerase (Amersham Laboratories, Buckinghamshire, United Kingdom). After an initial denaturation step of 5 min at 94°C, the samples were subjected to 30 cycles of denaturation (94°C, 30 s), annealing (52°C, 1 min), and extension (72°C, 60 s), followed by a single, final extension step of 5 min at 72°C. A 5-μl volume of each PCR product was subjected to submarine gel electrophoresis on a 1% agarose gel and visualized by staining with ethidium bromide.

Rep-PCR. Preparation of intact bacterial cells for whole-cell repetitive element sequence-based PCR (rep-PCR) has been described elsewhere (36). Briefly, cells were cultivated in Luria-Bertani broth, centrifuged, washed in 1 M NaCl, resuspended in 10 mM Tris–1 mM EDTA buffer (TE), and diluted in TE until the A_{600} equaled 1.00. A 1-μl aliquot of each cell suspension was used for

the PCR. For amplification of variable-length regions between interspersed repetitive elements, the PCR primer ERIC2 was used (33). The primer was obtained from Roth, Karlsruhe, Germany. Each 50-μl reaction mixture contained 25 pmol of the ERIC2 primer; 1 μl of a whole-cell suspension; 0.20 mM each dATP, dCTP, dGTP, and dTTP (Pharmacia LKB Biotechnology, Freiburg, Germany); 4 mM $MgCl_2$; and 2.5 U of *Taq* DNA polymerase (AmpliTaq; Perkin-Elmer, Vaterstetten, Germany) in a buffer containing 10 mM Tris-HCl (ph 8.3), 50 mM KCl, and 0.001% (wt/vol) gelatin. Thermal cycling reactions consisted of an initial denaturation step (95°C, 5 min) followed by 30 cycles of denaturation (94°C, 1 min), annealing (40°C, 1 min), and extension (72°C, 2 min), and a single, final extension step (72°C, 10 min). Reactions took place in a dedicated automated DNA thermal cycler (GeneAmp 2400; Perkin Elmer). Negative controls containing water in place of template DNA were run in parallel in each run.

An 8.0-μl volume of the PCR products was size fractionated by electrophoresis in a 1.5% agarose gel containing 1× TBE (Tris-boric acid-EDTA) buffer and 0.5 μg of ethidium bromide per ml and then visualized and photographed under UV light. The Boehringer VI marker was used as a size marker (Boehringer GmbH, Mannheim, Germany). A digital image of the gel was taken by a videocamera (INTAS, Göttingen, Germany). The digitized image was further analyzed by the GelCompar software package (Applied Maths, Kortrijk, Belgium) for IBM/PC computers. Calculation of the similarity matrix was done by the Pearson product-moment correlation coefficient method (30). The hierarchic clustering was achieved with the unweighted pair-group method with arithmetic averages clustering algorithm (30). Principal-component analysis of the rep-PCR-generated genomic fingerprints was also carried out with the GelCompar software.

DNA probes and Southern hybridization. Total bacterial DNAs of *E. coli* O101 isolates and control strains were isolated and restricted with *Bam*HI and *Bgl*II in accordance with the manufacturer's instructions. Hybridization experiments with digoxigenin-labeled *slt*-IB-, *slt*-IIB-, and *slt*-IIeB-specific DNA probes were performed under stringent conditions with the digoxigenin labeling and detection kit (Boehringer) as previously described (28).

Cloning and nucleotide sequence analysis. The A and B structural genes were cloned separately into vector pUC9 and subjected to *Taq* cycle sequencing. In parallel, PCR products were purified with the Prep-a-gene purification kit (Bio-Rad, Hercules, Calif.) as described by the manufacturer and then directly subjected to sequence analysis. Sequence analysis was performed with the M13/pUC sequencing and reverse sequencing oligonucleotides (Boehringer), internal primers were used to analyze the middle part of the DNA fragment. For sequencing of the PCR products, the respective oligonucleotides were used as sequencing primers. A total of 2 μg of the double-stranded DNA was subjected to *Taq* cycle sequencing reactions with the Prism Ready Reaction Dye Deoxy Terminator Cycle Sequencing kit (Applied Biosystems, Darmstadt, Germany) by following the manufacturer's instructions. Separation of sequencing products was performed on 7% denaturing polyacrylamide gels in a 373A automatic sequencer (Applied Biosystems). Nucleotide sequencing was carried out in triplicate and analyzed with the DNASIS program, version 2.0, from Hitachi Software, San Bruno, Calif.

