

# Emerging Multidrug-Resistant Hybrid Pathotype Shiga Toxin–Producing *Escherichia coli* O80 and Related Strains of Clonal Complex 165, Europe

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Enterohemorrhagic *Escherichia coli* serogroup O80, involved in hemolytic uremic syndrome associated with extraintestinal infections, has emerged in France. We obtained circularized sequences of the O80 strain RDEx444, responsible for hemolytic uremic syndrome with bacteremia, and noncircularized sequences of 35 O80 *E. coli* isolated from humans and animals in Europe with or without Shiga toxin genes. RDEx444 harbored a mosaic plasmid, pR444\_A, combining extraintestinal virulence determinants and a multidrug resistance–encoding island. All strains belonged to clonal complex 165, which is distantly related to other major enterohemorrhagic *E. coli* lineages. All *stx*-positive strains contained *eae*- $\xi$ , *ehxA*, and genes characteristic of pR444\_A. Among *stx*-negative strains, 1 produced extended-spectrum  $\beta$ -lactamase, 1 harbored the colistin-resistance gene *mcr1*, and 2 possessed genes characteristic of enteropathogenic and pyelonephritis *E. coli*. Because O80–clonal complex 165 strains can integrate intestinal and extraintestinal virulence factors in combination with diverse drug-resistance genes, they constitute dangerous and versatile multidrug-resistant pathogens.

Enterohemorrhagic *Escherichia coli* (EHEC), a subset of Shiga toxin–producing *E. coli* (STEC), are major foodborne pathogens responsible for outbreaks and sporadic cases of gastrointestinal diseases ranging from simple diarrhea to hemorrhagic colitis, characterized by bloody diarrhea. The

most serious complication, particularly in young children, is hemolytic uremic syndrome (HUS), defined by a combination of renal failure, thrombocytopenia, and hemolytic anemia (1). Post-STEC HUS is a major worldwide public health concern because it is the primary cause of acute renal failure in children (1). These clinical features result mainly from the action of the phage-encoded Shiga toxin (Stx), of which there are 2 types: Stx1, which has 3 subtypes, Stx1a, 1c, and 1d; and Stx2, which has 7 subtypes, Stx2a–g. In typical EHEC, adhesion to the intestinal epithelium is mediated by the locus of enterocyte effacement (LEE), a chromosomal pathogenicity island (PAI), shared with Enteropathogenic *E. coli* (EPEC) strains, which encodes a type III secretion system (T3SS), an adhesin called intimin, and its receptor Tir. Intimin, encoded by the *eae* gene, is a major virulence factor (VF) involved in the intimate attachment of typical EHEC to intestinal epithelium, causing characteristic attaching and effacing lesions. EHEC enterohemolysin (*ehxA*) is a pore-forming cytolysin carried by a plasmid involved in EHEC virulence. This plasmid, initially described as part of the O157 serogroup (pO157) (2), can carry 2 additional VFs, a catalase peroxidase, encoded by *katP*, and a serine protease, encoded by *espP*, which can cleave human coagulation factor V and might be involved in the development of hemorrhagic colitis (3).

Serogroup O157 is the predominant STEC serogroup worldwide, but non-O157 serogroups are increasingly associated with post-STEC HUS, and the unusual serogroup O80 is emerging in France and Europe. In 2016, O80 represented the second most frequent serogroup isolated in France, after serogroup O26 (4). This phenomenon is no longer restricted to France; strains of serotype O80:H2, all belonging to sequence type (ST) 301, have been identified in Spain (5), the Netherlands (6), and Switzerland (7).

This serogroup is unique for several reasons. First, it is always associated with multiple determinants of resistance

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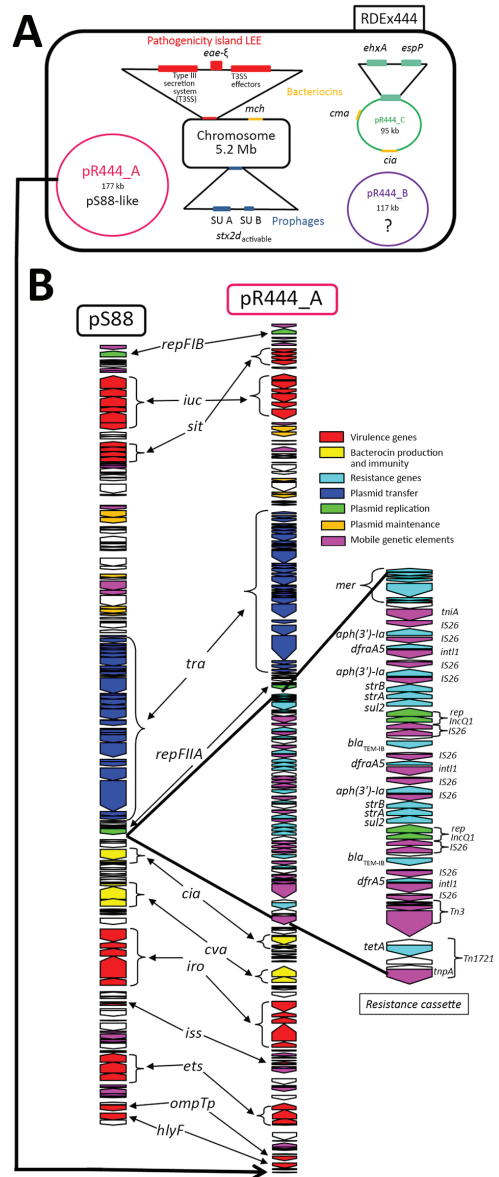
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(i.e., resistance to aminopenicillin, aminoglycoside, nalidixic acid, cotrimoxazole, tetracycline, or phenicols), whereas a resistance phenotype is uncommon among EHECs, which are generally fully susceptible to antibiotics, except for rare clones, such as the epidemic O104:H4 German clone carrying a *bla*<sub>CTX-M-15</sub> gene (8). Furthermore, unusual extraintestinal infections have recently been described for this serogroup (9,10), such as bacteremia, whereas EHEC is generally known to be a strictly intestinal pathogen. A recent case in the Netherlands illustrates the potential extreme pathogenicity of this serogroup; a 16-month-old boy died from multiorgan failure and extensive cerebral thrombotic microangiopathy attributable to an O80 Stx2d-producing *E. coli* strain (6). Finally, O80 EHEC appears to be a hybrid pathotype that combines intestinal VFs (Shiga toxin [*stx*], intimin [*eae*], enterohemolysin [*ehxA*]) and extraintestinal VFs (aerobactin [*iucC*]; salmochelin [*iroN*], an iron uptake protein encoded by *sit*ABCD; serum resistance protein [*issp*]; a putative secretion system I [*etsC*]; ompin [*ompTp*]; hemolysin [*hlyF*], and 2 bacteriocins [*cia* and *cva*]), suggesting the presence of a pS88-like plasmid (11). pS88 is a ColV plasmid, a key determinant of extraintestinal pathogenic *E. coli* virulence in poultry and humans. This plasmid is involved in neonatal meningitis (11) and could explain the occurrence of extraintestinal dissemination in these EHEC infections. The recent diffusion in Europe, high potential extraintestinal pathogenicity, and multidrug resistance (MDR) of this hybrid pathotype led us to further characterize these strains, which might represent a major public health concern.

**Methods**

We further characterized O80:H2 EHEC by fully sequencing a recent representative strain, called RDEx444. RDEx444 was responsible for a highly severe case of post-STECS HUS, complicated by bacteremia, in an 8-month-old infant in February 2016 in Bourg-en-Bresse, France. Initial symptoms were febrile diarrhea with signs of sepsis. Blood and stool cultures were positive for O80 EHEC, but urine cultures remained negative, suggesting gut translocation that led to bacteremia. The appearance of biologic signs of HUS with oliguria necessitated transfer to intensive care, several blood transfusions, and 5 days of peritoneal dialysis.

