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Case report

Fatal case of hemolytic-uremic syndrome in an adult due to a rare serogroup O91 Entero hemorrhagic *Escherichia coli* associated with a *Clostridium difficile* infection. More than meets the eye

Thomas Guillard [a,b,*], Anne Limelette [a,b], Elisabeth Le Magrex-Debar [a], Alain Wynckel [c], Malika Gouali [d], Patricia Mariani-Kurkdjian [e], Charlotte Guyot-Colosio [c], Christophe de Champs [a,b]

[a] *CHU Reims, Hôpital Robert Debré, Laboratoire de Bactériologie-Virologie-Hygiène, 51092 Reims, France*
[b] *UFR Médecine, SFR CAP-Santé, EA 4687, Université de Reims Champagne-Ardenne, 51092, Reims, France*
[c] *CHU Reims, Hôpital Maison Blanche, Service de Néphrologie, 51092, Reims, France*
[d] *Centre National de Référence des Escherichia coli, Shigella et Salmonella (CNR ESS) Unité de Recherche et d'Expertise des Bactéries Pathogènes Entériques, Institut Pasteur, 75724 Paris, France*
[e] *Centre National de Référence associé des Escherichia coli, Hôpital Robert Debré, 75019 Paris, France*

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ABSTRACT

Hemolytic-uremic syndrome due to enterohemorrhagic *Escherichia coli*, belonging to serogroup O91 has rarely been described. We report here a case of post-diarrheal HUS due to EHEC O91 in an elderly patient for whom diagnosis was delayed given a previously diagnosed *C. difficile* infection. This case highlights the usefulness of Shiga-toxin detection.

Enterohemorrhagic *Escherichia coli* (EHEC) are intestinal pathotypes of *E. coli* that cause various human infections such as benign diarrhea, bloody diarrhea and hemorrhagic colitis. These infections are the most typical outcome of EHEC, but in rare cases, some patients progress to hemolytic-uremic syndrome (HUS).

HUS is a thrombotic microangiopathy (TMA) characterized by non-immune hemolytic anemia, thrombocytopenia and acute renal failure. Infectious HUS remains a rare illness that occurs primarily in childhood or old age. Children younger than three are more likely to develop complications requiring hospitalization and kidney dialysis; the elderly (over 60) are more likely to die regardless of the clinical complications. In 2011, in the German EHEC outbreak, HUS occurred predominantly in adults (> 88%).[1]

EHEC are the human pathogenic subgroup of Shiga toxin-producing *E. coli* (STEC). Shiga-toxins (Stx1, Stx2 and their variants) are the main virulence factors. Most EHEC (typical EHEC) carry a locus of enterocyte effacement (LEE) pathogenicity island, which allows attaching, and effacing lesions (A/E) to occur on intestinal epithelial cells. LEE contains the *eae* gene encoding intimin. LEE-negative but Stx-producing strains are referred to as 'atypical EHEC' and can cause HUS.

The most common worldwide cause of Stx associated HUS is the EHEC O157 serogroup (serotype O157:H7), but other non-O157 serogroups are an emerging cause of EHEC infections.

We report a case of HUS due to an atypical EHEC O91 infection that had a fatal outcome. It was diagnosed, but unfortunately the diagnosis was delayed due to co-infection with *Clostridium difficile*.

1. Case report

On day 5 after an occlusive syndrome surgery, a 71-year-old man had acute diarrhea that was ascribed to *C. difficile* by characterization of its toxins A and B in the stool. Early outcome was favorable with an oral treatment of 1.5 g/day of metronidazole, allowing the patient's discharge on day 8.

* Corresponding author. Mailing address: Laboratoire de bactériologie-virologie-Hygiène, CHU Robert Debré, Rue du Général Koenig 51092 Reims cedex. Tel.: +33 3 26787702; Fax: +33 3 26784134.
E-mail address: tguillard@chu-reims.fr (T. Guillard).

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However, the patient's physical condition further deteriorated with acute bloody diarrhea (8 to 10 stools/day), and the patient was again hospitalized on day 17. According to his very recent past medical history of *C. difficile*, vancomycin 2 g daily was initiated. Blood tests showed white blood cells count of 15.7 G/L with 90.3% neutrophils, acute renal failure (ARF) with a creatinine of 200 μmol/L (72 μmol/L on day 5) and decreased platelets count of 153 G/L (328 G/L on day 5).