Analysis for virulence factors. The O101 isolates were investigated for enterotoxin genes, enterohemorrhagic *E. coli* (EHEC) hemolysin (Hly) the adhesion factor intimin (*eaeA*), and the enteropathogenic *E. coli* adherence factor by PCR. Samples were prepared in the same manner as described for amplification of the *slt* genes. Primers and PCR conditions for detection of heat-stable and heat-labile enterotoxins I were employed as described by Olsvik and Strockbine (23). Oligonucleotides and amplification protocols for detection of EHEC *hlyA* (27), *eaeA* (17), and the enteropathogenic *E. coli* adherence factor (9) have been published elsewhere. F107 fimbriae were detected by agglutination assay with anti-F107 antibodies following overnight growth at 37°C in a 10% CO_2 atmosphere on Iso-sensitest agar plates (Oxoid, Wesel, Germany) with 0.062% alizarine yellow (Fluka, Neu-Ulm, Germany)–0.125% eosin (35). Determination of the abilities of O101 strains to adhere to HEp-2 cells and the fluorescent actin staining test were performed as described by Knutton et al. (18) with minor modifications (17). The enterohemolytic phenotype of EHEC Hly (27) was investigated as previously described (2).

Nucleotide sequence accession numbers. The nucleotide sequences of the SLTs of O101 strains E-D42, E-D43, E-D53, and E-D68 were submitted to the EBI data library and assigned accession numbers X81415 to X81418.

RESULTS

PCR and Southern hybridization of SLTEC O101. Human *E. coli* O101 strain VUB-EH60 and porcine isolates E-D42, E-D43, E-D53, and E-D68 were analyzed by PCR with primers complementary to the published *slt* sequences. A positive PCR result was obtained only with primer pairs FK1-FK2 and FK8-FK9, which are complementary to *slt*-IIeB and *slt*-IIeA, respectively.

Southern hybridization experiments with *slt*-IIeB as a probe were performed with digested genomic DNAs of the five *E. coli* O101 strains under study, as well as with control strains possessing *slt*-I, *slt*-II, and *slt*-IIe sequences. The resulting patterns are shown in Fig. 1. No hybridization signal was observed for either SLT-I-producing control strain C600 (H19J) (lane 1) or SLT-II-producing strain C600 (933W) (lane 2). As shown in lanes 3 and 9, the porcine *E. coli* O138 and O139 strains demonstrated strong hybridization signals. DNA from human *E. coli* O101 isolate VUB-EH60 yielded a hybridization signal with a DNA fragment at approximately 5.1 kb (lane 4). The probe also recognized single fragments in the DNAs from the four porcine *E. coli* O101 strains (lane 5 to 8). O101:H^- strains E-D43 (lane 6), E-D53 (lane 7), and E-D68 (lane 8) showed a hybridization signal with a fragment of 5 kb. In contrast, the probe recognized a DNA fragment of approximately 7 kb from O101:H14 strain E-D42 (lane 5). When Southern hybridization experiments with the strains analyzed in Fig. 1 were performed with the *slt*-IB or the *slt*-IIB probe, hybridization was observed only with DNA of the *slt*-I or the *slt*-II control strain, respectively.