We performed sequencing of RDEx444 by using the PacBio single-molecule real-time method with RS II chemistry 2.4.0 (Pacific Biosciences, Menlo Park, CA, USA); we used 1 single-molecule real-time cell. De novo assembly was performed twice by using the HGAP pipeline (<https://github.com/PacificBiosciences/Bioinformatics-Training/wiki/HGAP>) and annotation by using the MAGE platform (<http://www.genoscope.cns.fr/agc/microscope/home/index.php>) (Figure 1). For this strain, we performed plasmid typing by using databases (PlasmidFinder 1.3 and

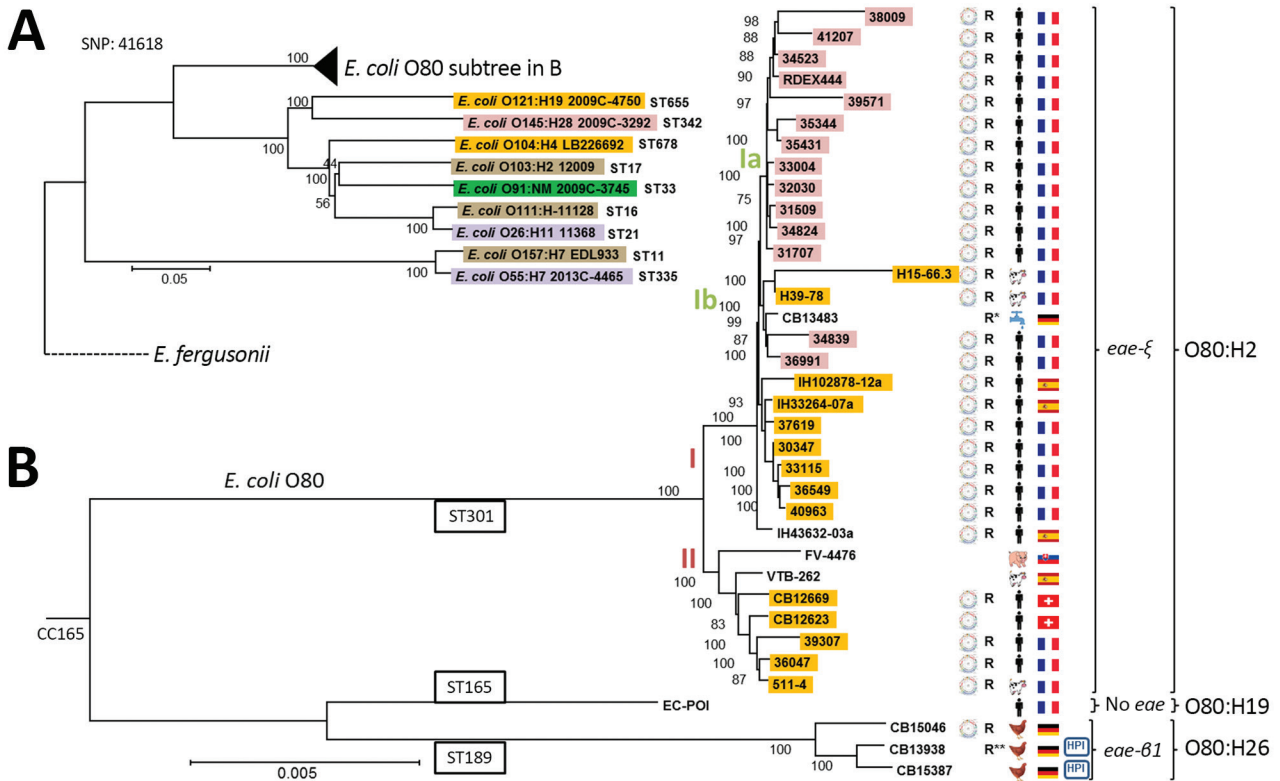


**Figure 1.** Genetic characterization of RDEx444, a strain of *Escherichia coli* serotype O80:H2 isolated in France in February 2016 and involved in hemolytic uremic syndrome with bacteremia, carrying both intestinal and extraintestinal virulence factors associated with (multidrug-resistance determinants (A) and genetic comparison between plasmid pS88 and mosaic plasmid pR444\_A (B). A) Four circularized contigs (chromosome of 5,256,050 bp and the 3 plasmids pR444\_A [176,500 bp], pR444\_B [117,090 bp], and pR444\_C [95,050 bp]) obtained by using PacBio (Pacific Biosciences, Menlo Park, CA, USA) sequencing of RDEx444 are schematically represented. The main virulence factors are presented as colored rectangles. Intestinal virulence factors are indicated in red for the locus of enterocyte effacement genes, blue for prophage-encoded Shiga toxin genes, and green for VFs carried by pR444\_C, a pO157-like plasmid. Bacteriocin genes (*mch*, *cia*, and *cma*) are indicated in yellow. B) Comparison of the sequences of pR444\_A and pS88, the plasmid of strain S88 involved in neonatal meningitis. LEE, locus of enterocyte effacement.

pMLST 1.4 [12] available on the Center for Genomic Epidemiology (CGE) website (<http://www.genomicepidemiology.org>). Plasmids were also characterized by S1 nuclease pulsed-field gel electrophoresis (PFGE) with Southern hybridization and conjugability of the largest confirmed by experiments using rifampin-resistant *E. coli* J53, as previously described (9).

We also sequenced a representative set of 35 O80 strains from various sources and countries using the Nextera kit (Illumina, San Diego, California, USA) to prepare the library. Sequencing was performed by using a MiSeq reagent kit V3 600 cycles (Illumina). This panel included human isolates from France (n = 21), Spain (n = 3), and Switzerland (n = 2), as well as animal and environmental strains isolated in France (n = 3), Spain (n = 1), Slovakia (n = 1), and Germany (n = 4) (Figure 2). Some of the human

strains from France and strains from Spain and Slovakia have been partially characterized previously (10). The strains from Spain and Slovakia were isolated during 1998–2012 and the strains from France during 2010–2016. The dates of isolation of the strains from Switzerland and Germany were not available. We performed assembly by using CLC Genomics Workbench software and SPAdes, also available on CGE website. The RAST server (<http://rast.nmpdr.org>) was used for genome annotation and the PHASTER web server (<http://phaster.ca>) to identify and annotate prophage sequences within bacterial genomes and plasmids. We established phylogeny by single-nucleotide polymorphism alignments between the contigs generated by CLC Genomics of O80 strains and 9 reference EHEC strain sequences of major serotypes available in GenBank (O157:H7 EDL933, O26:H11 11368, O111:H- 11128,



**Figure 2.** Phylogeny of 36 *Escherichia coli* serogroup O80 strains isolated from various sources and countries in Europe during 1998–2016 and their relationship to major enterohemorrhagic *E. coli* lineages. General phylogenetic tree rooted on *E. fergusonii* (GenBank accession no. NC\_011740), showing (A) the position of O80 strains among major enterohemorrhagic *E. coli* lineages (O157:H7 EDL933 NC\_002655.2, O26:H11 11368 NC\_013361.1, O111:H- 11128 NC\_013364.1, O103:H2 12009 NC\_013353.1, O55:H7 2013C-4465 CP015241, O91:NM 2009C-3745 JHGW00000000, O104:H4 LB226692 EO104H4LB.1, O145:H28 2009C-3292 JHH00000000, and O121:H19 2009C-4750 JHGL00000000) and (B) a focused view of clonal complex 165, including O80 strains. The highlighted strains carry the Shiga toxin genes; the subtype of Stx is indicated by a color code as follows: purple, *stx1a*; yellow, *stx2a*; pink, *stx2d<sub>activable</sub>*; brown, *stx1a2a*; dark green, *stx1a2b*. The presence of the pS88 like-plasmid is represented by using a plasmid scheme next to the strain number. R to the right of the plasmid indicates that the strain possesses  $\geq 2$  resistance genes that confer resistance to  $\beta$ -lactams, kanamycin, or cotrimoxazole (R\* indicates presence of additional extended-spectrum  $\beta$ -lactamase gene; R\*\* indicates presence of *mcr-1* gene conferring additional resistance to colistin). The strain origin (country and source of isolation) is represented by flags and human, animal, or water symbols. CC, clonal complex; HPI, high-pathogenicity island (presence of chromosomal locus encoding the siderophore yersiniabactin); SNP, single-nucleotide polymorphism; ST, sequence type. Scale bar indicates nucleotide substitutions per site.

