Haemolytic–uraemic syndrome with bacteraemia caused by a new hybrid *Escherichia coli* pathotype

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Abstract

We describe a new atypical Shiga-toxin-producing *Escherichia coli* (STEC) responsible for a severe episode of haemolytic–uraemic syndrome in an adult with a relapse associated with bacteraemia. This STECs train of serotype O80:H2 harboured *stx2c* and *stx2d* gene subtypes, the rare *eae* ξ, variant and a ColV plasmid with a conserved virulence plasmidic region involved in virulence of human and avian extraintestinal pathogenic *E. coli*. This atypical hybrid pathotype, which represents a new threat, is a further demonstration that STEC may be a recipient for extraintestinal virulence factors and raises again the question of antibiotic therapy during STEC infection.

Keywords: bacteraemia, ColV plasmid, extraintestinal virulence factors, haemolytic–uraemic syndrome, Shiga toxin-producing *Escherichia coli*

Original Submission: 7 February 2014; **Revised Submission:** 18 April 2014; **Accepted:** 24 April 2014

Article published online: 27 May 2014

New Microbe and New Infect 2014; **2:** 127–131

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Funding Information: None.

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Introduction

Among the intestinal pathogenic *Escherichia coli*, Shiga-toxin-producing *E. coli* (STEC) are major food-borne emerging pathogens that cause bloody diarrhoea, which may be complicated by the potentially fatal haemolytic–uraemic syndrome (HUS), an important cause of acute renal failure [1]. The main virulence factor of STEC is the phage-encoded cytotoxin called Shiga-toxin that exists as two main types—Stx1 and Stx2 [2]. In most cases, STEC also carry an enterocyte effacement pathogenicity island that causes the attaching and effacing lesions on infected epithelial cells. While other intestinal virulence factors have been described in STEC, extraintestinal manifestations are rare and virulence factors characteristic of extraintestinal pathogenic *E. coli* (ExPEC) have been rarely reported.

Case Report

In April 2013, a 39-year-old male was admitted to the intensive care unit because of afebrile generalized tonic–clonic seizure followed by coma without focal abnormalities. White-cell count was 10 200/mL, haemoglobin was 10 g/dL and platelet count was 25 200/mm^3 with indirect evidence of haemolysis. The electrolyte balance was normal. Cultures of cerebrospinal fluid, blood and urine were initially sterile. A computed tomographic brain scan revealed bilateral ischaemic lesions in posterior cerebral artery territory and diffuse cerebral oedema. An electroencephalographic study showed attenuation of background activity without spike-wave discharges. Furthermore, the patient received intravenous amoxicillin-clavulanate during the first 5 days of hospitalization for suspected aspiration pneumonia.

Oligoanuric acute renal failure with hypertension occurred 3 days after admission. The serum creatinine concentration increased up to 365 μmol/L. Blood tests revealed persistent thrombopenia and haemolytic anaemia (haemoglobin 6.3 g/dL) with schistocytes (3.5%). At the same time, the patient presented one episode of non-bloody diarrhoea. Stool cultures yielded a Shiga-toxin-2-producing *E. coli*, which confirmed the diagnosis of HUS. The patient required continuous veino-veinous haemofiltration (21 days) and received several erythrocyte, platelet and plasma transfusions.

Three weeks after his admission, while blood parameters were returning to normal, the patient again presented severe anaemia (haemoglobin, 6.1 g/dL) with thrombopenia (48 000/mm^3) and schistocytes (1.7%). He remained afebrile but two blood cultures yielded a Shiga-toxin 2-producing *E. coli*. A urine culture obtained 2 days before was negative for this pathogen, indicating that urine was unlikely to be the source of bacteraemia. Intravenous antibiotic therapy with piperacillin-tazobactam and amikacin was initiated and the patient received one plasmapheresis. As the STEC strain was still detected in stools, he was treated with oral azithromycin to suppress carriage. Stools became negative 6 days after this treatment.

Ten weeks after admission, the patient was alert and oriented and ischaemic lesions had completely regressed on the computed tomographic brain scan. Serum creatinine level was 44 μmol/L, haemoglobin level was 7.3 g/dL and platelet count was 178 000/mm^3.