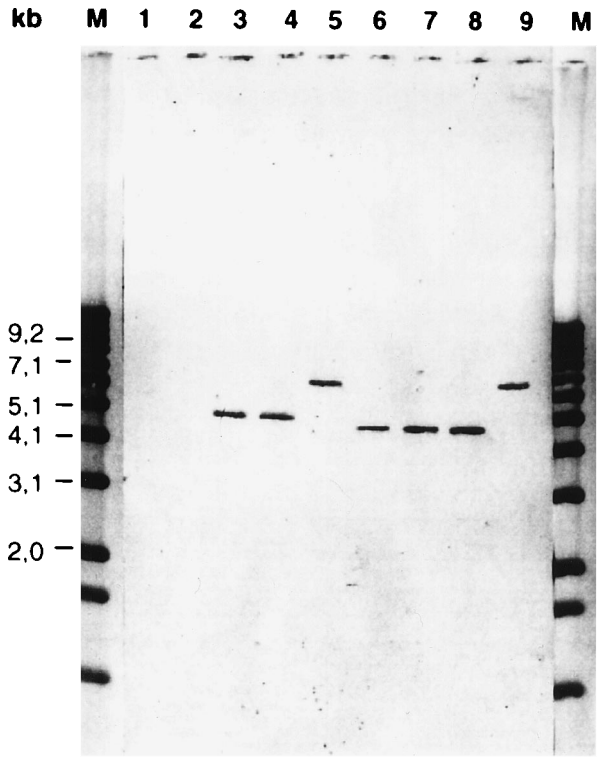

FIG. 1. Southern hybridization of *Bam*HI-*Bgl*II-restricted genomic DNA with a digoxigenin-labeled *slt*-IIeB probe. *E. coli* C600 (H19J) (lane 1), C600 (933W) (lane 2), E57 (O138) (lane 3), VUB-EH60 (O101) (lane 4), E-D42 (O101) (lane 5), E-D43 (O101) (lane 6), E-D53 (O101) (lane 7), E-D68 (O101) (lane 8), and 2458 (O139) (lane 9) are shown. Lanes M contained the 1-kb DNA ladder (Gibco-BRL).

Nucleotide sequence analysis of *slt* genes of *E. coli* O101. To subtype the *slt* genes present in the O101 strains more precisely, the A and B subunit genes resulting from PCR with primer pairs FK8-FK9 and FK1-FK2, respectively, were cloned into pUC9 and subjected to nucleotide sequence analysis. In parallel experiments, the PCR products were directly subjected to *Taq* cycle sequence analysis. Both methods gave identical results. The *slt* genes of human isolate VUB-EH60 showed complete homology to the published sequence (34) of SLTEC O139 strain S1191. Porcine *E. coli* O101:H14 isolate E-D42 exhibited a single nucleotide difference from the published

	31 50	81 120	301 320
SLT-IIeA	TQQSYVSSLN SIRTAISTPL	VYQERFDHLR LIIERNNLYV AGFVNTTTNT FYRFSDFAHI	NTAAAFLNRK SQSLYTTGEW
VUB-EH60	---------- ----------	---------- ---------- ---------- ----------	---------- ----------
E-D 42	---------Y ----------	-----I---- ---------- ---------- ----------	---------- --P-------
E-D 43	---------- ----------	---------- ---------- V--------- S---------	---------- --P-------
E-D 53	---------- ----------	---------- ---------- ---------- S---------	---------- --P-------
E-D 68	---------- ----------	---------- ---------- V--------- S---------	---------- --P-------

FIG. 2. Amino acid sequence changes in the O101 SLTs compared with SLT-IIeA. Human isolate VUB-EH60 showed 100% homology to SLT-IIeA, whereas porcine isolates E-D42, E-D43, E-D53, and E-D68 revealed single mutations at five sites within the A subunit. The B-subunit genes of all of the strains tested were identical to the published *slt*-IIeB sequence (34), except for strain E-D42, which exhibited a single nucleotide difference at position 187.

slt-IIeB sequence at position 187 (data not shown). Comparison of the nucleotide sequences present in the three porcine *E. coli* O101:H$^-$ strains revealed complete identity with the published *slt*-IIeB sequence. The A-subunit genes present in the *E. coli* O101 isolates revealed several single aberrations from the published *slt*-IIe DNA sequence but, nevertheless, showed more than 99% similarity to the *slt*-IIeA subunit gene. In Fig. 2, the sites of amino acid changes within the SLTs of the five O101 strains are shown and compared to the respective original SLT-IIeA regions.

Rep-PCR analysis of *E. coli* O101 strains. The primer corresponding to a conserved DNA sequence of ERIC elements annealed to genomic DNA and generated genomic fingerprints of each strain (Fig. 3). Rep-PCR DNA fingerprints clearly distinguished the *E. coli* O101 isolates from other SLTEC isolates. The patterns obtained from the human and porcine O101 strains were nearly congruent, whereas the fingerprints of the other SLTEC isolates demonstrated no similarity.

