FEMS Pathogens and Disease, 73, 2015, ftv076

doi: 10.1093/femspd/ftv076
Advance Access Publication Date: 26 September 2015
Short Communication

SHORT COMMUNICATION

Diarrhea, bacteremia and multiorgan dysfunction due to an extraintestinal pathogenic *Escherichia coli* strain with enteropathogenic *E. coli* genes

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One sentence summary: A novel isolate of *Escherichia coli* with features of extraintestinal and enteropathogenic strains was isolated from the blood of a man with multiorgan dysfunction following 3 weeks of diarrhea.

Editor: Eric Oswald

ABSTRACT

A 55-year-old man with well-controlled HIV had severe diarrhea for 3 weeks and developed multiorgan dysfunction and bacteremia due to *Escherichia coli*. The genome of the patient's isolate had features characteristic of extraintestinal pathogenic *E. coli* and genes distantly related to those defining enteropathogenic *E. coli*.

Keywords: case report; comparative genomics; virulence factors; type 3 secretion; hemolysin

Escherichia coli colonizes most neonates within hours of birth, likely during delivery (Bettelheim *et al.* 1974). *Escherichia coli* is notoriously dual natured. Most strains coexist with their host without incident, while others are highly pathogenic. The difference in the ability of strains to cause disease resides in specific genes encoding virulence factors, the acquisition of which often depends on horizontal transfer. The core genome shared among all *E. coli* strains amounts to only ~40% of its average 5000 gene total, while the total number of genes that exist in all *E. coli* strains is predicted to exceed 15 000 (Rasko *et al.* 2008; Touchon *et al.* 2009). While the nomenclature of *E. coli* remains in a state of flux as our understanding evolves, a variety of pathotypes have been described (Kaper, Nataro and Mobley 2004; Croxen *et al.* 2013).

Escherichia coli can cause disease at many sites outside the gastrointestinal tract, including the urinary tract, meninges, biliary tract, peritoneum, lungs, and skin and soft tissue (Russo and Johnson 2003). Strains that cause these infections often share several known and suspected virulence factors, and thus have been grouped into a single pathotype called extraintestinal pathogenic *E. coli* (ExPEC) (Russo and Johnson 2000). The known and suspected virulence factors common among ExPEC, whether from urinary tract infections (UTIs) or other sites, include capsule, iron-uptake systems and a variety of pili, most notably P fimbriae (Russo and Johnson 2000). Serotyping, randomly amplified polymorphic DNA genomic profiling, multilocus sequence typing and pulsed field gel electrophoresis reveal

Received: 4 May 2015; **Accepted:** 21 September 2015

that closely related strains from the same lineage can cause UTIs and infections at other sites (Johnson and Russo 2002; Johnson *et al*. 2013), providing the rationale that ExPEC is an appropriate category.

Strains typically associated with diarrheal illness include enteropathogenic (EPEC), enterohemorrhagic (EHEC), enterotoxigenic (ETEC), enteroaggregative (EAEC) and enteroinvasive *E. coli* (EIEC) (Kaper, Nataro and Mobley 2004; Croxen *et al*. 2013). The clinical syndrome caused by infection varies among these organisms, but diarrhea is the predominant symptom. Very rarely, isolates from *E. coli* pathotypes typically associated with enteric infections have caused infections outside the gastrointestinal tract. For example, case reports of UTIs due to diffuse-adhering *E. coli*, Shiga-toxin-producing *E. coli* (STEC) and EAEC have been reported (Tarr *et al*. 1996; Germani *et al*. 1997; Olesen *et al*. 2012). Bacteremia secondary to such strains is even less common, with only a few such reports in the literature. Recently, Herzog *et al*. (2014) reported a case of an EAEC strain causing UTI and bacteremia in a renal transplant patient on immunosuppressive therapy. There has also been a report of EIEC bacteremia, in a patient with AIDS (Bessesen *et al*. 1991). Here we report the draft genome sequence of a strain that caused bacteremia in an adult with severe diarrhea.

A 55-year-old man with well-controlled HIV was admitted to the Veterans Affairs Maryland Health Care System in Baltimore, Maryland, with a 3-week history of progressive diarrhea and cramping abdominal pain. By the time of presentation, he was having 10–15 watery, non-bloody bowel movements per day. The pain was diffuse throughout the abdomen. He denied nausea, vomiting, subjective fever and chills.