On day 19 the patient's physical condition worsened with the occurrence of a severe ARF (creatinine 600 μmol/L) and the appearance of neurologic disorders such as aphasia and tetraparesis. The computed-tomography scan of the brain was normal. Aggravated thrombocytopenia (77 G/L), hemolytic anemia with huge drop of haptoglobin (0.17 g/L) and presence of schizocytes led to the diagnosis of TMA. No disseminated intravascular coagulation was diagnosed. Given these symptoms, thrombotic thrombocytopenic purpura (TTP) was mentioned. Fractions of the complement were almost within the normal range. Testing for HBV, HCV, HIV, enteroviruses, herpes viruses, anti-nuclear factors, circulating anticoagulants antibodies, basal membrane antibodies, and anti-neutrophil cytoplasmic antibodies (ANCA) were negative. ADAMTS-13 activity was 70%. All together, these results eventually ruled out the diagnosis of TTP.

On day 19, *C. difficile* and its toxins were not detected in patient's stools. A search for EHEC in the stool was then rapidly performed and non-O157 *E. coli* isolates were detected. Using a homemade multiplex PCR, EHEC virulence factors were characterized in the stool and confirmed by screening many different colonies. The isolated strain carried the *stx2* gene, but not *stx1, eae* or *ehx* (encoding enterohemolysin). An agglutination test with anti-O157 antiserum was negative. Diagnosis of infectious HUS due to non-O157 EHEC was eventually established. The French National Reference Centre (FNRC) subsequently confirmed our molecular results and characterized the *E. coli* strain as the O91 serogroup. Daily plasmapheresis and hemodialysis were then initiated and the patient was transferred to the nephrology intensive care unit on day 20. Platelets and haptoglobin rapidly reached normal values. Unfortunately, renal function was not recovered and patient's neurological status worsened rapidly, requiring assisted ventilation. Brain magnetic resonance imaging showed multiple ischemic foci, both supra- and sub-tentorial. On day 24, the patient had an irreversible cardiovascular collapse during his dialysis and died.

2. Discussion

To our knowledge, O91 HUS cases are poorly reported in literature. Between 2002 and 2006 in Europe, only one case due to EHEC O91 was reported out of 454 HUS cases (including adults and children).[2] In Germany, between 1997 and 2007, of 96 EHEC O91 isolates, only 4 HUS cases were reported.[3] In France, epidemiological surveillance has shown the predominance of serogroup O157 EHEC infections in children since 1996.[4] However, HUS case frequency due to non-O157 serogroups has been growing (35% increase between 2002 and 2012). Only 3 pediatric O91 HUS cases were reported in 2011 (1/162) and 2012 (2/145).[5] Unfortunately, surveillance is conducted less frequently for Stx associated HUS in adults.

Microbiological diagnosis of EHEC infection is based on phenotypic and genotypic methods. Strains isolated on chromogenic medium for *E. coli* (CPS ID3, bioMérieux, Marcy l'Etoile, France) or CHROMagar STEC medium (CHROMagar Microbiology, Paris, France) can be identified using MALDI-TOF; pathogenicity can be assessed by molecular detection of the main virulence factors (*eae*, *stx1*, *stx2*, *ehx*). Molecular detection performed after enrichment of stool specimens or rectal swabs in Trypto-casein-soy (TCS) broth can be helpful.

In our case, EHEC etiology was confirmed by molecular characterization of the *stx2* gene carried by a strain isolated from stool. According to the Karmali *et al.* classification, most of the atypical strains belong to seropathotype C, including EHEC O91, but also EHEC O104 and O113. *E. coli* O104:H4 was responsible for the large outbreaks in 2011 in Germany.[1]

This case strikingly illustrates the importance of performing molecular characterization of Shiga toxins as soon as diarrhea begins and even more so when there is bloody diarrhea and/or thrombocytopenia. In our case, it is worth pointing out that EHEC diagnosis was delayed given the *C. difficile* diarrhea diagnosed 12 days previously, which raises the importance of the awareness of these symptoms during potential *C. difficile* relapse.

Several studies have shown that neutrophils might carry Shiga toxins. During *C. difficile* infection, neutrophils are increased. *C. difficile* toxins A and B, by virtue of their enterotoxic and cytotoxic activities, might have led to intestinal cell damages and neutrophils participating in Stx cell targeting might have played an aggravating role for our patient.

Contaminated ground beef, raw milk and dairy products account for about 75% of the infections, with the remainder being person-to-person, waterborne or animal contact sources. For our patient, the source of contamination based on epidemiological data has not been determined, but he lived in a rural area.

Non-O157 EHEC associated diarrhea can rapidly evolve into HUS. Differential diagnosis of acute diarrhea occurring in a hospitalized patient older than 65 years old will result in *C. difficile* detection, but the rapid screening for EHEC in a patient presenting with bloody diarrhea or TMA is of great importance. Elderly patients with HUS are also those with the highest rate of mortality. EHEC detection in children and adults with bloody diarrhea is a major public health issue because it will help to increase epidemiological knowledge about non-O157 infection and aid in the prophylactic and therapeutic management of severe complications such as HUS.

Notes

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