Two clusters were obtained by the Pearson product-moment correlation coefficient method and following unweighted pair-group method with arithmetic averages clustering (Fig. 4). One group contained all O101 isolates. Principal-component analysis also showed a distinct O101 group which clearly was different from all other groups and thus supporting the results obtained from unweighted pair-group method with arithmetic averages clustering (data not shown).

Analysis of virulence factors in SLT-IIe-producing *E. coli*. All *E. coli* O101 strains were tested by PCR with primers complementary to *lt*, *st*, EHEC Hly, *eaeA*, and the enteropathogenic *E. coli* adherence factor. These PCR analyses revealed no evidence for the presence of these virulence factors. In addition, all of the strains showed negative results in the assay for EHEC Hly, as well as in the fluorescent actin staining test, and were negative for F107 fimbriae.

DISCUSSION

Recently, SLT-IIe-producing *E. coli* strains of serogroup O101 have been isolated from a patient with diarrhea in Belgium (25) and from a patient with HUS in the United Kingdom (31). In both cases, the porcine origin of the infecting strain was not demonstrated, because no history of possible sources

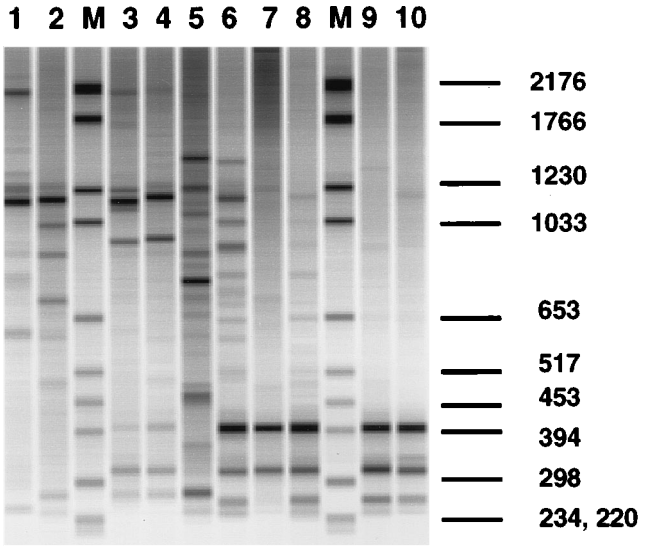

FIG. 3. Rep-PCR-generated DNA fingerprints obtained with primer ERIC2 from different *E. coli* isolates. An 8.0-μl portion of each rep-PCR mixture was loaded onto a 1.5% agarose gel. The resulting electrophoresis patterns of *E. coli* isolates EDL933 (lane 1), 76/5 (lane 2), HUS2/86 (lane 3), 4503/87 (lane 4), WF96 (lane 5), VUB-EH60 (lane 6), E-D53 (lane 7), E-D43 (lane 8), E-D42 (lane 9), and E-D68 (lane 10) are shown. Lanes M contained DNA molecular size markers (Boehringer VI marker); the sizes are in base pairs.