O103:H2 12009, O55:H7 2013C-4465, O91:NM 2009C-3745, O104:H4 LB226692, O145:H28 2009C-3292, and O121:H19 2009C-4750 [online Technical Appendix, <https://wwwnc.cdc.gov/EID/article/24/12/18-0272-Techappl.pdf>] using CSIPhylogeny 1.4 (13). The neighbor-joining tree was built by using MEGA 3.1 (<https://www.megasoftware.net>) using bootstraps calculated from 100 replicates. The maximum-likelihood method yielded the same results (online Technical Appendix Figure 1).

We performed multilocus sequence typing and identification of acquired antimicrobial resistance genes for all strains by using suitable databases available on the CGE website (SerotypeFinder 1.1 [14], MLST 1.8 [15], and ResFinder 3.0 [16]). Investigation of the resistome was completed by using the Resistance Gene Identifier at the Comprehensive Antibiotic Resistance Database website (<https://card.mcmaster.ca/analyze/rgi>). Moreover, 166 genes of intestinal and extraintestinal VFs (list available from authors) were assessed by local BLAST+ 2.2.31 analysis (<https://blast.ncbi.nlm.nih.gov>). Complete results are described in online Technical Appendix Figure 2. Nucleotide sequences of the 36 sequenced O80 strains (complete nucleotide sequence of RDEx444 and 35 draft nucleotide sequences) have been deposited in Genbank (project no. PRJNA449634); accession numbers are available in the online Technical Appendix.

## Results

RDEx444 can be considered to be representative of hybrid pathotype strains because it was responsible for an extraintestinal infection and showed similar virulence and resistance profiles as other strains described elsewhere (9,10). Complete sequencing yielded  $2.3 \times 10^9$  bp with 165,041 reads and an N50 (the length of the smallest contig among the set of the largest contigs that together cover >50% of the assembly) read length of 19,839 bp.

Four circularized contigs were obtained, including the chromosome of 5,256,050 bp, containing 5,146 coding sequences (CDSs), with an overall G+C content of 50.7% and 3 plasmids of 176,500 (pR444\_A), 117,090 (pR444\_B), and 95,050 bp (pR444\_C). The number and size of the RDEx444 plasmids were corroborated by S1 nuclease PFGE (data not shown). We compiled a schematic representation of the genetic content, including major VFs, of this strain (Figure 1, panel A).

As expected, RDEx444 belongs to serotype O80:H2 and ST301. Among the 12 prophage regions identified on its chromosome, this strain carries a complete Stx-converting bacteriophage of 43.9 kb. RDEx444 carries the *stx2d<sub>ac-tivable</sub>* variant, which has been shown to be predictive of severe clinical outcomes and progression to HUS (17). The Stx prophage is integrated into the chromosomal *yecE* gene, a known integration site (18,19), initially reported in a strain

producing Stx2e, encoded by the phage  $\phi$ P27 (20). The  $\approx$ 15-kb region between Stx subunit B and the phage integrase, comprising proteins for DNA replication and repression, shares strong homology with the phage  $\phi$ P27. RDEx444 is otherwise a typical EHEC, given that it harbors a complete LEE and does not possess atypical adhesins, such as autoagglutinating adhesion (*saa*) or aggregative factors (*aggA/R*). RDEx444 carries the rare variant of the intimin gene *eae- $\xi$*  (5), which has only very rarely been observed among other EHEC serotypes, but which is shared by all previously described O80 EHEC and EPEC isolates (5–7,9,10). Two other chromosomal traits of RDEx444 might also confer a selective advantage: 1) a region encoding microcin H47 (*mch A, S, X, B, C, D, E, and F*)  $\approx$ 35 kb downstream from the Stx prophage, and 2) a chromosomal mutation in DNA gyrase (*gyrA S83L*), which confers quinolone resistance.

Plasmid pR444\_C is 95,050 bp long with a G+C content of 49.8% and was predicted to contain 114 CDSs. Plasmid pR444\_C is comparable to pO157 because it contains enterohemolysin (*ehxA*) and serine protease (*espP*) but does not possess catalase peroxidase (*katP*). Moreover, 2 other colicins, Ia (*cia*) and M (*cma*), are also present on this plasmid.

Plasmid pR444\_B is 117,090 bp long with a G+C content of 46.5% and contains 135 CDSs and, unexpectedly, those for 3 tRNAs (threonine, asparagine, and tyrosine) redundant with those still present on the chromosome. This plasmid can be considered to be cryptic because it carries no drug resistance or virulence-associated genes, but several phage proteins are present. pR444\_B shares high homology (99% nucleotide identity and 89% coverage) with plasmid pECOH89 (21), encoding an extended-spectrum  $\beta$ -lactamase *bla<sub>CTX-M-15</sub>* identified in an *E. coli* strain and belonging to the family of phage-like plasmids. Members of this family are generally untypeable, nonconjugative, and cryptic plasmids, because no known virulence or resistance genes have been identified. Their function is unknown, but they all have strong homology to the *Salmonella*-specific bacteriophage SSU5 (21).

Plasmid pR444\_A is 176,500 bp long, with a G+C content of 51.8%, and carries 2 replicons: FII\_A and FIB\_1 (ST [F2:A:-B1]). We identified 202 CDSs. This plasmid carries virulence-associated genes characteristic of pS88 (described previously) and MDR genes, and thus can be considered to be a mosaic plasmid. We identified an MDR-encoding region (48,237 bp) in addition to the plasmid-related function (56,106 bp) and virulence-associated domain (72,157 bp), closely related to pS88 (99% nucleotide identity and 96% query coverage) (11). This MDR-encoding region contains genes encoding resistance tetracycline (*tetA*), trimethoprim (*dfpA5*), sulfonamide (*sul2*),  $\beta$ -lactam (*bla<sub>TEM-IB</sub>*), kanamycin (*aph[3']-Ia*), and streptomycin (*strA* and *strB*) and resistance against heavy metals such as mercury

(*mer* gene) (Figure 1, panel B). This resistance cassette has only been described for pO26-CRL<sub>125</sub> (100% nucleotide identity, 98% coverage) from an O26 EHEC but without VFs (22). Co-localization of extraintestinal VFs and MDR genes on the same plasmid was confirmed by Southern hybridization (data not shown). Moreover, we confirmed that this plasmid is conjugative, suggested by the presence on the annotation of an almost complete F-like transfer region, by successfully conjugating it with rifampin-resistant *E. coli* J53 (data not shown). Thus, this plasmid can diffuse by horizontal transfer.

Accordingly, this mosaic plasmid shows high homology with plasmid pS88, responsible for extraintestinal virulence in neonatal meningitis strains within which a resistance cassette has been integrated (Figure 1, panel B). Furthermore, pR444\_A also carries 2 bacteriocin genes (*cia* and *cva*), such as pS88, which might confer a selective advantage by promoting intestinal establishment and colonization by killing other *E. coli* and freeing up their ecologic niches (Figure 1, panel B).

We sequenced a panel of strains consisting of representative O80 strains from France and all O80 strains from outside of France available at the beginning of the study to establish the genetic relationship between O80 strains isolated in several countries in Europe and analyze the diversity of their genetic content. We obtained an average of 233 contigs, with a mean depth of coverage of 58X and a mean N50 of 72,312. Statistics of each sequenced genome are summarized in online Technical Appendix Table 1. Single-nucleotide polymorphism analysis (41,618 sites total) between the 36 sequenced O80 strains and 9 EHEC strains of other major serotypes (O157:H7, O26:H11, O111:H-, O103:H2, O55:H7, O91:NM, O104:H4, O145:H28, O121:H19) enabled us to establish a phylogenetic tree (Figure 2), which shows 2 main clusters. EHEC O157:H7 (EDL933) and O55:H7 (2013C-4465) group together, as expected by their common origin, demonstrated by Feng et al. (23), and are distantly related to the other major EHEC serotypes, including the O80 strains. However, O80 isolates clearly form a separate group, suggesting that O80 emerged independently from the other serogroups. All of the O80 strains belong to clonal complex 165 (CC165), containing ST301, ST165, and ST189 (Figure 2, panel B).

