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

P. Mariani-Kurkdjian¹,²,³, a, C. Lemaitre¹,²,³, a, P. Bides¹,²,³, D. Perez⁴, L. Boggini⁵, T. Kwon⁶ and S. Bonacorsi¹,²,³,*

1) Centre National de Référence associé Escherichia coli, Hôpital Robert-Debré, AP-HP, 2) IAME, UMR 1137, INSERM, 3) IAME, UMR 1137, Université Paris Diderot, Sorbonne Paris Cité, Paris, 4) Service de Réanimation, Centre hospitalier de Lons-le-Saunier, Lons-le-Saunier, 5) Service de Microbiologie, Centre hospitalier de Lons-le-Saunier, Lons-le-Saunier and 6) Service de Néphrologie, Hôpital Robert-Debré, AP-HP, Paris, France

**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 α 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, CoIV 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

**Corresponding author:** S. Bonacorsi, Service de Microbiologie, Hôpital Robert-Debré, 48 boulevard Sévérin, 75019 Paris, France

E-mail: stephane.bonacorsi@rdb.aphp.fr

**Funding Information:** None.

*These authors have contributed equally.

---

**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/mm⁶ 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/mm³) 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/mm³.

### 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, 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.ie/mlst/dbs/Ecoli). Pulsed-field gel electrophoresis of XbaI-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 size.
molecular weight (ranging from 150 kbp to 300 kbp), which hybridized with iroN and etsC probes on Southern blot (Fig. 1). The main characteristics of the O80:H2 STEC strain are presented in Table 1.

**Discussion**

Here, we report the isolation of a new hybrid pathotype of STEC serotype O80:H2. A first unusual feature of this case report is bacteraemia because, to our knowledge, only six cases have been previously described during HUS [6–11]. Besides rare cases of STEC infection with bacteraemia, several cases of urinary tract infection have been reported since the first report in 1996 of O103:H2 STEC [12]. Among all these reports, very few searches for ExPEC virulence factors were performed.

The O157:H7 STEC, the most frequent clonal group involved in HUS does not harbour extraintestinal virulence traits. However, combined virulence pathotypes have been previously described in other serotypes. In 2011, the O104:H4 STEC, causing an outbreak of HUS in Europe, combined Shiga-toxin Stx2a production, enteroaggregative genes and two loci encoding the ExPEC siderophores aerobactin and yersiniabactin [13]. The high pathogenicity island encoding yersiniabactin is known to be disseminated among intestinal pathogenic E. coli including certain clonal subgroups of STEC [14,15]. Aerobactin has been found in more than 75% of enteroaggregative E. coli strains but also in several STEC [14]. In contrast, the iro locus encoding salmochelins is extremely rare in intestinal E. coli [14].

This is the first time that a conserved virulence plasmidic region characteristic of ExPEC has been described in one STEC strain. This region is a key genetic determinant in ExPEC strains virulent in humans and poultry [4,5]. Plasmids containing conserved virulence plasmidic regions have been especially described in neonatal meningitis strains and are associated with high levels of bacteraemia which favour the blood–brain barrier passage [5,16]. The siderophores encoded by conserved virulence plasmidic region play a key role in this pathophysiological step; however, several genes of as yet unknown function are still under investigation [17,18]. Our results blur the classical distinction between intestinal and extraintestinal pathogenic E. coli. Surprisingly, although very