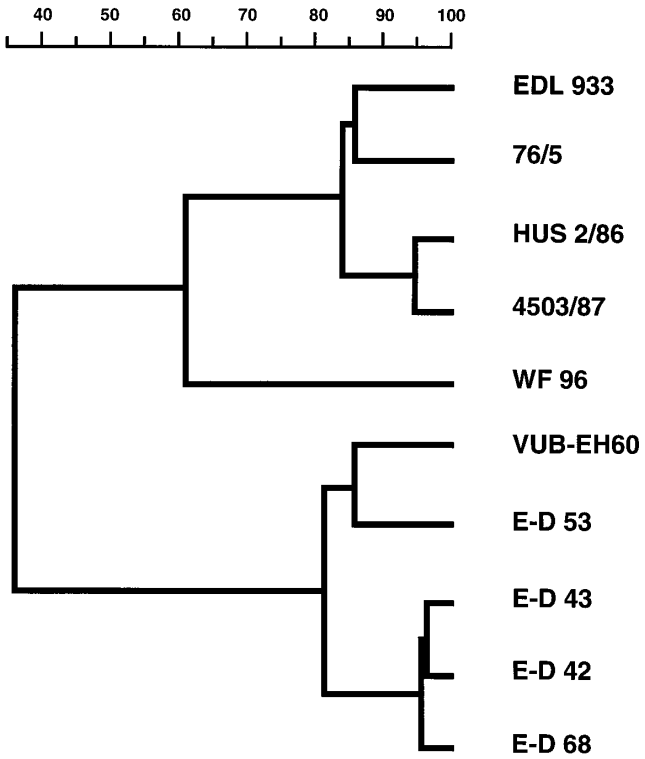

FIG. 4. Dendrogram derived from ERIC2 rep-PCR data with the GelCompare software package. The similarity matrix was calculated by the Pearson product-moment correlation method, and hierarchic clustering was achieved with the unweighted pair-group method with arithmetic averages clustering algorithm. The similarity scale is above the dendrogram.

of infection was available for the patients. However, SLTEC strains have been isolated from healthy pigs in Italy, suggesting that pigs are a natural reservoir of O101 SLTEC and that pork products may be contaminated with and transmit pathogenic strains (6). In this study, the SLTEC O101 strains from humans and pigs were compared by rep-PCR, which revealed a high degree of genetic relatedness. This makes the porcine origin of human O101 SLTEC strain VUB-EH60 very likely. The rep-PCR method proved to be a valuable and powerful tool for epidemiological analysis. We chose the Pearson correlation coefficient and not a band-based similarity coefficient (e.g., the coefficient of Jaccard or Dice) for numerical comparison, because the Pearson coefficient has proved to be the most objective and reliable measure of similarity (32). This coefficient is by far the most robust one, as it does not rely on subjective band detection and matching criteria, because it compares entire densitometric curves rather than band characteristics.

We demonstrated that the structural *slt* genes of the O101 strains were either identical or nearly identical to that previously published for the *slt*-IIe genes of the O139 SLTEC strains typically associated with edema disease (34). Comparison of the nucleotide sequence of the *slt*-IIe genes with those of *slt*-II showed 94% homology and 80% homology for the A and B subunits, respectively. SLT-IIe preferentially binds to globotetraosylceramide, whereas SLT-II produced by human and bovine strains binds predominantly to globotriaosylceramide. The impact of the distribution of globotriaosylceramide on the clinical manifestations of SLTEC infection has been the subject of several studies (3, 5, 26). Site-directed mutagenesis of the B subunit of *slt*-IIe changes the target distribution of SLT-IIe in piglets (5). Conversely, little is known regarding the distribution of globotetraosylceramide in human tissues, which could have an important role in determining the localization and intensity of toxin-induced damage during infections with SLT-IIe-producing *E. coli*. Only recently was it shown that SLT-I, SLT-II, and SLT-IIe bind efficiently to human erythrocytes in vitro (3). Interestingly, SLT-IIe binds to human erythrocytes with even higher efficiency than does SLT-I or SLT-II (3). Erythrocytes seem either to be a vehicle for SLT or to compete with SLT receptors on endothelial cells. Boyd et al. (5) have shown that erythrocyte binding of SLT-IIe prolonged toxin circulation after intravenous injection into pigs. However, those investigators could not demonstrate protection of the experimental pigs against toxin-mediated damage of endothelial cells (5).

Although SLTEC strains pathogenic to pigs often harbor genes that encode the production of adhesins (1, 35, 37) and enterotoxins (20, 37), the O101 isolates described here were negative in PCR analysis for the genes for heat-stable and heat-labile enterotoxins I and did not produce the F107 fimbriae found in O139 strains that cause edema disease (1, 35). In addition, other virulence characteristics of human SLTEC, such as the presence of the *eaeA* gene (38), positivity in the fluorescent actin staining test on HEp-2 cells (18), and production of the EHEC Hly (27), could not be detected. The absence of the *eaeA* determinant and of the ability to adhere to Hep-2 cells has been reported for SLTEC strains associated with hemorrhagic colitis or HUS and belonging to serogroups such as O18, O91, O113, O117, and O145 (19). Similarly, SLTEC strains associated with edema disease in pigs do not cause attaching and effacing lesions and do not possess the *eaeA* gene, but some of them express fimbrial adhesins and attach to the intestinal mucosa in a fashion similar to that displayed by enterotoxigenic *E. coli* (1). Further investigation of the adhesion properties of O101 strains is needed.