His HIV had been treated for the past six years with atazanavir and lopinavir/ritonavir, with an undetectable viral load and recent CD4 count of 271 mm^{-3}. He had chronic hepatitis C infection. He was a current tobacco user and had a remote history of cocaine use.

His vital signs and oxygen saturation were normal. Orthostatic blood pressure and pulse were not recorded. He was cachectic and ill appearing with dry mucous membranes. His abdomen was soft, but tender in all quadrants. His peripheral white blood cell count was elevated (Table S1, Supporting Information) with 91% neutrophils and 2% band forms. He was anemic and had hyponatremia (120 mEq/L) and acute kidney injury. His liver function parameters were also abnormal with marked hyperbilirubinemia.

Computed tomography scan without intravenous contrast revealed non-specific gallbladder wall thickening and mild ascites. Magnetic resonance cholangiopancreatography showed no abnormality of the biliary tree. Later in his hospitalization, he also underwent liver biopsy, which revealed moderate to marked activity with early cirrhosis (modified Batts–Ludwig Classification: Grade 3–4, Stage 3–4) in addition to marked chronic cholestasis.

Fecal *Clostridium difficile* toxin gene PCR was negative. Fecal tests for leukocytes and ova and parasites were also negative. Stool culture revealed heavy growth of an oxidase-negative, non-lactose fermenting Gram-negative bacillus, but tests for *Salmonella, Shigella, Yersinia, Campylobacter, Aeromonas, Plesiomonas* and *Vibrio* species were negative and the isolate was discarded.

At presentation, he received multiple liters of intravenous fluid and his antiretroviral medication was held in consideration of his hepatic insult. Blood cultures drawn in the emergency department grew a non-lactose fermenting Gram-negative rod. A suspected diagnosis of *Salmonella* bacteremia was excluded when biochemical testing of the blood isolate indicated *E. coli* (API® 20E code 5144502 Biomerieux, Hazelwood, MO). Although the strain did not ferment lactose, it converted tryptophan to indole and displayed lysine decarboxylase activity.

Antibiotic therapy with piperacillin-tazobactam was initiated on admission. Based on sensitivities of the blood isolate, antibiotic coverage was narrowed to ciprofloxacin. The patient's diarrhea resolved, his metabolic parameters began to return to baseline and his antiretroviral medications were resumed. He was discharged 7 days after his presentation to finish a 2-week course of oral ciprofloxacin. On follow-up 1 month later, he felt well, his liver enzymes and total bilirubin were normal. His creatinine had improved, but remained elevated.

In view of the patient's bacteremia, we tested the ability of the strain isolated from blood (VACI-14) to invade epithelial cells using a standard gentamicin protection assay (Donnenberg, Donohue-Rolfe and Keusch 1989). As controls, we included a human commensal isolate and an EIEC isolate (Donnenberg, Donohue-Rolfe and Keusch 1989). We detected minimal invasive ability in the VACI-14 isolate compared to the EIEC strain (Fig. S1, Supporting Information).

To gain further insight into the apparent ability of the blood isolate to cause both severe diarrhea and bacteremia, we determined its draft genome sequence. Genomic DNA was extracted from the isolate using the Sigma GenElute genomic kit (Sigma-Aldrich) and sequenced using Illumina MiSeq sequencing of a paired-end library at the University of Maryland School of Medicine, Institute for Genome Sciences, Genome Resource Center with standard operating procedures (http://www.igs.umaryland.edu/resources/grc/). The paired-end Illumina reads were subsampled to an approximate genome coverage of 120-fold, and the subsampled reads were assembled using MaSuRCA v.1.9.2 (Zimin *et al*. 2013). The assembly statistics are as follows: total number of contigs, 109; number of bases in assembly, 5241 165; average contig length, 48 084; N50, 232 331 bp; 97.07% of the bases in assembly are included in contigs >10 kb. Overall, the draft assembly quality is excellent for these types of comparative analyses. The resulting assembly was compared to representative genomes from each of the pathotypes and phylogenetic lineages of *E. coli* and *Shigella* species. A total of 41 genomes were aligned with Mugsy (Angiuoli and Salzberg 2011). Homologous blocks present in each genome were concatenated with the bx-python toolkit (https://bitbucket.org/james˙taylor/bx-python); the total amount of conserved sequence in the core genome was ∼2.5 Mb. Columns that contained one or more gaps in the alignment were removed with Mothur (Schloss *et al*. 2009), and a maximum-likelihood tree was inferred with RAxML v.7.2.8 (Stamatakis 2006); bootstrap support values were calculated with 100 replicates. The resulting phylogeny was visualized with FigTree v.1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/) (Fig. 1). The genomic sequence of this isolate has been deposited in GenBank under accession number LASE00000000.