**TABLE 1. Genetic background of the new hybrid pathotype Escherichia coli O80:H2**

| Gene                | Description                                                                 | Presence |
|---------------------|-----------------------------------------------------------------------------|----------|
| stx1/stx2          | Shiga-toxin 1                                                               | Negative |
| stx2/stx2c         | Shiga-toxin 2 variant                                                        | Positive |
| ssa                 | Intimin (adhesin)                                                           | Positive |
| EAgfA               | Enterohaemolysin A                                                          | Positive |
| saa                 | Shiga-toxin-producing E. coli autoagglutination adhesin                     | Negative |
| rfa1                | Enterohaemorrhagic E. coli factor for adherence                             | Positive |
| rfa2                | Translocated intimin receptor protein                                        | Positive |
| espP                | Extracellular serine protease                                                | Positive |
| fbgA                | Major subunit of bundle-forming pili                                         | Negative |
| aggR                | Subunit of aggregative adherence fimbria AAFI                              | Negative |
| aggA                | Master regulator for a package of enteroaggregative E. coli plasmid (pAA)   | Negative |
| pic                 | Virulence genes of enteropathogenic E. coli                                 | Negative |
| sepA                | Pic protein with mucinase activity involved in the intestinal colonization    | Negative |
| sigA                | Siga protein, IgA protease-like homologue                                   | Negative |
| estA                | Enteroaggregative E. coli heat-stable enterotoxin                           | Negative |
| sepA, sigA          | Virulence genes of extraintestinal pathogenic E. coli                       | Negative |
| inA                 | Yersiniabactin siderophore (receptor)                                       | Negative |
| iroN                | Salmochelin siderophore (receptor)                                          | Positive |
| iucA                | Aerobactin siderophore (receptor)                                           | Positive |
| setA                | Iron uptake system StABCD                                                   | Positive |
| cvaA                | Colicin V                                                                   | Positive |
| iss                 | Increased serum survival gene                                               | Positive |
| etsC                | Type I secretion system                                                     | Positive |
| bfpA                | L-haemolysin (pore-forming toxin)                                            | Positive |
| ompt                | Outer membrane protein (ompT)                                                | Positive |
| mchF                | ABC transporter MchF (bacteriocin)                                          | Positive |
| pofA, popGII1, popGII | Virulence genes of extraintestinal pathogenic E. coli                  | Negative |
| cnf1                | Cytotoxic necrotizing factor 1                                               | Negative |
| hla                 | Haemagglutinin                                                               | Negative |
| hleB                | ABC iron transporter                                                        | Negative |
| tsh                 | Temperature-sensitive haemagglutinin                                         | Negative |
| sat                 | Serine protease auto transporter                                              | Negative |
| vca                 | Vacuolating autotransporter                                                  | Negative |
| cdt                 | Cytolethal distending toxin                                                  | Negative |
| cadC/cadD8          | Colicin B                                                                   | Negative |
close in terms of virulence gene content and chromosomal backbone, the three isolates differed in their ColV plasmid size. The reasons for such apparent plasticity of the extraintestinal virulence-associated plasmid between faecal and blood isolates remain to be determined.

The serotype of our strain appears also to be atypical, as O80:H2 STEC strains have rarely been described [2,3]. The European Food Safety Authority reported in their Scientific Opinion published in 2013 that O80 is an HUS-associated serotype. However, among 777 HUS cases in Europe between 2007 and 2010, only two were related to O80 STEC strains [19]. The subtype of stx2 genes in HUS-associated O80 strains has not been previously described. Stx2d has been reported to be one of the more potent toxins, similar to Stx2a [20], and may in part explain the severe presentation of our case. The potential role of the Stx2d and Stx2c combination in severe presentations remains to be studied. Interestingly, our strain harboured an infrequent intimin type that was described for the first time in two O80 STEC strains isolated from cattle [3]. Moreover, examining the EcMLST database, only two E. coli strains of serotypes O80 and O4, respectively, belong to the sequence type 301.

Finally, our case raises again the unsolved question of antibiotic therapy during STEC infections. Here, we demonstrated that a plasmid characteristic of ExPEC could be transferred into an intestinal pathogenic E. coli genetic background. This plasmid probably contributes to the virulence, especially to establish bacteraemia. It is no longer recommended to treat STEC infections with antibiotics because such treatment has been associated with a higher rate of subsequent HUS [2,21]. However, this novel hybrid pathotype may represent a new threat and the risk of bacteraemia may lead to a dramatic outcome in the context of HUS. Among antibiotics, it has been shown that azithromycin, in contrast to other antibiotics, is able to significantly reduce Shiga-toxin production in vitro [22] and to lower the frequency of long-term carriage [23]. In our case, earlier azithromycin treatment may have prevented the HUS relapse.

The genetic plasticity of E. coli allows multiple gene combinations that result in phenotypic diversification and the emergence of new hypervirulent pathogens such as E. coli O80:H2. This strain may represent a threat in terms of public health requiring a redefinition of the place of antibiotic treatment during STEC infection.

Conflict of Interest

None declared.