Almost all of the O80 strains (32/36), including RDEx444, belong to the ST301 clonal group of serotype O80:H2. All of these isolates have EHEC markers, including genes encoded by the LEE, containing the rare variant of intimin *eae-ξ*, *ehxA*, and *stx2a* or *stx2d* genes, except for 4 strains missing the *stx* gene (CB13483, IH43632-03a, FV-4476, and VTB262) (online Technical Appendix Figure 2). However, the presence of the other EHEC markers in these 4 strains (*eae*, *ehxA/espP*, or *katP*) suggests that they were initially STEC and underwent subsequent

elimination of the *stx* gene. This finding led us to search for a scar of the Stx-converting bacteriophage at the insertion site described in the RDEx444 strain (*yecE*). We first searched for the contig carrying the *yecE* gene. Then, we used the Phaster webserver system to detect phage regions, which were finally blasted against RDEx444. We found scars of ≈31 kb in the human strain from Spain (IH43632-03a) and scars of ≈17 kb in the porcine strain from Slovakia (FV-4476), similar to the RDEx444 Stx-converting bacteriophage (92% and 94% nucleotide identity and 40% and 56% coverage, respectively) (data not shown). We found no scars for the other 2 strains, suggesting either complete prophage excision or insertion at another site.

All strains carrying the *stx* gene (28/36) also possess VFs typical of pS88, and all but 1 (CB12623) also carry genes conferring resistance to ≥1 of penicillin, tetracycline, kanamycin, or cotrimoxazole. The consistent association between extraintestinal VFs typical of pS88 and MDR genes, irrespective of the source or country of isolation, might suggest the presence of a mosaic plasmid, such as in the RDEx444 strain. Although it lacks pS88 markers, the water strain from Germany (CB13483) is nevertheless multidrug resistant and carries an extended spectrum β-lactamase gene (*bla*<sub>CTX-M-1</sub>), as well as the *mphA* gene, which confers resistance to azithromycin, the only antimicrobial drug that can be used for intestinal decontamination of EHEC (24; online Technical Appendix Figure 2).

Among our panel, 3 STEC strains from cattle in France clearly belong to this ST301 clonal group. Cattle might thus represent an animal reservoir for these hybrid pathotype strains, given that the isolates possess exactly the same VFs (*eae*, genes of T3SS, *ehxA*, *stx*, and determinants of the pS88-like plasmid) and resistance genes as human EHECs.

Two clusters (I and II) can be distinguished within the ST301 group we describe. The main difference between these 2 groups concerns the presence of the unknown cryptic plasmid (pR444\_B) in RDEx444. All strains of cluster I (n = 25) possess ≥85% of the genetic determinants of this plasmid of unknown function. BLAST results are depicted for each strain in online Technical Appendix Table 2. Conversely, no strain of group II (n = 7), except 1, has this plasmid. No strains of ST165 or ST189 carry it either.

Within cluster I, the pS88-like plasmid carries 2 distinct gene profiles, showing its plasticity. The 12 Stx2d<sub>acti-vable</sub> EHECs isolated from humans in France (designated as subcluster Ia in Figure 2) carry the most complete form of the plasmid, identical to pS88. The aerobactin iron-uptake system, encoded by *iuc* genes, and the type I secretion system, encoded by *etsC*, are absent from the pS88-like plasmid of the subcluster Ib strains.

The second clonal group, ST165, is formed by 1 strain (EC\_POI) of serotype O80:H19, which is devoid of all VF and resistance genes. This isolate might reflect the ancestral

origin from which serogroup O80 EHEC strains were derived after the acquisition of diverse VFs.

Finally, clonal group ST189 consists of the 3 avian strains of serotype O80:H26 from Germany. None has *stx* or *ehxA* genes, but all have a complete LEE with the variant  $\beta 1$  of the intimin gene (*eae*). However, none of these strains carries the plasmid *bfp* gene of typical EPEC (online Technical Appendix Figure 2). One strain (CB13938) has a region of  $\approx 27$  kb at the same insertion site (*yecE* gene) that shares homology with RDEx444 Stx-converting bacteriophage (88% nucleotide identity, 16% coverage), suggesting a potential scar of Stx-converting phage. Thus, this strain could have been originally an EHEC which secondarily lost its *stx* gene. No similar regions were found in the other 2 strains of this group. Otherwise, 1 strain (CB15046) has several VFs, confirming the presence of the pS88-like plasmid with MDR genes. The other 2 strains (CB15387 and CB13938) carry the locus encoding the siderophore yersiniabactin (*fyuA*), also called the high-pathogenicity island; these 2 strains also have pyelonephritis-associated pili with Pap-GII adhesin (online Technical Appendix Figure 2). These strains constitute another type of hybrid pathotype with intestinal and extraintestinal VFs. Although none of them has VFs typical of pS88 plasmids, CB13938 is multidrug-resistant and carries the recently described *mcr-1* plasmid gene (25), conferring resistance to colistin. Blast analysis performed with the contig containing *mcr-1* (10,119 bp) shows strong homology (100% coverage, 99% identity) with 2 chromosomal insertion sites previously described in strains Mb1323 and Mb1506 (26).

## Discussion

We deciphered the molecular characteristics of O80:H2–CC165 EHEC, an emerging hybrid pathotype diffusing throughout Europe. This pathotype is armed to spread by means of a conjugative plasmid combining extraintestinal virulence with resistance to nearly all major classes of antibiotics, improved by the presence of several plasmid and chromosomally encoded bacteriocins, such as colicines I, V, M, and H47. We used the same criteria of MDR as a recent study in England (27) (*bla*<sub>TEM-1</sub>, *strA-strB*, *sul1/sul2/dfrA*, and *tetA*) and showed that 93% (26/28) of O80 STEC have this genotypic resistance profile, whereas only 5% of the strains identified within the O157 and O26 serogroups in the study in England had such a profile. MDR observed with this hybrid pathotype might complicate patient care, and the use of antimicrobial drugs during EHEC infections is still a subject of debate (28). However, the occurrence of invasive infections, such as bacteremia during EHEC infections, with this clone warrants antimicrobial treatment for such infections. In a previous study, the observed Stx rate was

lower with a combination of azithromycin and ceftriaxone assays relative to basal secretion, and we proposed this association for the treatment of such infections (10).

Such a troubling plasmid has never been identified in human EHEC isolates. The only example of a similar mosaic plasmid was reported for *S. enterica* serovar Kentucky, in which an AmpC  $\beta$ -lactamase gene (*bla*<sub>CMY-2</sub>) was integrated into a pS88-like plasmid (29). The insertion of an MDR-encoding island in a pS88-like plasmid containing extraintestinal virulence genes is particularly worrisome. Massive and inappropriate use of veterinary antibiotics, such as tetracycline, in food-animal production promotes antimicrobial drug resistance among animals, known to be reservoirs for STEC. This practice can select and favor the spread of such MDR plasmids in human EHECs. Tetracycline still represented 36.5% of the tonnage of veterinary antibiotic use in 2015 in France (30). In our panel, all the sequenced O80 STEC strains carry the *tetA* gene, conferring resistance to tetracycline. Thus, large veterinary use of this drug might favor the selection of these hybrid strains and increase their diffusion.

We indicated a potential reservoir of these hybrid pathotype strains when we identified 3 O80:H2 strains isolated from cattle that carry the same VFs and resistance genes as human strains. However, the presence of the CC165 strains in chickens suggest that this clonal complex is also adapted to poultry. An initial description of pS88-like plasmids in avian pathogenic *E. coli* strains reinforces this hypothesis (31). Moreover, the environmental survival of this clone in these potential reservoirs might be enhanced because of the resistance to mercury shared by all but 1 strain (36047), all carriers of the pS88-like plasmid, irrespective of their origin. Such resistance to heavy metals has been rarely described in EHEC strains (22).

