

Hemolytic Uremic Syndrome in a 65-Year-Old Male Linked to a Very Unusual Type of *stx*_{2e}- and *eae*-Harboring O51:H49 Shiga Toxin-Producing *Escherichia coli*

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We report on a 65-year-old male patient with a Shiga toxin-producing *Escherichia coli* O51:H49 gastrointestinal infection and sepsis associated with hemolytic uremic syndrome (HUS) with a fatal outcome. The strains isolated harbored *stx*_{2e} and *eae*, a very unusual and new virulence profile for an HUS-associated enterohemorrhagic *E. coli*.

CASE REPORT

A 65-year-old male patient had undergone unrelated allogeneic hematopoietic stem cell transplantation for a myelodysplastic syndrome. Immunosuppressive therapy posttransplantation included 300 mg cyclosporine daily with a trough level of 150 to 300 µg/liter. Antithymoglobulin was given on days -3 to -1, and 30 mg methotrexate was given on days 1, 3, and 6 as graft-versus-host disease (GVHD) prophylaxis. Neutrophil engraftment occurred on day 21 after transplantation. On day 33, clinical signs of acute GVHD grade II with whole body exanthema and severe diarrhea occurred. The immunosuppressive therapy was extended with 2 mg prednisone/kg of body weight daily and 3 mg budenofalk three times a day (TID). At the same time, virological investigations revealed a low-level replication of cytomegalovirus (CMV) in blood and intestine without histological proof. Therefore, antiviral treatment with valganciclovir was given for 21 days. After clinical improvement on day 40, the patient was discharged from the hospital. As the GVHD improved, the immunosuppressive treatment with prednisone was gradually reduced to 0.4 mg/kg daily, and cyclosporine was changed to 3 mg prograf twice daily (BID) due to side effects, such as renal insufficiency and hypertension.

On day 120, the patient was again hospitalized because of severe watery diarrhea and abdominal pain for 8 days, without fever or vomiting. No special dietary habits were identified. Upon admission, blood pressure was stable, he was afebrile, and the abdomen was tender without defense. Blood tests revealed a white blood cell count (WBC) of 3.8×10^9 /liter, thrombocytopenia (74×10^9 /liter), an elevated creatinine level (124 µmol/liter), and a slightly elevated C-reactive protein (CRP) level (11.2 mg/liter). C-reactive protein increased to 46.7 mg/liter on day 129. CMV PCR in plasma was again positive. Stool analysis showed no growth of *Salmonella* species, *Campylobacter* species, or *Shigella* species, and *Clostridium difficile* antigen was also negative. Biopsy specimens from the colon showed changes consistent with active GVHD. Therefore, the dose of prednisone was increased to 2 mg/kg daily, and asymptomatic CMV replication was treated with 5 mg/kg intravenous ganciclovir BID.

On day 130, as the diarrhea persisted, an intra-arterial steroid

application into the arteria mesenterica superior and inferior was given with moderate clinical effect. A screening multiplex PCR (Luminex Molecular Diagnostics Inc., Toronto, Canada) analyzing the stool for 15 gastroenteritis pathogens revealed the presence of Shiga toxin-producing *Escherichia coli* (STEC). Initially, following current recommendations, antibiotic treatment was withheld (1). However, as the patient's physical condition further deteriorated with persistent and severe diarrhea, treatment with 500 mg azithromycin daily was initiated (2). Blood cultures taken before administration of azithromycin turned positive on day 131, with growth of Gram-negative rods, later identified as STEC. A combination therapy with 1 g meropenem TID and 500 mg azithromycin daily was established.

On day 135, the patient got confused. Blood tests showed hemolysis, rising creatinine, and aggravated thrombocytopenia, leading to the diagnosis of hemolytic uremic syndrome (HUS). The patient received plasmapheresis and hemodialysis in the intensive care unit. Under the established therapy, an initial stabilization with improvement of the status was achieved.

On day 140, the patient left the hospital on his request against medical advice. He received further antibiotic treatment with azithromycin and levofloxacin. The immunosuppressive therapy was continued with 80 mg methylprednisolone daily and 3 mg tacrolimus TID. He died a few days later.

Two STEC strains were isolated from the stool (strain P13-119, at day 130) and the blood (strain P13-131, at day 131) of this patient. The strains were serotyped by using standard methods at the Robert Koch Institute, Wernigerode, Germany, and further tested for Shiga toxin production using an enzyme immunoassay

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TABLE 1 Profile of chromosomal and plasmid-located non-*stx* putative virulence genes within the two O51:H49 STEC strains isolated from the stool (P13-119) and the blood (P13-131) of the patient and an unrelated O51:H49 EPEC strain (P10-2234)