Analysis of seven standard genes indicates that this strain belongs to multilocus sequence type 12 (https://cge.cbs.dtu.dk/services/MLST/). Phylogenetic analysis based on conservation of core *E. coli* genome revealed that the strain belongs to a lineage that includes other members of the ExPEC pathotype (highlighted in red and indicated by an asterisk in Fig. 1). Indeed, features characteristic of ExPEC, including complete sets of genes for P fimbriae and hemolysin and the *kpsM* gene used to identify group II capsule loci (Johnson and O'Bryan 2004), were present (Table 1). The predicted serotype of the strain, as inferred *in silico* from the sequence

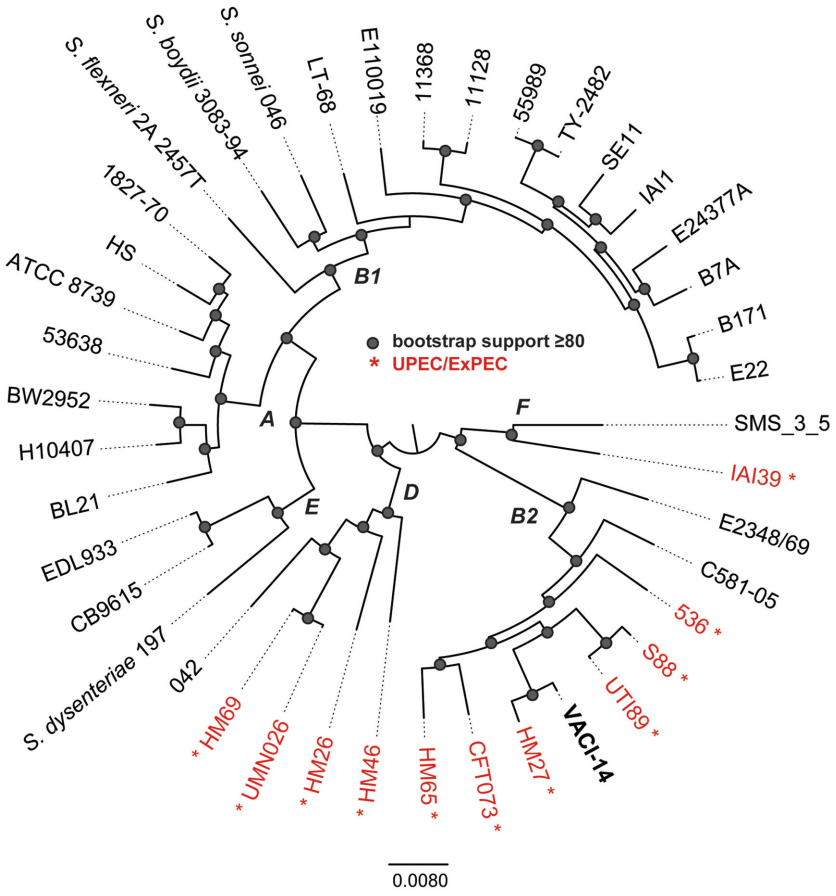

Figure 1. Phylogenomic analysis of 41 *E. coli* and *Shigella* genomes that represent members of each of the sequenced pathotypes and phylogenetic groups. All genomes were aligned using Mugsy (Angiuoli and Salzberg 2011) and homologous blocks were concatenated as previously described (Hazen *et al.* 2013). A phylogenetic tree was inferred with RAxML v7.2.8 (Stamatakis 2006) on this alignment, with 100 bootstrap replicates and nodes that have bootstrap support ≥80 are indicated by a shaded circle. Letters at nodes indicate the *E. coli* phylogroup as previously defined by MLST or phylogenomic analyses. Isolates indicated in red are from urinary tract sources of isolation.

(https://cge.cbs.dtu.dk/services/SerotypeFinder/), is O4:H1. Surprisingly, the results of this analysis also revealed that the isolate has features defining EPEC strains, including the presence of locus of enterocyte effacement (LEE) genes encoding the ability to produce the attaching and effacing (A/E) phenotype and the absence of *stx1* and *stx2* genes encoding Shiga toxins, which are found in addition to the LEE in EHEC strains (Kaper 1996). However, some essential LEE genes were not identified and the others displayed low degrees of sequence similarity, raising doubt as to whether the T3SS is fully functional in this isolate. Interestingly, genes distantly related to some *bfp* genes encoding the bundle-forming pilus (BFP) were also present. Additional common ExPEC and tEPEC virulence factors such as other iron acquisition systems and non-LEE-encoded effectors were not found in the VACI-14 strain.