We also detected an O80:H19–CC165 strain devoid of virulence genes, which might represent the ancestral precursor of CC165, and from which these hybrid pathotype strains might have been derived. This strain could be used for tracing the genetic history of this clone in future studies.

Our genetic description of the emerging hybrid pathotype *E. coli* O80:H2, associated with O80-related strains, reveals the outstanding capacity of O80–CC165 to acquire the combination of virulence genes involved in intestinal and extraintestinal pathogenicity and genes conferring broad antibiotic resistance, including extended-spectrum  $\beta$ -lactamase-encoding genes and those most recently identified, such as *mcr-1*. O80–CC165 strains, which are able to integrate multiple VFs with various consequences, MDR genes that encompass nearly all classes, and bacteriocins, represent a serious threat because of their exceptional versatility and should therefore be closely monitored in all countries in Europe.

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## References

- Garg AX, Suri RS, Barrowman N, Rehman F, Matsell D, Rosas-Arellano MP, et al. Long-term renal prognosis of diarrhea-associated hemolytic uremic syndrome: a systematic review, meta-analysis, and meta-regression. *JAMA*. 2003;290:1360–70. <http://dx.doi.org/10.1001/jama.290.10.1360>
- Schmidt H, Karch H, Beutin L. The large-sized plasmids of enterohemorrhagic *Escherichia coli* O157 strains encode hemolysins which are presumably members of the *E. coli* alpha-hemolysin family. *FEMS Microbiol Lett*. 1994;117:189–96.
- Brunder W, Schmidt H, Karch H, EspP, a novel extracellular serine protease of enterohaemorrhagic *Escherichia coli* O157:H7 cleaves human coagulation factor V. *Mol Microbiol*. 1997;24:767–78. <http://dx.doi.org/10.1046/j.1365-2958.1997.3871751.x>
- Bruyand M. Surveillance du syndrome hémolytique et urémique post-diarrhéique chez les enfants de moins de 15 ans en France en 2016 [cited 2016 Feb 16]. [http://invs.santepubliquefrance.fr/content/download/138859/500759/version/1/file/Bilan\\_SHU\\_2016.pdf](http://invs.santepubliquefrance.fr/content/download/138859/500759/version/1/file/Bilan_SHU_2016.pdf)
- Blanco M, Blanco JE, Mora A, Dahbi G, Alonso MP, González EA, 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-ξ*). *J Clin Microbiol*. 2004;42:645–51. <http://dx.doi.org/10.1128/JCM.42.2.645-651.2004>
- Wijnsma KL, Schijvens AM, Rossen JWA, Kooistra-Smid AMDM, Schreuder MF, van de Kar NCAJ. Unusual severe case of hemolytic uremic syndrome due to Shiga toxin 2d-producing *E. coli* O80:H2. *Pediatr Nephrol*. 2017;32:1263–8. <http://dx.doi.org/10.1007/s00467-017-3642-3>
- Fierz L, Cernela N, Hauser E, Nüesch-Inderbinen M, Stephan R. Characteristics of Shigatoxin-producing *Escherichia coli* strains isolated during 2010–2014 from human infections in Switzerland. *Front Microbiol*. 2017;8:1471. <http://dx.doi.org/10.3389/fmicb.2017.01471>
- Bielaszewska M, Mellmann A, Zhang W, Köck R, Fruth A, Bauwens A, et al. Characterisation of the *Escherichia coli* strain associated with an outbreak of haemolytic uraemic syndrome in Germany, 2011: a microbiological study. *Lancet Infect Dis*. 2011;11:671–6. [http://dx.doi.org/10.1016/S1473-3099\(11\)70165-7](http://dx.doi.org/10.1016/S1473-3099(11)70165-7)
- Mariani-Kurkdjian P, Lemaître C, Bidet P, Perez D, Boggini L, Kwon T, et al. Haemolytic-uraemic syndrome with bacteraemia caused by a new hybrid *Escherichia coli* pathotype. *New Microbes*. 2014;2:127–31. <http://dx.doi.org/10.1002/nmi2.49>
- Soysal N, Mariani-Kurkdjian P, Smail Y, Liguori S, Gouali M, Loukiadis E, et al. Enterohemorrhagic *Escherichia coli* hybrid pathotype O80:H2 as a new therapeutic challenge. *Emerg Infect Dis*. 2016;22:1604–12. <http://dx.doi.org/10.3201/eid2209.160304>
- Peigne C, Bidet P, Mahjoub-Messai F, Plainvert C, Barbe V, Médigue C, 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;77:2272–84. <http://dx.doi.org/10.1128/IAI.01333-08>
- Carattoli A, Zankari E, García-Fernández A, Voldby Larsen M, Lund O, Villa L, et al. In silico detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. *Antimicrob Agents Chemother*. 2014;58:3895–903. <http://dx.doi.org/10.1128/AAC.02412-14>
- Kaas RS, Leekitcharoenphon P, Aarestrup FM, Lund O. Solving the problem of comparing whole bacterial genomes across different sequencing platforms. *PLoS One*. 2014;9:e104984. <http://dx.doi.org/10.1371/journal.pone.0104984>
- Joensen KG, Tetzschner AMM, Iguchi A, Aarestrup FM, Scheutz F. Rapid and easy in silico serotyping of *Escherichia coli* isolates by use of whole-genome sequencing data. *J Clin Microbiol*. 2015;53:2410–26. <http://dx.doi.org/10.1128/JCM.00008-15>
- Wirth T, Falush D, Lan R, Colles F, Mensa P, Wieler LH, et al. Sex and virulence in *Escherichia coli*: an evolutionary perspective. *Mol Microbiol*. 2006;60:1136–51. <http://dx.doi.org/10.1111/j.1365-2958.2006.05172.x>
- Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, et al. Identification of acquired antimicrobial resistance genes. *J Antimicrob Chemother*. 2012;67:2640–4. <http://dx.doi.org/10.1093/jac/dks261>
- Bielaszewska M, Friedrich AW, Aldick T, Schürk-Bulgrin R, Karch H. Shiga toxin activatable by intestinal mucus in *Escherichia coli* isolated from humans: predictor for a severe clinical outcome. *Clin Infect Dis*. 2006;43:1160–7. <http://dx.doi.org/10.1086/508195>
- Serra-Moreno R, Jofre J, Muniesa M. Insertion site occupancy by *stx*<sub>2</sub> bacteriophages depends on the locus availability of the host strain chromosome. *J Bacteriol*. 2007;189:6645–54. <http://dx.doi.org/10.1128/JB.00466-07>
- Bonanno L, Loukiadis E, Mariani-Kurkdjian P, Oswald E, Garnier L, Michel V, et al. Diversity of Shiga toxin-producing *Escherichia coli* (STEC) O26:H11 strains examined via *stx* subtypes and insertion sites of Stx and EspK bacteriophages. *Appl Environ Microbiol*. 2015;81:3712–21. <http://dx.doi.org/10.1128/AEM.00077-15>
- Recktenwald J, Schmidt H. The nucleotide sequence of Shiga toxin (Stx) 2e-encoding phage phiP27 is not related to other Stx phage genomes, but the modular genetic structure is conserved. *Infect Immun*. 2002;70:1896–908. <http://dx.doi.org/10.1128/IAI.70.4.1896-1908.2002>
- Falgenhauer L, Yao Y, Fritzenwanker M, Schmiedel J, Mirzalioglu C, Chakraborty T. Complete genome sequence of phage-like plasmid pECO89, encoding CTX-M-15. *Genome Announc*. 2014; 2:e00356–14. <http://dx.doi.org/10.1128/genomeA.00356-14>
- Venturini C, Hassan KA, Roy Chowdhury P, Paulsen IT, Walker MJ, Djordjevic SP. Sequences of two related multiple antibiotic resistance virulence plasmids sharing a unique IS26-related molecular signature isolated from different *Escherichia coli* pathotypes from different hosts. *PLoS One*. 2013;8:e78862. <http://dx.doi.org/10.1371/journal.pone.0078862>
- Feng P, Lampel KA, Karch H, Whittam TS. Genotypic and phenotypic changes in the emergence of *Escherichia coli* O157:H7. *J Infect Dis*. 1998;177:1750–3. <http://dx.doi.org/10.1086/517438>

