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CASE REPORT

Diarrheagenic enteroaggregative *Escherichia coli* causing urinary tract infection and bacteremia leading to sepsis

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Abstract We report a case of a 55-year-old immunocompromised female who presented to the emergency department with severe diarrhea and vomiting following travel to the Philippines. Stool bacteriology revealed a mixed infection involving an enteropathogenic *Escherichia coli* and two distinct strains of enteroaggregative *Escherichia coli* (EAEC). During hospitalization, urine and blood culture tested positive for one of the diarrheagenic EAEC strains, necessitating urinary catheterization, intensive care, and antimicrobial treatment with trimethoprim–sulfamethoxazole, followed by meropenem. Although known to occasionally cause urinary tract infections, EAEC have not been previously associated with sepsis. Our report highlights the potential of EAEC to cause severe extraintestinal infections.

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Introduction

Enteroaggregative *Escherichia coli* (EAEC) is recognized as an etiological agent of acute and persistent diarrhea in people in developing countries and in travelers visiting less developed areas of the world, as well as in immunocompromised individuals [1, 2]. In addition, the pathogenic potential of EAEC has been demonstrated by the emergence of food-borne outbreaks, most notably in Germany in 2011 [3]. Recently, EAEC has been associated with an outbreak of urinary tract infections [4], emphasizing the need to increase understanding of the extraintestinal properties of EAEC. Here, we describe the case of a diarrheal EAEC associated with urinary tract infection and bacteremia leading to sepsis, and characterize this isolate by its adherence pattern to HEp-2 cells, pulsed-field gel electrophoresis (PFGE), virulence and virulence-associated gene analysis, serotyping, multilocus sequence typing (MLST), and antibiotic susceptibility testing.

Case presentation

A 55-year-old woman was admitted to the emergency unit of her local general hospital because of persistent diarrhea and vomiting. The patient, immunosuppressed [Tacrolimus (Prograf®) 0.5 mg 2×/day, Mycophenolate Mofetil (CellCept®) 500 mg 2×/day] since a renal transplantation 7 years previously, was well until 15 days prior to admission, when gastroenteritic symptoms started 5 days after

she returned from Manila. She was hemodynamically stable, but hypotonic, dehydrated, and anuric as of 3 days before admission. Urinary catheterization was initiated upon admission. Her blood pressure was 86/44 mmHg, her pulse was 66/min, and her white blood cell count (WBC) was 11.6 × 10^9/l. Moreover, her serum creatinine was 1,212 µmol/l (13.7 mg/dl), serum urea was 35.6 mmol/l (99.7 mg/dl), glomerular filtration rate (GFR) was 3.4 ml/min, and phosphorus was 3.6 mmol/l (11.1 mg/dl), so continuous venovenoushemodialysis and filtration (CVVHDF) was started. No blood culture but a urine culture was drawn upon admission, yielding 10^4 colony-forming units (CFU)/ml by three different contaminating bacteria. Despite the anuric kidney failure, a low serum potassium value of 2.7 mmol/l (10.5 mg/dl) was observed, consistent with the severe and long-standing gastroenteritis. Substitution of liquids and electrolytes was initiated, and dialysis was stopped on day 1, when renal function resumed. Standard bacteriology [5] of three stool samples performed between day 4 and day 5 yielded negative results concerning the *Salmonella*, *Shigella, Campylobacter*, *Mycobacterium* sp., as well as for theenzyme-linked immunosorbent assay (Novitec, HiSS Diagnostics GmbH, Freiburg, Germany) used for detecting *Clostridium difficile* toxins A/B. Parasitological analysis revealed *Blastocystis hominis*, a facultative pathogen. Gastroscopy and colonoscopy on day 4 showed erosive gastritis and unspecific inflammation of the colon. Biopsies revealed eosinophilic ileitis and colitis, but no pathogen. Consequently, antiparasitic therapy with metronidazole was administered on day 5. On day 6, a further stool sample was analyzed by multiplex polymerase chain reaction (PCR) for diarrheagenic *E. coli* (DEC), including enteropathogenic (EPEC), enteroaggregative (EAEC), Shiga toxin-producing (STEC), enteroinvasive (EIEC), and enterotoxic *E. coli* (ETEC), using the commercial *E. coli* DEC Primer Mix (Statens Serum Institut, Hillerød,Denmark). In addition, single PCRs aimed at either the EAEC virulence plasmid pCVD432 [6] or the conserved usher gene *agg-3C* [7] were performed. That last sample tested positive for EPEC and two distinct strains of EAEC, EAEC-30-I and EAEC-30-II, respectively (Fig. 1a, lanes "stool day 6"). Both EAEC isolates were additionally characterized by the pattern of adherence to HEp-2 as described by Karch et al. [8], and they showed the "stacked-brick" characteristic of EAEC (data not shown).