References

1. Kaper JB, Nataro JP, Mobley HL. Pathogenic Escherichia coli. Nat Rev Microbiol 2004; 2: 123–140.
2. Tarr PI, Gordon CA, Chandler WL. Shiga-toxin-producing Escherichia coli and haemolytic uraemic syndrome. Lancet 2005; 365: 1073–1086.
3. Blanco M, Blanco JE, Mora A et al. Serotypes, virulence genes, and intimin types of Shiga toxin (verotoxin)-producing Escherichia coli isolates from cattle in Spain and identification of a new intimin variant gene (eae-xi). J Clin Microbiol 2004; 42: 645–651.
4. Johnson TJ, Siek KE, Johnson SJ, Nolsen LR. DNA sequence of a CoLV plasmid and prevalence of selected plasmid-encoded virulence genes among avian Escherichia coli strains. J Bacterial 2006; 188: 745–758.
5. Peigne C, Bidet P, Mahjour-Messafi F et al. The plasmid of Escherichia coli strain S88 (O45.K1.H7) that causes neonatal meningitis is closely related to avian pathogenic E. coli plasmids and is associated with high-level bacteremia in a neonatal rat meningitis model. Infect Immun 2009; 77: 2272–2284.
6. Buvens G, De Rauw K, Roisin S et al. Verocytotoxin-producing Escherichia coli O128ab:H2 bacteremia in a 27-year-old male with haemolytic–uraemic syndrome. J Clin Microbiol 2013; 51: 1633–1635.
7. Chiurichiu C, Furrincieli A, Santostefano M, Fusaroli M, Remuzzi G, Ruggenenti P. Adult non-diarrhoeal haemolytic–uraemic syndrome associated with Shiga toxin Escherichia coli O157:H7 bacteremia and urinary tract infection. Am J Kidney Dis 2003; 41: E4.
8. Krishnan C, Fitzgerald VA, Dabin SJ, Belme RJ. Laboratory investigation of outbreak of hemorrhagic colitis caused by Escherichia coli O157:H7. J Clin Microbiol 1987; 25: 1043–1047.
9. Liemann T, Salo E, Rinhamen-Finne R et al. Shiga toxin-producing Escherichia coli serotype O78:H4(+) in family, Finland, 2009. Emerg Infect Dis 2012; 18: 577–581.
10. Nguyen QV, Hochstrasser L, Chuard C, Hachler H, Regamey C, Descombres E. Adult haemolytic-uraemic syndrome associated with urosepsis due to Shigatoxin-producing Escherichia coli O138:H. Ren Fail 2007; 29: 747–750.
11. Raymond D, Blanchetti MB, Burnens A, Lior H. Haemolytic–uraemic syndrome. Lancet 1994; 343: 1042–1043.
12. Starr M, Bennett-Wood V, Bigham AK et al. Haemolytic–uraemic syndrome following urinary tract infection with enterohemorrhagic Escherichia coli: case report and review. Clin Infect Dis 1998; 27: 310–315.
13. Rohde H, Qin J, Cui Y et al. Open-source genomic analysis of Shiga-toxin-producing E. coli O104:H4. N Engl J Med 2011; 365: 718–724.
14. Okabe IN, Stalsiack IC, Soars EH, Macfarlane LR, Torres AG. Molecular epidemiology of the iron utilization genes of enteraggregative Escherichia coli. J Clin Microbiol 2004; 42: 36–44.
15. Schubert S, Rakin A, Karsh H, Carniel E, Hesseman J. Prevalence of the “high-pathogenicity island” of Yersinia species among Escherichia coli strains that are pathogenic to humans. Infect Immun 1998; 66: 480–485.
16. Lemaître C, Mahjour-Messafi F, Dupont D et al. A conserved virulence plasmid region contributes to the virulence of the multiresistant Escherichia coli meningitis strain S286 belonging to phylogenetic group C. PLoS One 2013; 8: e74423.
17. Lemaître C, Bidet P, Benoist JF et al. The ssbl gene harbored by the CoLV plasmid of an Escherichia coli neonatal meningitis strain is an auxiliary virulence factor boosting the production of siderophores through the Shikimate pathway. J Bacterial 2014; 196: 1343–9.
18. Lemaître C, Bidet P, Bingen E, Bonacorsi S. Transcriptional analysis of the Escherichia coli CoLV-ia plasmid pS88 during growth in human serum and urine. BMC Microbiol 2012; 12: 115.
19. EFSA Panel on Biological Hazards (BIOHAZ). Scientific opinion on VTEC-seropathotype and scientific criteria regarding pathogenicity assessment. EFSA J 2013; 11: 313B.
20. Fuller CA, Pellino CA, Flagler MJ, Strasser JE, Weiss AA. Shiga toxin subtypes display dramatic differences in potency. *Infect Immun* 2011; 79: 1329–1337.

21. Wong CS, Mooney JC, Brandt JR et al. Risk factors for the hemolytic uremic syndrome in children infected with *Escherichia coli* O157:H7: a multivariable analysis. *Clin Infect Dis* 2012; 55: 33–41.

22. Bielaszewska M, Idelevich EA, Zhang W et al. Effects of antibiotics on Shiga toxin 2 production and bacteriophage induction by epidemic *Escherichia coli* O104:H4 strain. *Antimicrob Agents Chemother* 2012; 56: 3277–3282.

23. Nitschke M, Sayk F, Hartel C et al. Association between azithromycin therapy and duration of bacterial shedding among patients with Shiga toxin-producing enteroaggregative *Escherichia coli* O104:H4. *JAMA* 2012; 307: 1046–1052.