24. Jost C, Bidet P, Carrère T, Mariani-Kurkdjian P, Bonacorsi S. Susceptibility of enterohaemorrhagic *Escherichia coli* to azithromycin in France and analysis of resistance mechanisms. *J Antimicrob Chemother.* 2016;71:1183–7. <http://dx.doi.org/10.1093/jac/dkv477>
25. Liu Y-Y, Wang Y, Walsh TR, Yi L-X, Zhang R, Spencer J, et al. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. *Lancet Infect Dis.* 2016;16:161–8. [http://dx.doi.org/10.1016/S1473-3099\(15\)00424-7](http://dx.doi.org/10.1016/S1473-3099(15)00424-7)
26. Donà V, Bernasconi OJ, Pires J, Collaud A, Overesch G, Ramette A, et al. Heterogeneous genetic location of *mcr-1* in colistin-resistant *Escherichia coli* isolates from humans and retail chicken meat in Switzerland: emergence of *mcr-1*-carrying IncK2 plasmids. *Antimicrob Agents Chemother.* 2017;61:e01245–17. <http://dx.doi.org/10.1128/AAC.01245-17>
27. Day M, Doumith M, Jenkins C, Dallman TJ, Hopkins KL, Elson R, et al. Antimicrobial resistance in Shiga toxin-producing *Escherichia coli* serogroups O157 and O26 isolated from human cases of diarrhoeal disease in England, 2015. *J Antimicrob Chemother.* 2017;72:145–52. <http://dx.doi.org/10.1093/jac/dkw371>
28. Freedman SB, Xie J, Neufeld MS, Hamilton WL, Hartling L, Tarr PI; Alberta Provincial Pediatric Enteric Infection Team (APPETITE). Shiga toxin-producing *Escherichia coli* infection, antibiotics, and risk of developing hemolytic uremic syndrome: a meta-analysis. *Clin Infect Dis.* 2016;62:1251–8. <http://dx.doi.org/10.1093/cid/ciw099>
29. Fricke WF, McDermott PF, Mammel MK, Zhao S, Johnson TJ, Rasko DA, et al. Antimicrobial resistance-conferring plasmids with similarity to virulence plasmids from avian pathogenic *Escherichia coli* strains in *Salmonella enterica* serovar Kentucky isolates from poultry. *Appl Environ Microbiol.* 2009;75:5963–71. <http://dx.doi.org/10.1128/AEM.00786-09>
30. Agence Nationale de Sécurité Sanitaire de l'Alimentation, de l'Environnement et du Travail (ANSES). Suivi des ventes de médicaments vétérinaires contenant des antibiotiques en France en 2015 [cited 2016 Feb 16]. <https://www.anses.fr/fr/system/files/ANMV-Ra-Antibiotiques2015.pdf>
31. Johnson TJ, Siek KE, Johnson SJ, Nolan LK. DNA sequence of a ColV plasmid and prevalence of selected plasmid-encoded virulence genes among avian *Escherichia coli* strains. *J Bacteriol.* 2006;188:745–58. <http://dx.doi.org/10.1128/JB.188.2.745-758.2006>

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# Emerging Multidrug-Resistant Hybrid Pathotype Shiga Toxin–Producing *Escherichia coli* O80 and Related Strains of Clonal Complex 165, Europe

## Technical Appendix

### GenBank Accession Numbers

We established phylogeny by single-nucleotide polymorphism alignments between the contigs generated by CLC Genomics of O80 strains and 9 reference EHEC strain sequences of major serotypes available in GenBank: O157:H7 EDL933 (accession no. NC\_002655.2), O26:H11 11368 (NC\_013361.1), O111:H- 11128 (NC\_013364.1), O103:H2 12009 (NC\_013353.1), O55:H7 2013C-4465 (CP015241), O91:NM 2009C-3745 (JHGW00000000), O104:H4 LB226692 (EO104H4LB.1), O145:H28 2009C-3292 (JHHD00000000), and O121:H19 2009C-4750 (JHGL00000000).

Nucleotide sequences of the 36 sequenced O80 strains (complete nucleotide sequence of RDEx444 and 35 draft nucleotide sequences) have been deposited in Genbank: Project PRJNA449634 under accession numbers: QBCG00000000, QBCH00000000, QBCI00000000, QBCJ00000000, QBCK00000000, QBCL00000000, QBCM00000000, QBCN00000000, QBCO00000000, QBCP00000000, QBCQ00000000, QBCR00000000, QBCS00000000, QBCT00000000, QBCU00000000, QBCV00000000, QBCW00000000, QBCX00000000, QBCY00000000, QBCZ00000000, QBDA00000000, QBDB00000000, QBDC00000000, QBDD00000000, QBDE00000000, QBDF00000000, QBDG00000000, QBDH00000000, QBDI00000000, QBDJ00000000, QBDK00000000, QBDL00000000, QBDM00000000, QBDN00000000, QBDO00000000, QBDP00000000.

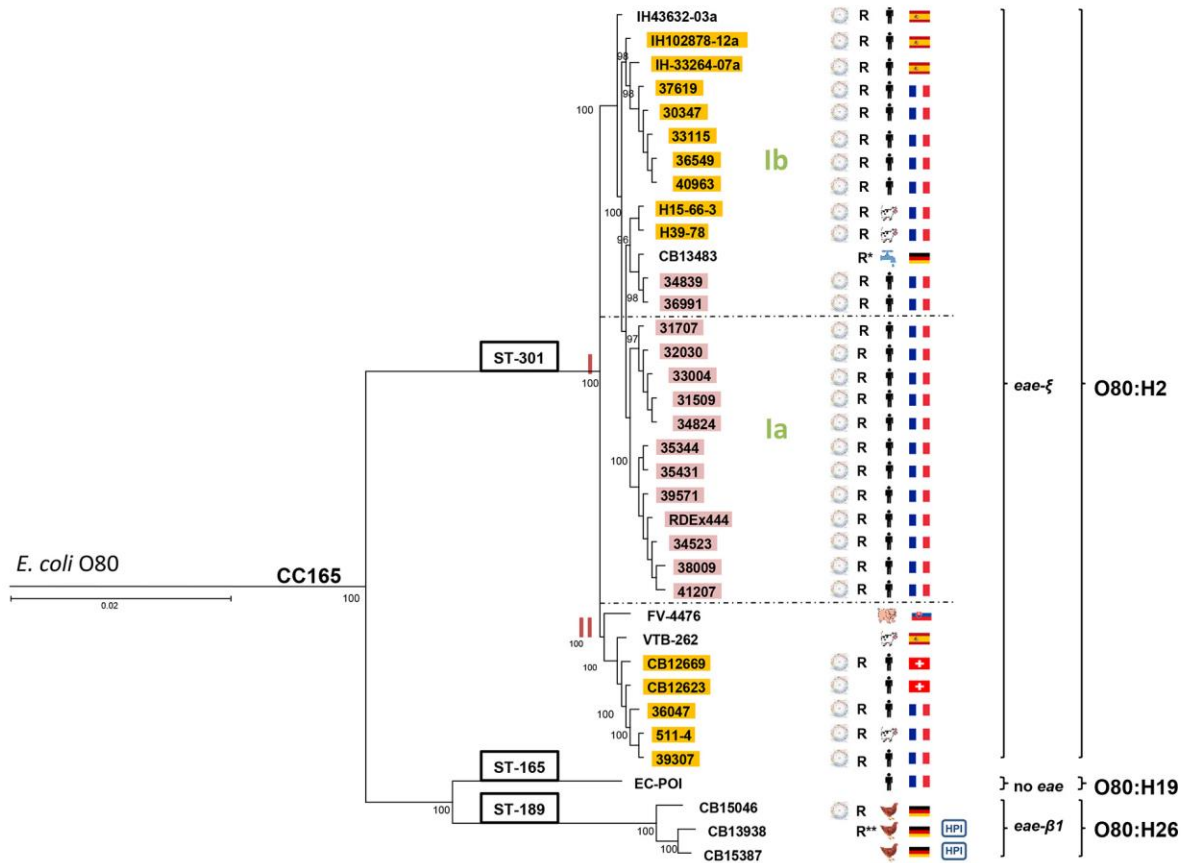
**Technical Appendix Table 1.** Statistic of sequenced genomes