Laboratory Results

Three isolates of STEC, successively recovered from stools and blood cultures, were found to harbour *stx2c*, *stx2d*, *hlyA* and *eae* genes. The *eae* ξ, variant was identified by a specific PCR [3]. Enteroaggregative *E. coli* virulence factors *aggr*, *pic* and *astA* were negative. All the isolates harboured the O80 antigen (*E. coli* antisera; Statens Serum Institut, Copenhagen, Denmark) and were resistant to aminopenicillins, cotrimoxazole, nalidixic acid and kanamycin.

As the STEC strain was also isolated from blood cultures, major virulence factors of ExPEC were sought by PCR. A first screening indicated the presence of genes encoding salmochelin (*iroN*) and aerobactin (*iucC*), whose combination suggested the presence of a conserved virulence plasmidic region characteristic of ColV plasmids described in ExPEC strains [4,5]. The presence of *ompT*$_p$, *etsC*, *iss*, *hlyF*, *sitA* and *cvaA* together with *iroN* and *iucC*, which are considered to be a signature of this region, were identified in the three isolates.

All isolates were assigned to the phylogenetic group A with a PCR-based method and to the sequence type 301 constitutive of STc 165 using the multilocus sequence typing Achtman scheme (mlst.ucc.i.e/mlst/dbs/Ecoli). Pulsed-field gel electrophoresis of *Xba*I-restricted DNA showed that the three isolates were genetically related (Fig. 1). Furthermore, oligonucleotide microarray results with 392 probes (Clondiag, Alere, France) were identical for the three strains (data not shown). Pulsed-field gel electrophoresis of S1 nuclease-digested DNA showed the presence of four plasmids for each strain; three of similar sizes in all isolates plus one of high

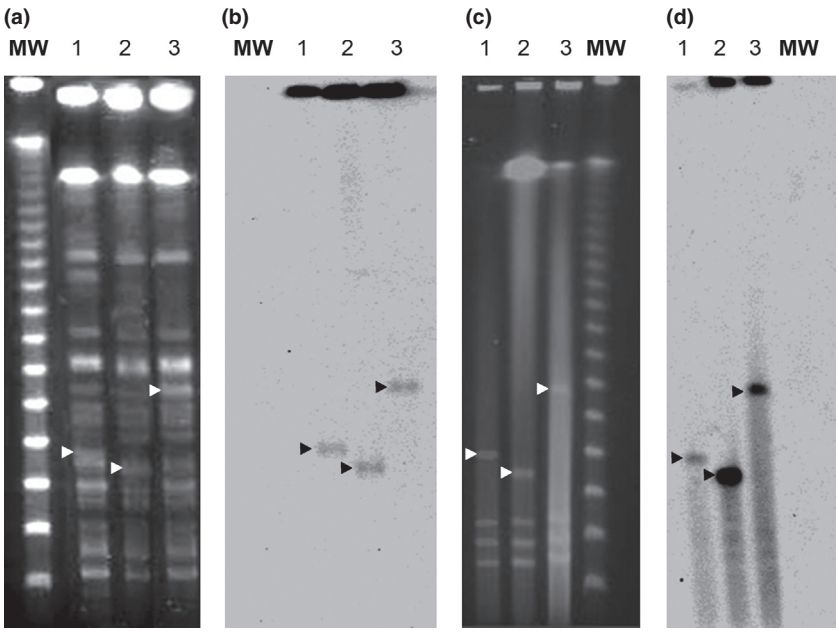

FIG. 1. Pulsed-field gel electrophoresis of *Xba*I-restricted DNA (a) and of S1 nuclease-digested DNA (c) with the corresponding hybridizations by Southern blot (b and d, respectively) of the three Shiga-toxin-producing O80:H2 *Escherichia coli* isolates. MW, molecular weight: lane 1, first strain isolated from stool; lane 2, strain isolated from blood culture; and lane 3, second strain isolated from stool. White arrows indicate plasmids and black arrows indicate plasmid bands hybridizing with *etsC* probe.