Gene	Description	Presence or absence of gene ^a		
		P13-119	P13-131	P10-2234
α - <i>hlyA</i>	α -Hemolysin	–	–	–
EHEC- <i>hlyA</i>	EHEC hemolysin	–	–	–
<i>eae</i>	Intimin	+ (beta)	+ (beta)	+ (alpha)
<i>iha</i>	Iron-regulated gene A homologue adhesion	–	–	–
<i>efa-1</i>	EHEC factor for adherence	–	–	–
<i>saa</i>	Auto-agglutinating adhesin	–	–	–
<i>fedA</i>	Major subunit of F18 fimbrial adhesin	–	–	–
<i>pup</i> cluster	P fimbriae	–	–	–
<i>aggR</i>	Transcriptional activator	–	–	–
<i>sfpA</i>	Major subunit of Sfp fimbriae	–	–	–
<i>lpfAO26</i>	Major subunit of long polar fimbriae	+	+	–
<i>lpfAO113</i>	Major subunit of long polar fimbriae	–	–	–
<i>lpfAO157-OI141</i>	Major subunit of long polar fimbriae	–	–	–
<i>lpfA O157-OI154</i>	Major subunit of long polar fimbriae	–	–	–
<i>bfpA</i>	Major subunit of bundle-forming pili	–	–	–
<i>katP</i>	Catalase-peroxidase	–	–	–
<i>espP</i>	Serine protease	–	–	–
<i>etpD</i>	Type II secretion system	–	–	–
<i>cnf1</i>	Cytotoxic necrotizing factor	–	–	–
<i>vat</i>	Vacuolating autotransporter toxin	–	–	+
<i>traT</i>	Serum resistance	+	+	–
<i>terE</i>	Marker for the cluster encoding tellurite resistance	–	–	–
<i>irp2</i>	Marker for the cluster encoding an iron uptake system	+	+	–
<i>fyuA</i>	Yersiniabactin receptor	+	+	+

^a +, gene is present; –, gene is absent.

(ProSpect Shiga-toxin *E. coli* test; Remel, Kansas, USA) and the Vero cell test (3). Shiga toxin genes were subtyped by PCR. Furthermore, the *stx* subtype was confirmed by sequencing (4). Genes encoding non-Shiga toxin putative virulence factors of STEC, including toxins, adhesins, and plasmid-located genes (Table 1), were searched for by different PCR systems (3, 5–7). The phylogenetic groups of the isolates were determined as described previously (8). Multilocus sequence typing (MLST) was done in accordance with the *E. coli* MLST website (<http://mlst.ucc.ie/mlst/dbs/Ecoli>). Pulsed-field gel electrophoresis (PFGE) of the isolates was performed by following the CDC PulseNet protocol.

The two STEC strains were of serotype O51:H49, belonged to sequence type (ST) 20, *E. coli* phylogenetic group B1, showed a sorbitol-fermenting phenotype, and harbored the combination of the virulence genes *stx*_{2e} and *eae* (subtype beta). Further molecular characterization results are summarized in Table 1. Both strains produced Stx as detected by the Vero cell assay (P13-119 Stx titer, 1:128; P13-131 Stx titer, 1:32) but not by the enzyme-linked immunosorbent assay (ELISA). In addition, the strains were indistinguishable by the PFGE analysis.

Pathogenicity of STEC is associated with various virulence factors; the most important represent the Shiga toxins (Stxs). These toxins can be subdivided into two main groups, Stx1 and Stx2 (9). STEC strains pathogenic for humans tend to feature Stx2 and additional virulence traits, such as the adhesion factor intimin (10, 11). Of the seven Stx2 variants described so far, the representatives of the Stx2acd group (*stx*_{2a}, *stx*_{2c}, *stx*_{2d}), which are genetically closely related, are reported to be strongly associated with HUS in

patients (4, 10). The highly pathogenic subgroup of STEC that causes severe human disease is also called enterohemorrhagic *E. coli* (EHEC). Recently, a collection of representative HUS-associated enterohemorrhagic *E. coli* (HUSEC) (www.ehec.org) was established (12).

Here, we provide clinical and microbiological evidence for a very unusual STEC O51:H49 infection leading to HUS in a 65-year-old male patient. Three consecutive patient serum samples, which were additionally tested for concurrent or recent infection by *E. coli* O157, were negative for immunoglobulin M and immunoglobulin G anti-O157 lipopolysaccharide antibodies. This result further underlined the etiological link of the isolated STEC strains to the HUS of this patient. Therefore, STEC O51:H49 is proposed to be integrated into the HUSEC strain and data collection (12).

The fatal course in this patient is probably due to the severe intestinal GVHD that on one hand is associated with a disrupted integrity of the mucosal membrane and on the other hand requires intensive immunosuppression leading to a decreased innate and adaptive host immune response that both favor the penetration of enteric bacteria.

So far, *E. coli* O51:H49 was described to be associated with atypical enteropathogenic *E. coli* (EPEC) (13–15) and with an *stx*_{2e}- and *eae* β 1-harboring isolate from a wild boar (16). In this clinical case, both strains, isolated from the feces and blood of this patient and indistinguishable from each other, harbored *stx*_{2e} and *eae* genes, a new virulence profile for an HUSEC strain. Due to the presence of the high-pathogenicity island (HPI) encoding an iron uptake system, the two isolates do not seem to be of husbandry or

pork origin, which are usual reservoirs for *stx*_{2e}-positive but *irp2*-negative strains (6).

Stx_{2e}-producing *E. coli* strains have only occasionally been isolated from humans. The majority of the patients had uncomplicated diarrhea or were asymptomatic carriers (6, 10, 17, 18). Stx_{2e}-producing *E. coli* strains are challenging for routine microbiological diagnostics, since most of the ELISAs do not detect Stx_{2e} (19). Molecular-based standard methods, however, usually detect the *stx*_{2e} subtype (19). Although human infections with *stx*_{2e}-harboring STEC strains are rare, their outcome can be very severe.

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