SLTEC O101 strains appear to be nonpathogenic to pigs, since they have been isolated from healthy animals (6) but not from diseased animals. Many SLTEC serotypes which have been firmly associated with human disease, including the most dangerous serotype, O157:H7, seem to be innocuous to adult cattle (6, 13). If this is also true for O101 SLTEC strains, it could have important epidemiologic and hygienic implications in the preparation of food from porcine sources. Further investigations are required to evaluate the frequency of SLT-IIe-producing *E. coli* in human infections. To address this issue, it is important that PCR primers and DNA probes complementary to SLT-IIe DNA sequences be included in the tools for laboratory diagnosis of SLTEC infections to correctly identify these strains.

ACKNOWLEDGMENTS

We thank J. Heesemann, Würzburg, Germany, for helpful discussions and B. Plaschke for excellent technical assistance. We also thank T. S. Whittam, Department of Biology, Pennsylvania State University, for critical reading of the manuscript and J. R. Lupski, Baylor College of Medicine, Houston, Tex., for making unpublished material available.

This work was supported by the Deutsche Forschungsgemeinschaft (Ka 717-2).

REFERENCES

1. **Bertschinger, H. U., M. Bachmann, C. Mettler, A. Pospischil, E. M. Schraner, M. Stamm, T. Sydler, and P. Wild.** 1990. Adhesive fimbriae produced in vivo by *Escherichia coli* O139:K12(B):H1 associated with enterotoxaemia in pigs. Vet. Microbiol. **25:**267–281.
2. **Beutin, L., M. A. Montenegro, I. Orskov, F. Orskov, J. Prada, S. Zimmermann, and R. Stephan.** 1989. Close association of verotoxin (Shiga-like toxin) production with enterohemolysin production in strains of *Escherichia coli*. J. Clin. Microbiol. **27:**2559–2564.
3. **Bitzan, M., S. Richardson, C. Huang, M. Petric, and M. A. Karmali.** 1994. Evidence that verotoxins (Shiga-like toxins) from *Escherichia coli* bind to P blood group antigens of human erythrocytes in vitro. Infect. Immun. **62:** 3337–3347.
4. **Böhm, H., and H. Karch.** 1992. DNA fingerprinting of *Escherichia coli* O157:H7 strains by pulsed-field gel electrophoresis. J. Clin. Microbiol. **30:** 2169–2172.
5. **Boyd, B., G. Tyrrell, M. Maloney, C. Gyles, J. Brunton, and C. Lingwood.** 1993. Alteration of the glycolipid binding specificity of the pig edema toxin from globotetraosyl to globotriaosyl ceramide alters in vivo tissue targetting and results in a verotoxin 1-like disease in pigs. J. Exp. Med. **177:**1745–1753.
6. **Caprioli, A., A. Nigrelli, R. Gatti, M. Zavanella, A. M. Blando, F. Minelli, and G. Donelli.** 1993. Characterisation of verocytotoxin-producing *Escherichia coli* isolated from pigs and cattle in northern Italy. Vet. Rec. **133:**323–324.
7. **Dobrescu, L.** 1983. New biological effect of edema disease principle (*Escherichia coli* neurotoxin) and its use as an in vitro assay for this toxin. Am. J. Vet. Res. **44:**31–34.
8. **Dorn, C. R.** 1988. Hemorrhagic colitis and hemolytic uremic syndrome caused by *Escherichia coli* in people consuming undercooked beef and unpasteurized milk. J. Am. Vet. Med. Assoc. **193:**1360–1361. (Letter.)
9. **Franke, J., S. Franke, H. Schmidt, A. Schwarzkopf, L. H. Wieler, G. Baljer, L. Beutin, and H. Karch.** 1994. Nucleotide sequence analysis of enteropathogenic *Escherichia coli* (EPEC) adherence factor probe and development of PCR for rapid detection of EPEC harboring virulence plasmids. J. Clin. Microbiol. **32:**2460–2463.
10. **Franke, S., F. Gunzer, L. H. Wieler, G. Baljer, and H. Karch.** 1995. Construction of recombinant Shiga-like toxin-IIv (SLT-IIv) and its use in monitoring the SLT-IIv antibody status of pigs. Vet. Microbiol. **43:**41–52.
11. **Gannon, V. P., and C. L. Gyles.** 1990. Characteristics of the Shiga-like toxin produced by *Escherichia coli* associated with porcine edema disease. Vet. Microbiol. **24:**89–100.
12. **Gannon, V. P., C. Teerling, S. A. Masri, and C. L. Gyles.** 1990. Molecular cloning and nucleotide sequence of another variant of the *Escherichia coli* Shiga-like toxin II family. J. Gen. Microbiol. **136:**1125–1135.
13. **Griffin, P. M., and R. V. Tauxe.** 1991. The epidemiology of infections caused by *Escherichia coli* O157:H7, other enterohemorrhagic *E. coli*, and the associated hemolytic uremic syndrome. Epidemiol. Rev. **13:**60–98.
14. **Gunzer, F., and H. Karch.** 1993. Expression of A and B subunits of Shiga-like toxin II as fusions with glutathione *S*-transferase and their potential for use in seroepidemiology. J. Clin. Microbiol. **31:**2604–2610.
15. **Gyles, C. L., S. A. De Grandis, C. MacKenzie, and J. L. Brunton.** 1988. Cloning and nucleotide sequence analysis of the genes determining verocy-

