Life-threatening *Escherichia coli* cellulitis in patients with haematological malignancies

S. Sunder,1,2 E. Haguenoer,2,3 D. Bouvet,2,3 S. Lissandre,4 A. Bree,5 D. Perrotin,2,6 E. Helloin,5 P. Lanotte,2,3 C. Schouler5 and A. Guillon2,6

1Service de Médecine Interne et Maladies Infectieuses, CHRU de Tours, France
2Université François Rabelais de Tours, France
3Service de Bactériologie–Virologie, CHRU de Tours, France
4Service d’Hématologie et Thérapie Cellulaire, CHRU de Tours, France
5INRA, UR 1282 Infectiologie Animale et Santé Publique, Nouzilly, France
6Service de Réanimation Médicale, CHRU de Tours, France

Cellulitis due to *Escherichia coli* is rare and usually secondary to a cutaneous portal of entry. Skin and soft tissue infections (SSTI) secondary to *E. coli* bacteraemia have been reported exclusively in immunodeficient patients. Here, we report two cases of serious cellulitis secondary to *E. coli* bacteraemia in patients with haematological malignancies. Both isolated strains belonged to phylogenetic group B2 and harboured some of the main virulence factor genes commonly found in extra-intestinal pathogenic *E. coli* (ExPEC), including *neuC*, *iro* and *fimH*. Cellulitis due to *E. coli* seems to be linked to the immunocompromised status of patients rather than to a highly virulent clone. Nevertheless, some of the virulence factors appear to be important because both isolates belong to phylogenetic group B2. This aetiology should be considered in SSTI in patients with haematological malignancies.

**Introduction**

Cellulitis is an acute spreading infection of the skin, extending more deeply than erysipelas to reach subcutaneous tissues. Although most cases of cellulitis are caused by group A streptococci, a number of other micro-organisms may be responsible for this disease, including other β-haemolytic streptococci, *Staphylococcus aureus*, *Haemophilus influenzae* in children, *Capnocytophaga canimorsus*, following a dog or cat bite, and *Pseudomonas aeruginosa* (Stevens et al., 2005). Cellulitis due to *Escherichia coli* is rare and less documented. Uropathogenic *E. coli* and other strains that cause extra-intestinal infections are grouped under the term extra-intestinal pathogenic *E. coli* (ExPEC) (Russo & Johnson, 2000). Recently, a Slovenian team (Petkovsek et al., 2009) studied the virulence factor profile of *E. coli* isolated from skin and soft tissue infections (SSTI). They found that these strains exhibited a remarkable virulence potential, comparable to that of *E. coli* strains isolated from urinary tract infections and cases of bacteraemia. However, in all cases, the portal of entry was cutaneous (surgical wounds, foot ulcers, fistulae, traumatic wounds, etc.). No reported cases of cellulitis were secondary to an *E. coli* bloodstream infection.

Here, we report two cases of cellulitis associated with bacteraemia without pyomyositis caused by *E. coli* in patients with haematological malignancies (multiple myeloma and chronic lymphoid leukaemia). In both cases, the phenotypic and molecular characteristics of the isolates were determined.

**Case reports**

**Case one**

A 71-year-old man was admitted to intensive care unit (ICU) in January 2010 for septic shock. He had a medical history of multiple myeloma from 2008, which was treated with dexamethasone (40 mg day⁻¹ twice a week for 2 months) for cytopenia, with a recent asymptomatic recurrence. The patient reported 2 days of progressive fever with pain, swelling and erythema of the upper right limb. He was initially admitted in a secondary health care centre and treated with a fluid loading of 3500 ml, continuous injection of norepinephrine, 2 g of ceftriaxone, 280 mg of gentamicin and 200 mg of ketoprofen. On admission to the ICU, the upper right limb was erythematous, bullous and tender; there was no superficial wound and no palpable axillary node. Vital signs were: Glasgow coma scale 10, blood pressure 76/28 mmHg with anuria, pulse 96 beats min⁻¹ and a temperature...
of 37.7 °C. Laboratory investigations revealed a white cell count of 1.1 × 10^9 l^{-1} with neutropenia (0.7 × 10^9 l^{-1}), platelets 18 × 10^9 l^{-1}, haemoglobin 5.8 g dl^{-1}, serum creatinine 198 μmol l^{-1}, procalcitonin 8.21 ng ml^{-1} and prothrombin time was prolonged. Blood gas showed metabolic acidosis with lactate 5.4 mmol l^{-1}. Antimicrobial therapy was modified for imipenem (1 g every 8 h) and gentamicin. Axial CT image of the right limb showed diffuse superficial soft-tissue swelling; there was no evidence of local fluid collection or abscess formation in the deep musculature, nor subcutaneous air or bone destruction. A surgical exploration of the upper right limb showed no extension of infection to the fascia. Cellulitis without primary superficial skin lesion was diagnosed. The patient died on the same day. *E. coli* was isolated from a skin biopsy culture without any other aerobic or anaerobic bacteria. Moreover, only one blood culture was positive for *E. coli*. Both isolates were susceptible to β-lactams (aminopenicillins, carboxypenicillins, cephalosporins and penems), aminoglycosides and quinolones. The isolates were only resistant to co-trimoxazole. The molecular analysis was only performed on the isolate from skin biopsy. Unfortunately, the isolate from blood was not preserved because the positive blood culture was performed in another hospital before the admission of the patient in the ICU.