PFGE [9] confirmed that they represented two distinct isolates (Fig. 1b, lanes "stool day 6"). Hence, in total, the patient was infected by three distinct DECs, one EPEC, and two EAECs.

Respiratory problems occurred on day 11, and pneumocystis pneumonia was suspected based on radiological examination. Therapy with trimethoprim–sulfamethoxazole

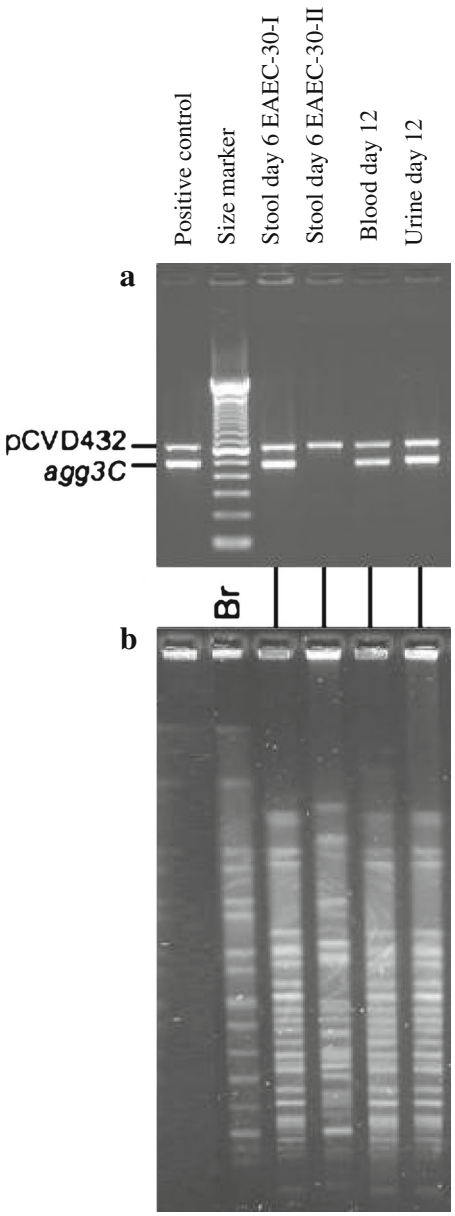

Fig. 1 Results from polymerase chain reactions (PCRs) aimed at enteroaggregative *Escherichia coli* (EAEC) marker genes (**a**) and from pulsed-field gel electrophoresis (PFGE) (**b**) performed using DNA from purified strains from indicated patient samples. PCRs for pCVD432 and for *agg3C* were performed as single reactions, and the two amplicons were mixed for each strain, including controls, and electrophoresed as a pool. Lane designations are the same for **a** and **b**, except for the size markers. *Br Salmonella* serovar Braenderup standard

was started. On day 12, the patient developed progressive azotemia and a status judged as clearly septic considering full immunosuppression—as indicated by hypotension (79/40 mmHg), hypothermia (35.4 °C), tachypnea (25/min), leukocytosis (WBC 33 × 10^9/l), and a C-reactive protein (CRP) of 91 mg/l—and was transferred to the intensive care unit (ICU), where she needed catecholamines for hemodynamic support. CVVHDF was reinstalled. On day 12, blood

Table 1 Characteristics of the enteroaggregative *Escherichia coli* (EAEC) and enteropathogenic *Escherichia coli* (EPEC) isolates from the urinary tract infection (UTI)/sepsis patient

Strain	Origin	Serotype		MLST	Virulence or adherence factors					STEC/EPEC adherence factors			
					stx1/2	*nle*B	ETEC-LT	ETEC-ST	*bfpA*	*eae*	EAF	*iha*	*saa*
EAEC-30 I	Stool, urine, blood	O176	NT	3067									
EAEC-30 II	Stool	O12	H4	484									
EPEC-1327	Stool	O56	H6	3071						+			

Strain	pAA plasmid											Chromosome					Resistance profile
				Adhesins				Toxins									
	pCVD 432	*agg*R	*aap*	*agg*3C	*agg*4A	*agg*A	*aaf*A	*ast*A	*sat*	*sep*A	*pet*	*pic*	*sig*A	*aai*C	*air*	*eil*A	
EAEC-30 I	+	+	+	+				+				+		+	+		AM, SMZ, TMP
EAEC-30 II	+	+	+		+					+				+		+	AM, S, SMZ, TMP
EPEC-1327								+								+	SMZ intermediate