A/E activity and localized adherence are defining phenotypes of typical EPEC strains (Kaper 1996). However, as the patient's isolate rapidly destroyed the tissue culture cells used in these assays (not shown), we were unable to test for either of these phenotypes. Strains that produce BFP also often exhibit autoaggregation when grown under conditions that induce BFP expression (Bieber *et al.* 1998). We observed such aggregates after incubation for three hours at 37°C in Dulbecco's Modified Eagle Medium, although they were smaller than those produced by the prototype EPEC strain, E2348/69 (Fig. S2, Supporting Information).

Here, we report the unique case of a patient with severe, protracted diarrhea and multiorgan dysfunction with bacteremia due to an ExPEC strain that also featured distant orthologs of genes characteristic of EPEC. We presume that the non-lactose-fermenting Gram-negative rod that dominated his stool culture was the same strain, although this cannot be confirmed. While chronic diarrhea is a well-described complication of EPEC infection (Rothbaum *et al.* 1982), the disease occurs almost exclusively in infants and bacteremia is exceptional. We attribute the unusual presentation of chronic diarrhea and bacteremia due to *E. coli* to a combination of host and bacterial factors.

The patient's immunocompromised status, as evidenced by his low CD4 count, cachexia and cirrhosis, likely played a role in his severe illness. $CD4^{-/-}$ mice infected with *Citrobacter rodentium*, which shares the A/E genes and phenotype with EPEC, suffer rapid mortality and splenic and hepatic abscesses, indicating systemic spread of the bacteria (Bry and Brenner 2004). In animal models, protein malnutrition can facilitate translocation of bacteria across the intestinal mucosa (Deitch *et al.* 1987). Cirrhosis, a well-recognized risk factor for numerous serious infections, contributes to poor pathogen clearance by a variety of mechanisms (Christou, Pappas and Falagas 2007).

We believe that the patient's acute kidney injury and hyponatremia were due to volume loss secondary to his severe and prolonged diarrhea. Similarly, it is possible that

Table 1. Potential virulence features identified in VACI-14.