totoxin production in a porcine edema disease isolate of *Escherichia coli*. Microb. Pathog. **5:**419–426.
16. **Jackson, M. P., R. J. Neill, A. D. O'Brien, R. K. Holmes, and J. W. Newland.** 1987. Nucleotide sequence analysis and comparison of the structural genes for Shiga-like toxin I and Shiga-like toxin II encoded by bacteriophages from *Escherichia coli* 933. FEMS Microbiol. Lett. **44:**109–114.
17. **Karch, H., H. Böhm, H. Schmidt, F. Gunzer, S. Aleksic, and J. Heesemann.** 1993. Clonal structure and pathogenicity of Shiga-like toxin-producing, sorbitol-fermenting *Escherichia coli* O157:H$^-$. J. Clin. Microbiol. **31:**1200–1205.
18. **Knutton, S., T. Baldwin, P. H. Williams, and A. S. McNeish.** 1989. Actin accumulation at sites of bacterial adhesion to tissue culture cells: basis of a new diagnostic test for enteropathogenic and enterohemorrhagic *Escherichia coli*. Infect. Immun. **57:**1290–1298.
19. **Louie, M., J. De Azavedo, R. Clarke, A. Borczyk, H. Lior, M. Richter, and J. Brunton.** 1994. Sequence heterogeneity of the *eae* gene and detection of verotoxin-producing *Escherichia coli* using serotype-specific primers. Epidemiol. Infect. **112:**449–461.
20. **Meyer, T., and H. Karch.** 1989. Genes coding for Shiga-like toxin and heat-stabile enterotoxin in porcine strains of *Escherichia coli*. FEMS Microbiol. Lett. **49:**115–119.
21. **O'Brien, A. D., M. A. Karmali, and S. M. Scotland.** 1994. A proposal for rationalizing the nomenclature of the *Escherichia coli* cytotoxin, p. 147–149. *In* M. A. Karmali and A. G. Goglio (ed.), Recent advances in verocytotoxin-producing *Escherichia coli* infections. Elsevier Science B.V., Amsterdam.
22. **O'Brien, A. D., and G. D. LaVeck.** 1983. Purification and characterization of a *Shigella dysenteriae* 1-like toxin produced by *Escherichia coli*. Infect. Immun. **40:**675–683.
23. **Olsvik, O., and N. A. Strockbine.** 1993. PCR detection of heat-stable, heat-labile, and Shiga-like toxin genes in Escherichia coli, p. 271–276. *In* D. H. Persing, T. F. Smith, F. C. Tenover, and T. J. White (ed.), Diagnostic molecular microbiology—principles and applications. American Society for Microbiology, Washington, D.C.
24. **Paton, A. W., J. C. Paton, M. W. Heuzenroeder, P. N. Goldwater, and P. A. Manning.** 1992. Cloning and nucleotide sequence of a variant Shiga-like toxin II gene from *Escherichia coli* OX3:H21 isolated from a case of sudden infant death syndrome. Microb. Pathog. **13:**225–236.
25. **Pierard, D., L. Huyghens, S. Lauwers, and H. Lior.** 1991. Diarrhoea associated with *Escherichia coli* producing porcine oedema disease verotoxin. Lancet **338:**762. (Letter.)
