Hemolytic uremic syndrome in a 65 year-old male linked to a very unusual type of stx2e and eae harboring O51:H49 Shiga-toxin producing Escherichia coli

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Running Title: HUS linked to an unusual STEC

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Abstract

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 *stx2e* and *eae*, a very unusual and new virulence profile for a 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 post-transplantation included ciclosporin 300 mg daily with a trough level of 150-300 µg/l. On day -3 to -1 anti-thymoglobuline and on day 1, 3 and 6 methotrexate 30 mg was given 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 2mg/kg daily prednisone and budesonide 3 mg 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 0.4mg/kg daily and ciclosporin was changed to prograf 3 mg 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 since 8 days without fever or vomiting. No special dietary habits were identified. Upon admission, blood pressure was stable and he was afebrile, the abdomen was tender without defecation. Blood tests revealed a white blood cell count (WBC) of 3.8 × 10^9/l, thrombocytopenia (74 × 10^9/l), an elevated creatinine (124 µmol/l) and slightly elevated C-reactive protein (CRP, 11.2 mg/l). C-reactive protein increased to 46.7 mg/l 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. Biopsies from the colon showed changes consistent with active GvHD. Therefore, the dose of prednisone was increased to 2mg/kg daily and asymptomatic CMV replication was treated with intravenous ganciclovir 5 mg/kg 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 azithromycin 500mg 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 meropenem 1 gr/tid and azithromycin 500 mg/day was established.

On day 135, the patient got confused. Blood tests showed hemolysis, rising creatinine, aggravated thrombocytopenia, leading to the diagnosis of hemolytic-uremic syndrome (HUS). The patient received plasmapheresis and hemodialysis at the intensive care unit. Under the established therapy, an initial stabilization with improvement of the status could be 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 tacrolimus 3 mg 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 (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 (3,5,6,7). 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 ST 20, *E. coli*
phylogenetic group B1, showed a sorbitol-fermenting phenotype and harbored the
combination of the virulence genes *stx2e* and *eae* (subtype beta). Further molecular
characterisation results are summarised in Table 1. Both strains produced Stx as detected by
the Verocell assay (P13-119 Stx titer 1:128; P13-131 Stx titer 1:32) but not by the 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 (*stxa2a, stx2c, stx2d*), which are
genetically closely related, are reported to be strongly associated with HUS in patients
(10,12). 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 (13).

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
test 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 (13).

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 to atypical enteropathogenic E. coli (EPEC) (14,15,16) and to a stx2e and eaeβ1 harboring isolate from a wild boar (17). In this clinical case, both strains, isolated from feces and blood of this patient, and indistinguishable for each other, harbored stx2e and eae genes, a new virulence profile for a 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 stx2e positive but irp2 negative strains (6).

Stx2e-producing E. coli strains have only occasionally been isolated from humans. The majority of the patients had uncomplicated diarrhea or were asymptomatic carriers (6,10,18,19). Stx2e-producing E. coli strains are challenging for routine microbiological diagnostics, since most of the ELISA assays do not detect Stx2e (20). Molecular-based standard methods, however, usually detect the stx2e subtype (20). Even though human infections with stx2e harboring STEC strains are rare, their outcome can be very severe.

Acknowledgments

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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)

<table>
<thead>
<tr>
<th>Gene</th>
<th>Description</th>
<th>P13-119</th>
<th>P13-131</th>
<th>P10-2234</th>
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<tbody>
<tr>
<td>α-hlyA</td>
<td>α-hemolysin</td>
<td>-</td>
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<td>EHEC-hlyA</td>
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<td>eae</td>
<td>intimin</td>
<td>+ (beta)</td>
<td>+ (beta)</td>
<td>+ (alpha)</td>
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<td>iha</td>
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<td>yersiniabactin receptor</td>
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</table>

+, gene is present; -, gene is absent