Gene[1]	Associated pathotype	Protein	peptide Identity (%)[2]	BLAST e-value[3]	Reference Protein ID
hlyC	ExPEC	hemolysin-activating lysine-acyltransferase	100	2E-99	AAS93636.1
hlyA	ExPEC	Hemolysin, chromosomal	100	0	WP_001620144.1
hlyB	ExPEC	Alpha-hemolysin translocation ATP-binding protein	100	0	KGM64139.1
hlyD	ExPEC	Hemolysin secretion protein D, chromosomal	69	0	WP_000860472.1
kpsM	ExPEC	KpsM	97	3E-169	AAN82146.1
kpsT	ExPEC	KpsT	90	2E-124	AAN82145.1
papH	ExPEC	PAP fimbrial minor pilin protein	100	2E-108	KEN78663.1
papC	ExPEC	Outer membrane usher protein papC	99	0	CAM84417.1
papD	ExPEC	Chaperone protein papD	99	1E-176	WP_001758925.1
papK	ExPEC	Fimbrial adapter papK	87	1E-13	ENB84384.1
papK	ExPEC	Fimbrial adapter papK	100	9E-45	WP_029404503.1
papE	ExPEC	Fimbrial protein papE	100	5E-124	WP_001621802.1
papF	ExPEC	Minor pilin subunit PapF	100	5E-124	WP_001621802.1
papK	ExPEC	Fimbrial adapter papK	100	2E-41	WP_029404503.1
papK	ExPEC	Fimbrial adapter papK	87	1E-13	ENB84384.1
papD	ExPEC	Chaperone protein papD	100	5E-178	WP_000265729.1
papC	ExPEC	Outer membrane usher protein papC	100	0	EOV01938.1
papH	ExPEC	PAP fimbrial minor pilin protein	98	1E-138	KEN81350.1
prsA	ExPEC	PRS fimbrial major pilin protein	100	4E-121	EFJ72838.1
–	ExPEC	TonB-dependent siderophore receptor	99	0	WP_021542660.1
flu	ExPEC	Antigen-43	100	0	WP_021517784.1
flu	ExPEC	Antigen-43	100	0	EQN15149.1
–	ExPEC	Yersiniabactin biosynthetic protein	99	0	WP_021521384.1
tia	ETEC	Tia adhesin	99	8E-179	EFJ81905.1
sen	EIEC	shET2 enterotoxin, N-terminal region family protein	99	2E-112	CDL47274.1
espF	EPEC, EHEC	LEE-encoded effector EspF	46	>1	YP_002331392.1
–	EPEC, EHEC	Component of T3SS	27	>1	YP_002331393.1
escF	EPEC, EHEC	T3SS structure protein EscF	34	0.7	YP_002331394.1
	EPEC, EHEC		35	0.21	
espB	EPEC, EHEC	Translocon EspB	NSM	NSM	YP_002331396.1
espD	EPEC, EHEC	Translocon EspD	35	0.15	YP_002331397.1
espA	EPEC, EHEC	Translocon EspA	NSM	NSM	YP_002331398.1
speL	EPEC, EHEC	Secretion switching protein SpeL	NSM	NSM	YP_002331399.1
escD	EPEC, EHEC	T3SS structure protein EscD	31	>1	YP_002331400.1
eae	EPEC, EHEC	Intimin	41	7E-96	YP_002331401.1
cesT	EPEC, EHEC	Chaperone CesT	29	0.25	YP_002331402.1
tir	EPEC, EHEC	Translocated intimin receptor Tir	36	>1	YP_002331403.1
map	EPEC, EHEC	LEE-encoded effector Map	26	0.16	YP_002331404.1
cesF	EPEC, EHEC	Chaperone CesF	29	>1	YP_002331405.1
espH	EPEC, EHEC	LEE-encoded effector EspH	29	0.51	YP_002331406.1
sepQ	EPEC, EHEC	T3SS structure protein SepQ	35	2.2	YP_002331407.1
–	EPEC, EHEC	Hypothetical protein	28	0.14	YP_002331408.1
–	EPEC, EHEC	Hypothetical protein	28	>1	YP_002331409.1
escN	EPEC, EHEC	Translocator EscN	40	1E-79	YP_002331410.1
escV	EPEC, EHEC	Translocator EscV	33	5E-84	YP_002331411.1
mpc	EPEC, EHEC	Regulator Mpc	32	>1	YP_002331412.1
espZ	EPEC, EHEC	LEE-encoded effector EspZ	32	0.68	YP_002331413.1
rorf8	EPEC, EHEC	Chaperone of T3SS Rorf8	24	>1	YP_002331414.1
escJ	EPEC, EHEC	T3SS structure protein EscJ	26	0.38	YP_002331415.1
sepD	EPEC, EHEC	Secretion switching protein SepD	29	>1	YP_002331416.1
escC	EPEC, EHEC	T3SS structure protein EscC	29	4E-20	YP_002331417.1
cesD	EPEC, EHEC	Chaperone CesD	44	0.007	YP_002331418.1
grlA	EPEC, EHEC	Positive regulator GrlA	29	0.008	YP_002331419.1
grlR	EPEC, EHEC	Negative regulator GrlR	56	>1	YP_002331420.1
–	EPEC, EHEC	Hypothetical protein	39	6E-28	YP_002331421.1
escU	EPEC, EHEC	Secretion system apparatus protein SsaU	26	3E-30	YP_002331422.1
escT	EPEC, EHEC	T3SS structure protein EscT	31	0.69	YP_002331423.1
escS	EPEC, EHEC	T3SS structure protein EscS	38	0.006	YP_002331424.1
escR	EPEC, EHEC	Type III secretion system protein	40	3E-35	YP_002331425.1
–	EPEC, EHEC	Component of T3SS	39	>1	YP_002331426.1
–	EPEC, EHEC	Component of T3SS	45	>1	YP_002331427.1
–	EPEC, EHEC	Component of T3SS	50	>1	YP_002331428.1

Table 1. (*Continued*)