Strain number	No. of contigs	N50	No. of bases	Coverage (X)
38009	202	106 974	5 426 079	147.6
41207	211	107 035	5 467 265	79.4
34523	265	55 705	5 426 710	32.6
39571	228	84 038	5 476 022	119.3
35344	203	83 962	5 343 804	70.2
35431	269	43 457	5 314 749	31.8
33004	246	56 663	5 401 414	37.9
32030	237	67 378	5 486 203	43
31509	186	83 971	5 379 976	53.1
34824	281	39 286	5 366 586	31.3
31707	413	49 584	5 380 230	39.6
H15-66.3	185	121 183	5 457 940	123.9
H39-78	216	102 553	5 449 344	60.3
CB13483	250	45 176	5 302 150	37.4
34839	192	99 988	5 433 192	100.1
36991	292	38 410	5 457 973	31.4
IH102878-12a	197	88 042	5 554 738	67
IH33264-07a	242	49 429	5 277 481	41.3
37619	253	52 463	5 308 653	35.6
30347	194	84 601	5 358 355	48.1
33115	259	49 396	5 343 896	36.8
36549	206	71 156	5 375 868	59.9
40963	383	33 063	5 468 507	35.2
IH43632-03a	189	77 642	5 252 308	48.5
FV-4476	166	75 655	5 224 753	72.1
VTB-262	126	102 083	5 005 084	72.1
CB12669	191	88 417	5 317 409	65.4
CB12623	302	33 724	5 223 037	37.8
39307	176	102 609	5 503 620	106.7
36047	284	37 908	5 263 027	37.7
511-4	232	85 373	5 443 854	57.2
EC-POI	56	183 356	4 744 330	62.7
CB15046	290	35 034	5 225 546	34.8
CB13938	319	38 745	5 310 718	44.2
CB15387	215	56 890	5 169 961	33.8

**Technical Appendix Table 2.** Distribution of sequences homologous to the pR444\_B plasmid among 08O:H2 hybrid STEC strains

Strain number	Contig number	Max score	Total score	Query cover	Identity
38009	91	91650	91789	42%	99%
	30	73706	1.091e+05	50%	100%
41207	19	91650	91789	42%	99%
	10	73719	1.091e+05	50%	99%
34523	99	73582	1.089e+05	50%	100%
	100	64467	64537	29%	99%
	214	26325	26394	12%	99%
39571	66	56604	56604	26%	99%
	45	49125	49195	22%	99%
	85	42492	45301	20%	99%
	40	35349	52330	24%	99%
35344	6	73584	1.089e+05	50%	100%
	34	54010	54079	25%	99%
	7	15254	17994	8%	99%
35431	26	12213	12282	5%	100%
	66	73582	1.089e+05	50%	100%
	48	53963	54033	25%	99%
	151	15254	17990	8%	99%
33004	166	12213	12282	5%	100%
	67	73656	1.090e+05	50%	99%
	115	65237	65467	30%	99%
	149	26256	26326	12%	99%
32030	44	91580	2.033e+05	93%	99%
31509	101	91613	94490	43%	99%
	24	73582	1.089e+05	50%	100%
34824	5	73582	1.089e+05	50%	100%
	47	64247	67055	31%	99%
	121	23736	23805	11%	99%
31707	36	73577	1.089e+05	50%	99%
	37	64379	67186	31%	99%
	117	26478	26547	12%	99%
H15-66-3	29	91563	2.033e+05	93%	99%
H39-78	42	91563	2.033e+05	93%	99%
CB13483	44	73538	2.027e+05	93%	99%
34839	4	91569	2.033e+05	93%	99%
36991	64	73538	1.536e+05	71%	99%
	49	26299	49982	23%	99%
IH102878-12a	7	73538	2.033e+05	93%	99%
IH-33264-07a	77	54769	1.270e+05	58%	99%
	124	36808	76261	35%	99%
37619	13	65442	1.049e+05	48%	99%
	109	35318	88540	40%	99%
30347	30	54894	94314	43%	99%
	52	32221	33229	15%	99%
	106	26559	26559	12%	99%
	101	26380	29564	13%	99%
	146	9858	9858	4%	100%
33115	29	66279	1.027e+05	47%	99%
	59	36684	64757	29%	99%
	111	26559	26559	12%	99%
36549	41	36662	64735	29%	99%
	40	35323	68797	31%	99%
	99	33883	33883	15%	99%
	21	26559	26559	12%	99%
40963	58	36684	64757	29%	99%
	98	35323	68747	31%	99%
	78	33883	33883	15%	99%
	200	26559	26559	12%	99%
IH43632-03a	32	51182	2.069e+05	95%	99%
	FV-4476	114	4678	4678	2%
VTB-262	73	3613	3880	1%	99%
	22	3164	4103	2%	94%
	8	2106	2106	1%	91%
	65	4676	4676	2%	100%
	44	3965	3965	2%	95%
	27	3158	4098	2%	94%
	32	2106	2106	1%	91%
30	1256	2765	1%	99%	

Strain number	Contig number	Max score	Total score	Query cover	Identity
CB12669	11	4676	4676	2%	100%
	44	3965	3965	2%	95%
	128	3531	3531	1%	99%
	43	3164	4103	2%	94%
	119	1256	2765	1%	99%
CB12623	30	4676	4676	2%	100%
	61	3164	4103	2%	94%
39307	23	57483	1.705e+05	83%	98%
	5	4676	4676	2%	100%
36047	244	4676	4676	2%	100%
	174	3164	4103	2%	94%
	15	2372	2372	1%	96%
	50	2100	2100	1%	91%
511-4	129	4676	4676	2%	100%
	76	3164	4103	2%	94%
	14	2372	2372	1%	96%
	149	2106	2106	1%	91%
Ec-POI	7	1256	2138	1%	99%
	6	3169	4258	2%	94%
CB15046	221	4340	4340	2%	98%
	64	3609	3609	1%	100%
	55	3164	4103	2%	94%
	190	2309	2309	1%	97%
CB13938	41	4617	4617	2%	100%
	165	4340	4340	2%	98%
	40	3164	4103	2%	94%
	73	2817	2817	1%	100%
CB15387	93	4340	4340	2%	98%
	65	3609	3609	1%	100%
	40	3164	4103	2%	94%



**Technical Appendix Figure 1.** Phylogeny (maximum likelihood method) of 36 O80 *E. coli* isolated from various sources and European countries between 1998 and 2016. The highlighted strains carry the Shiga toxin genes and the subtype of Stx is indicated by a color code as follows: purple, *stx1a*; yellow, *stx2a*; pink, *stx2d<sub>activable</sub>*; brown, *stx1a2a*; dark green, *stx1a2b*. The presence of the pS88 like-plasmid is represented using a plasmid scheme next to the strain number. The “R” to the right of the plasmid indicates that the strain possesses at least two resistance genes that confer resistance to betalactams, kanamycin, or cotrimoxazole. R\*: additional extended spectrum betalactamase (ESBL) gene. R\*\*: additional resistance to colistin (*mcr-1* gene). The strain origin (country and source of isolation) is represented by flags and human/animal/water symbols. HPI: presence of chromosomal locus encoding the siderophore yersiniabactin, also called the High Pathogenicity Island.