26. **Samuel, J. E., L. P. Perera, S. Ward, A. D. O'Brien, V. Ginsburg, and H. C. Krivan.** 1990. Comparison of the glycolipid receptor specificities of Shiga-like toxin type II and Shiga-like toxin type II variants. Infect. Immun. **58:**611–618.
27. **Schmidt, H., L. Beutin, and H. Karch.** 1995. Molecular analysis of the plasmid-encoded hemolysin of *Escherichia coli* O157:H7 strain EDL933. Infect. Immun. **63:**1055–1061.
28. **Schmidt, H., M. Montag, J. Bockemühl, J. Heesemann, and H. Karch.** 1992. Shiga-like toxin-related cytotoxins in *Citrobacter freundii* strains from humans and beef samples. Infect. Immun. **61:**534–543.
29. **Schmitt, C. K., M. L. McKee, and A. D. O'Brien.** 1991. Two copies of Shiga-like toxin II-related genes common in enterohemorrhagic *Escherichia coli* strains are responsible for the antigenic heterogeneity of the O157:H$^-$ strain E32511. Infect. Immun. **59:**1065–1073.
30. **Sneath, P. H., and R. R. Sokal.** 1973. Numerical taxonomy. The principles and practice of numerical classification. W. H. Freeman & Co., San Francisco.
31. **Thomas, A., T. Cheasty, H. Chart, and B. Rowe.** 1994. Isolation of Vero cytotoxin-producing *Escherichia coli* serotypes O9ab:H$^-$ carrying VT2 variant gene sequences from a patient with haemolytic uraemic syndrome. Eur. J. Microbiol. Infect. Dis. **13:**1074–1076.
32. **Vauterin, L., J. Swings, and K. Kersters.** 1993. Protein electrophoresis and classification, p. 251–313. *In* M. Goodfellow and A. G. O'Donnell (ed.), Handbook of new bacterial systematics. Academic Press, London.
33. **Versalovic, J., T. Koeuth, and J. R. Lupski.** 1991. Distribution of repetitive DNA sequences in eubacteria and application to fingerprinting of bacterial genomes. Nucleic Acids Res. **19:**6823–6831.
34. **Weinstein, D. L., M. P. Jackson, J. E. Samuel, R. K. Holmes, and A. D. O'Brien.** 1988. Cloning and sequencing of a Shiga-like toxin type II variant from *Escherichia coli* strain responsible for edema disease of swine. J. Bacteriol. **170:**4223–4230.
35. **Wittig, W., R. Prager, M. Stamm, W. Streckel, and H. Tschäpe.** 1994. Expression and plasmid transfer of genes coding for the fimbrial antigen F107 in porcine *Escherichia coli* strains. Int. J. Med. Microbiol. Virol. Parasitol. Infect. Dis. **281:**130–134.
36. **Woods, C. R., J. Versalovic, T. Koeuth, and J. R. Lupski.** 1993. Whole cell repetitive element sequence-based polymerase chain reaction allows rapid assessment of clinical relationships of bacterial isolates. J. Clin. Microbiol. **31:**1927–1931.
37. **Woodward, M. J., and C. Wray.** 1990. Nine DNA probes for detection of toxin and adhesin genes in *Escherichia coli* isolated from diarrhoeal disease in animals. Vet. Microbiol. **25:**55–65.
38. **Yu, J., and J. B. Kaper.** 1992. Cloning and characterization of the *eae* gene of enterohemorrhagic *Escherichia coli* O157:H7. Mol. Microbiol. **6:**411–417.