Gene[1]	Associated pathotype	Protein	peptide Identity (%)[2]	BLAST e-value[3]	Reference Protein ID
–	EPEC, EHEC	Component of T3SS	35	0.19	YP_002331429.1
ler	EPEC, EHEC	Transcriptional regulator Ler	44	0.004	YP_002331430.1
–	EPEC, EHEC	Hypothetical protein	39	>1	YP_002331431.1
espG	EPEC, EHEC	LEE-encoded effector EspG	NSM	NSM	YP_002331432.1
bfpA	EPEC	Bundlin	NSM	NSM	NSM
bfpG	EPEC	BfpG	NSM	NSM	NSM
bfpB	EPEC	BfpB	26	2E-34	NP_053067.1
bfpC	EPEC	BfpC	NSM	NSM	NSM
bfpU	EPEC	BfpU	NSM	NSM	NSM
bfpD	EPEC	BfpD	31	3E-49	NP_053070.1
bfpE	EPEC	BfpE	30	3E-31	NP_053071.1
bfpF	EPEC	BfpF	32	7E-21	NP_053072.1
bfpP	EPEC	Prepilin peptidase	28	1E-19	NP_053073.1
bfpH	EPEC	BfpH	28	6E-07	NP_053074.1
bfpI	EPEC	BfpI	NSM	NSM	NSM
bfpJ	EPEC	BfpJ	NSM	NSM	NSM
bfpK	EPEC	BfpK	NSM	NSM	NSM
bfpL	EPEC	BfpL	NSM	NSM	NSM
perA	EPEC	PerA	37	3E-18	NP_053086.1
perB	EPEC	PerB	NSM	NSM	NSM
perC	EPEC	PerC	NSM	NSM	NSM

[1]"–" indicates that there is no meaningful gene name or that the gene has more than one name.
[2]NSM – no significant match.
[3]The BLAST analysis is based on the TBLASTN algorithm.

his liver derangements were also secondary to prolonged hypovolemia, exacerbated by chronic hepatitis C-associated liver disease and cirrhosis. However, cholestasis associated with bacteremia may have contributed to the clinical presentation.

The genomic sequence of this patient's isolate reveals features characteristic of both ExPEC and EPEC. The core genome of this isolate is most similar to other ExPEC isolates associated with urinary tract and other systemic infections. Indeed, this isolate had many features associated with ExPEC including capsule and iron-acquisition systems likely to have facilitated its survival in the blood. How it crossed from the intestinal lumen into the bloodstream is unclear. The fact that we did not observe evidence of cellular invasion is difficult to interpret, as we later observed that it caused extensive damage to tissue culture cells. This rapid cell destruction may be due to *E. coli* hemolysin, which could facilitate breach of the epithelial barrier (Trifillis *et al.* 1994). In contrast to the core genome sequence, the accessory genome of the patient's isolate includes highly divergent versions of genes found in EPEC and additional genes found in other diarrheagenic *E. coli*. These findings highlight the dynamic nature of these pathogens.

As noted above, hybrid strains of *E. coli* have been reported previously. Most notably, a 2011 outbreak in Germany due to a hybrid STEC-EAEC sickened 3816 people with 54 deaths (Frank *et al.* 2011). Genomic comparison with previously cultured isolates indicated that the outbreak was caused by a hybrid EAEC strain that developed increased potential for systemic illness following acquisition of the bacteriophage encoding Shiga toxin 2 (Rasko *et al.* 2011).

Previously, a patient with severe diarrhea and bacteremia due to what would now be called an atypical (*bfp*-) EPEC strain was described (Bratoeva *et al.* 1994). Although it produced uncharacterized pili, the presence of *pap* genes encoding P-fimbriae or other features characteristic of ExPEC was not reported. Genes characteristic of intestinal pathogenic *E. coli* have previously been noted among strains isolated from patients with UTIs (Toval *et al.* 2014). As revealed by its phylogenetic history, the strain described in the current report may have evolved from an ExPEC strain already capable of causing systemic infection, by the acquisition of mobile genetic elements distantly related to those encoding BFP and A/E ability, as has been described more generally in EPEC (Hazen *et al.* 2013). Indeed, EPEC strains closely related to ExPEC have been described previously (Hazen *et al.* 2013). However, characteristic ExPEC virulence genes are not present in those strains (data not shown). While bacteremia due to a hybrid ExPEC-EPEC strain appears to be rare, the total burden of such complex *E. coli* strains on human health remains to be determined.

SUPPLEMENTARY DATA

Supplementary data are available at FEMSPD online.

FUNDING

This work was supported by Public Health Service grants U19 AI090873 and U19 AI110820 from the National Institute of Allergy and Infectious Diseases.

ACKNOWLEDGEMENTS

The authors are grateful to Ms. Marianne Norfolk, MT (ASCP) for expert assistance in diagnostic microbiology.

Conflict of interest. None declared.

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Diarrhea, bacteremia and multiorgan dysfunction due to an extraintestinal pathogenic Escherichia coli strain with enteropathogenic E. coli genes

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2015-09-26