Antibiotic disks used for susceptibility testing: *AM* ampicillin, *AMC* amoxicillin–clavulanic acid, *CF* cephalothin, *CTX* cefotaxime, *TZ* ceftazidime, *CIP* ciprofloxacin, *NA* nalidixic acid, *GM* gentamicin, *K* kanamycin, *S* streptomycin, *TE* tetracycline, *C* chloramphenicol, *SMZ* sulfamethoxazole, *TMP* trimethoprim

NT non-typable

and urine samples tested positive for *E. coli* using BacT-Alert and Vitek 2 (bioMérieux, Marcy, L'Etoile, France). Subcultures tested positive by PCR for EAEC marker genes pCVD432 and *agg*3C (Fig. 1a, lanes "urine/blood day 12") and PFGE analysis demonstrated that the isolates from urine and blood were indistinguishable from the diarrheagenic isolate EAEC-30-I (Fig. 1b). This strongly suggested that isolate EAEC-30-I had colonized the urinary tract and entered the bloodstream, causing urinary tract infection and bacteremia leading to sepsis. Meropenem therapy was, thus, initiated, while trimethoprim–sulfamethoxazole was discontinued. Treatment with metronidazole was stopped on day 20 and meropenem on day 25. The patient was released on day 26 in a cachectic and weak condition. However, renal function had resumed on day 14 and diarrhea had stopped on day 15.

The EAEC stool isolates as well as the EPEC isolate were characterized by the serotyping of O and H antigens, performed by using standard methods at the National Reference Laboratory for *Escherichia coli*, Federal Institute for Risk Assessment, Berlin, Germany.

MLST was performed as described by Wirth et al. [10]. Alleles and sequence types (STs) were assigned in accordance with the *E. coli* MLST website (http://mlst.ucc.ie/).

Furthermore, the isolates were investigated for the presence of 26 different virulence factors or virulence-associated genes harbored by *E. coli* as described in previous studies [11, 12] and the results are summarized in Table 1.

All three isolates were subjected to antibiotic susceptibility testing by the disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) protocols [13]. The antibiotics (Becton–Dickinson, Sparks, MD, USA) tested are listed in the footnote to Table 1.

The EPEC isolate belonged to serotype O56:H6 and was assigned to the allelic profile ST3071. Its virulence profile was *eae*, *ast*A, and *eil*A. Antibiotic susceptibility profiling revealed a reduced susceptibility to sulfamethoxazole. The isolate EAEC-30-I belonged to serotype O176:H non-typable, was assigned to the allelic profile ST3067, and had a virulence profile of pCVD432, *agg*R, *aap*, *agg*3C, *ast*A, *pic*, *aai*C, and *air*. It was resistant to ampicillin, sulfamethoxazole, and trimethoprim. Isolate EAEC-30-II was typed O12:H4, ST484, and carried the virulence genes pCVD432, *agg*R, *aap*, *agg*4A, *sep*A, *aai*C, and *eil*A. Its antibiotic resistance profile included ampicillin, streptomycin, sulfamethoxazole, and trimethoprim.

Discussion

EAEC is an important etiologic agent of diarrhea and increasingly recognized as a global emerging pathogen and as a potential threat to public health. However, there are few studies that explore the role of EAEC in extraintestinal infections. Recently, EAEC has been confirmed as a pathogenic agent of urinary tract infections in an outbreak in Denmark [4]. In a follow up study, the authors reported that the involved EAEC outbreak strain showed enhanced adherence to the uroepithelium [14]. Notably, the virulence gene *pic*, which is one of the marker genes distinguishing EAEC-30-I from the diarrheal EAEC-30-II isolate analyzed in this study, has been described in 95 % of the strains of this outbreak, as well as in various uropathogenic *E. coli* (UPEC) strains [15]. *pic* has been reported to be a stimulant of host intestinal mucus secretion as well as to possess mucinase activity, and is thought to be involved in

the process of urinary tract infection in UPEC [16]. Conceivably, *pic* may be the reason for the dissemination of EAEC-30-I from the intestine to the urinary tract in our patient. Further investigation is needed to establish the possible role of *pic* as well as other EAEC-specific virulence factors in the uropathogenity of EAEC. Furthermore, the role of EAEC in urinary tract infections, especially in catheterized patients and patients with EAEC-associated diarrhea, warrants increased awareness by clinicians.

In conclusion, this case describes the dissemination of an EAEC isolate, originating from a mixed intestinal infection, to the urinary tract and bloodstream of an immunocompromised, catheterized patient. Finally, we suggest that certain subtypes of EAEC are both diarrheagenic and extraintestinal pathogens, and that their extraintestinal virulence potential includes urinary tract infection as well as bacteremia and sepsis.

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Conflict of interest None declared.

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