Strain number	Strain origin	Cluster number (Figure 2)	Clinical features	Resistance genes																Virulence factors																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																											
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				BATEM1B	blaCTX-M-1	aac(2)-Ia	aac(2)-IIa	catA	catB	catC	catD	catE	catF	catG	catH	catI	catJ	catK	catL	catM	catN	catO	catP	catQ	catR	catS	catT	catU	catV	catW	catX	catY	catZ	catAA	catAB	catAC	catAD	catAE	catAF	catAG	catAH	catAI	catAJ	catAK	catAL	catAM	catAN	catAO	catAP	catAQ	catAR	catAS	catAT	catAU	catAV	catAW	catAX	catAY	catAZ	catBA	catBB	catBC	catBD	catBE	catBF	catBG	catBH	catBI	catBJ	catBK	catBL	catBM	catBN	catBO	catBP	catBQ	catBR	catBS	catBT	catBU	catBV	catBW	catBX	catBY	catBZ	catCA	catCB	catCC	catCD	catCE	catCF	catCG	catCH	catCI	catCJ	catCK	catCL	catCM	catCN	catCO	catCP	catCQ	catCR	catCS	catCT	catCU	catCV	catCW	catCX	catCY	catCZ	catDA	catDB	catDC	catDD	catDE	catDF	catDG	catDH	catDI	catDJ	catDK	catDL	catDM	catDN	catDO	catDP	catDQ	catDR	catDS	catDT	catDU	catDV	catDW	catDX	catDY	catDZ	catEA	catEB	catEC	catED	catEE	catEF	catEG	catEH	catEI	catEJ	catEK	catEL	catEM	catEN	catEO	catEP	catEQ	catER	catES	catET	catEU	catEV	catEW	catEX	catEY	catEZ	catFA	catFB	catFC	catFD	catFE	catFF	catFG	catFH	catFI	catFJ	catFK	catFL	catFM	catFN	catFO	catFP	catFQ	catFR	catFS	catFT	catFU	catFV	catFW	catFX	catFY	catFZ	catGA	catGB	catGC	catGD	catGE	catGF	catGG	catGH	catGI	catGJ	catGK	catGL	catGM	catGN	catGO	catGP	catGQ	catGR	catGS	catGT	catGU	catGV	catGW	catGX	catGY	catGZ	catHA	catHB	catHC	catHD	catHE	catHF	catHG	catHH	catHI	catHJ	catHK	catHL	catHM	catHN	catHO	catHP	catHQ	catHR	catHS	catHT	catHU	catHV	catHW	catHX	catHY	catHZ	catIA	catIB	catIC	catID	catIE	catIF	catIG	catIH	catII	catIJ	catIK	catIL	catIM	catIN	catIO	catIP	catIQ	catIR	catIS	catIT	catIU	catIV	catIW	catIX	catIY	catIZ	catJA	catJB	catJC	catJD	catJE	catJF	catJG	catJH	catJI	catJJ	catJK	catJL	catJM	catJN	catJO	catJP	catJQ	catJR	catJS	catJT	catJU	catJV	catJW	catJX	catJY	catJZ	catKA	catKB	catKC	catKD	catKE	catKF	catKG	catKH	catKI	catKJ	catKK	catKL	catKM	catKN	catKO	catKP	catKQ	catKR	catKS	catKT	catKU	catKV	catKW	catKX	catKY	catKZ	catLA	catLB	catLC	catLD	catLE	catLF	catLG	catLH	catLI	catLJ	catLK	catLL	catLM	catLN	catLO	catLP	catLQ	catLR	catLS	catLT	catLU	catLV	catLW	catLX	catLY	catLZ	catMA	catMB	catMC	catMD	catME	catMF	catMG	catMH	catMI	catMJ	catMK	catML	catMN	catMO	catMP	catMQ	catMR	catMS	catMT	catMU	catMV	catMW	catMX	catMY	catMZ	catNA	catNB	catNC	catND	catNE	catNF	catNG	catNH	catNI	catNJ	catNK	catNL	catNM	catNO	catNP	catNQ	catNR	catNS	catNT	catNU	catNV	catNW	catNX	catNY	catNZ	catOA	catOB	catOC	catOD	catOE	catOF	catOG	catOH	catOI	catOJ	catOK	catOL	catOM	catON	catOO	catOP	catOQ	catOR	catOS	catOT	catOU	catOV	catOW	catOX	catOY	catOZ	catPA	catPB	catPC	catPD	catPE	catPF	catPG	catPH	catPI	catPJ	catPK	catPL	catPM	catPN	catPO	catPP	catPQ	catPR	catPS	catPT	catPU	catPV	catPW	catPX	catPY	catPZ	catQA	catQB	catQC	catQD	catQE	catQF	catQG	catQH	catQI	catQJ	catQK	catQL	catQM	catQN	catQO	catQP	catQQ	catQR	catQS	catQT	catQU	catQV	catQW	catQX	catQY	catQZ	catRA	catRB	catRC	catRD	catRE	catRF	catRG	catRH	catRI	catRJ	catRK	catRL	catRM	catRN	catRO	catRP	catRQ	catRR	catRS	catRT	catRU	catRV	catRW	catRX	catRY	catRZ	catSA	catSB	catSC	catSD	catSE	catSF	catSG	catSH	catSI	catSJ	catSK	catSL	catSM	catSN	catSO	catSP	catSQ	catSR	catSS	catST	catSU	catSV	catSW	catSX	catSY	catSZ	catTA	catTB	catTC	catTD	catTE	catTF	catTG	catTH	catTI	catTJ	catTK	catTL	catTM	catTN	catTO	catTP	catTQ	catTR	catTS	catTT	catTU	catTV	catTW	catTX	catTY	catTZ	catUA	catUB	catUC	catUD	catUE	catUF	catUG	catUH	catUI	catUJ	catUK	catUL	catUM	catUN	catUO	catUP	catUQ	catUR	catUS	catUT	catUU	catUV	catUW	catUX	catUY	catUZ	catVA	catVB	catVC	catVD	catVE	catVF	catVG	catVH	catVI	catVJ	catVK	catVL	catVM	catVN	catVO	catVP	catVQ	catVR	catVS	catVT	catVU	catVV	catVW	catVX	catVY	catVZ	catWA	catWB	catWC	catWD	catWE	catWF	catWG	catWH	catWI	catWJ	catWK	catWL	catWM	catWN	catWO	catWP	catWQ	catWR	catWS	catWT	catWU	catWV	catWW	catWX	catWY	catWZ	catXA	catXB	catXC	catXD	catXE	catXF	catXG	catXH	catXI	catXJ	catXK	catXL	catXM	catXN	catXO	catXP	catXQ	catXR	catXS	catXT	catXU	catXV	catXW	catXX	catXY	catXZ	catYA	catYB	catYC	catYD	catYE	catYF	catYG	catYH	catYI	catYJ	catYK	catYL	catYM	catYN	catYO	catYP	catYQ	catYR	catYS	catYT	catYU	catYV	catYW	catYX	catYY	catYZ	catZA	catZB	catZC	catZD	catZE	catZF	catZG	catZH	catZI	catZJ	catZK	catZL	catZM	catZN	catZO	catZP	catZQ	catZR	catZS	catZT	catZU	catZV

**Technical Appendix Figure 2.** Summary of complete sequencing data of the 36 O80 strains isolated in various European countries between 1998 and 2016. The strain origin (country and source of isolation) is represented by flags and human/animal/water symbols as in Figure 2. Clinical features are indicated as follows: HUS for hemolytic uremic syndrome, BD for bloody diarrhea, NA for not applicable (animal strains), UK for unknown, and C for healthy carrier. Gray boxes indicate the presence of resistance genes, virulence genes, or the presence of cryptic plasmids similar to pR444\_B. Resistance genes are classified by antibiotic family: BL for betalactams, AS for aminoglycoside, SXT for cotrimoxazole, TET for tetracycline, CHL for phenicols, MCRL for macrolide, CT for colistin, and AN for nalidixic acid and numbers indicate the variant of the resistance genes. \*variant *bla<sub>CTX-M-1</sub>* of extended spectrum betalactamase (ESBL) gene. Bacteriocins are indicated in yellow and the type of virulence factor is indicated by a color code as follows: purple, toxin; green, adhesion factor; light purple, hemolysin; beige, protectin; pink, iron uptake system.