**Case 2**

A 53-year-old man was hospitalized in November 2009 following 3 days of fever with cutaneous eruption, ten days after receiving chemotherapy (rituximab + dexamethasone + endoxan) for lymphoid leukaemia (stage C). The patient received co-trimoxazole as a prophylaxis (400/80 mg daily). On admission, his temperature was 39.2 °C, blood pressure 86/58 mmHg and pulse 108 beats min^{-1}. He had three erythematous skin lesions: on his left shoulder, left thigh and left ankle. There was no arthritis (the joints were painless, non-inflammatory and motilities were preserved). There was no intravascular device. Laboratory investigations found a white blood cell count of 1.1 × 10^9 l^{-1} with neutropenia of 0.04 × 10^9 l^{-1}, platelets 58 × 10^9 l^{-1}, haemoglobin 8.5 g dl^{-1}, serum creatinine 86 μmol l^{-1}, and C-reactive protein 275 mg l^{-1}. Blood pressure was rapidly normalized after loading of 1000 ml of physiological serum. He was treated intravenously with ceftiraxone (1 g daily) and amikacin (1200 mg) in the emergency department and was transferred to the haematology department. Twenty-four hours after admission, vancomycin (2 g daily) was introduced and an injection of granulocyte colony-stimulating factor (G-CSF) was given. *E. coli* was isolated from two different blood cultures with a time-to-positivity under 16 h. Among the β-lactams tested, the strain was resistant to aminopenicillins and carboxypenicillins and susceptible to cephalosporins and penems. It was also susceptible to aminoglycosides and quinolones and was resistant to co-trimoxazole. Urine was sterile. Cutaneous echography revealed no abscess. A skin biopsy was performed. Aerobic and anaerobic cultures remained sterile after 5 days of incubation. Histology confirmed the diagnosis of cellulitis without evidence of bacteria or mycelium. Colonoscopy did not reveal any digestive injury. Due to the positivity of the blood culture, antimicrobial therapy was changed to a combination of ciprofloxacin and amoxicillin–clavulanic acid. The outcome was favourable with regression of apleasia.

**Microbiological study**

Blood cultures in both aerobic and anaerobic conditions were performed with the BACTEC blood culture system (Becton Dickinson). Cultures of skin biopsies were performed in Schaedler broth with vitamin K3 (Oxoid) and on various agar plates (trypticase soy agar supplemented with 5% horse blood, 5% blood Columbia agar and chocolate agar plus PolyViteX (Oxoid)), incubated in different atmospheres (aerobic, anaerobic and atmosphere with 5% CO₂, respectively) for 5 days. Antimicrobial susceptibility was determined by the agar diffusion method performed on Mueller–Hinton blood culture isolates or by the Vitek system (bioMérieux) for skin biopsy samples. Susceptibility test results were interpreted according to the French guidelines of the Antibiogram Committee of the French Society of Microbiology (CA-SFM, 2010).

Following the consent of the patients or their family, the two strains of *E. coli* were deposited at the Biological Resource Centre of INRA (Institut National de la Recherche Agronomique, France) under accession numbers CIRM-BP-494 (skin biopsy isolate from patient 1) and CIRM-BP-495 (blood isolate from patient 2). The following analyses were performed for each isolate: determination of the phylogenetic group, using a triplex PCR (Clermont et al., 2000); determination of serotype by conventional serotyping; and determination of the genotype by multilocus sequence typing (MLST) (Wirth et al., 2006).

PCRs were performed to determine the presence of genes encoding virulence factors representative of the main classes of identified ExPEC virulence determinants, including adhesins (*papC, sfa/foc, afa, eae, fimH* and its variant *fimAv*), toxins (*hlyA, cdt1, cdt2 and ccrf1*), iron capture systems (*iutA, iroN, iroB and iucD*), invasin (*ibeA*) and protectin (*neuC*), as well as a gene encoding an autotransporter (*tsh*) (Johnson et al., 2001; Lefort et al., 2011).

Both isolates belonged to phylogenetic group B2. The results of tests for virulence gene carriage are presented in Table 1. Both isolates were positive for some classical ExPEC-associated virulence genes. Both isolates possessed genes coding for fimbrines (*P. fimbriae* and/or type 1 fimbriae), iron capture systems (*Iro system and/or aerobactin*) and the capsular antigen K1. Only the isolate from patient 2 possessed the *ibeA* gene. Serotypes of isolates were O1 (patient 1) and O18 (patient 2). MLST showed two different isolates, exhibiting two different combinations of alleles among the seven sequenced loci, with no alleles in common. These patterns correspond to sequence type (ST) ST357 for patient 1 and to ST95 for patient 2.
**Table 1. Characteristics of studied Escherichia coli**

<table>
<thead>
<tr>
<th>Patient (source)</th>
<th>Phages</th>
<th>ST*</th>
<th>Phylogenetic group</th>
<th>O type</th>
<th>Fimbriae/Adhesin</th>
<th>Iron capture system</th>
<th>Invasin or proteob</th>
<th>Autoantibody</th>
<th>Toxins</th>
<th>Autotransporter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin biopsy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- **Characteristics**: papC, afimbrial adhesion; sfa/foc, invasin or proteob; fimH, type 1 fimbriae; fimAv, type 1 fimbriae avian pathogenic variant; iutA, receptor of the iroB ferric aerobactin/iroN iucD siderophore systems; ihaA, haemolysin; cnf1, cytotoxic necrotizing factor 1; cdt1/2, cytolethal distending toxin; tsh, autotransporter.

**Discussion**

Here, we report two cases of serious cellulitis caused by *E. coli* in patients with haematological malignancies. SSTIs caused by Gram-negative bacteria, notably *E. coli*, are not common and present as primary skin infections with a cutaneous portal of entry (Moet *et al.*, 2007). Neither of our patients had any cutaneous lesions. We supposed that the skin infection was secondary to bloodstream infection from a digestive source (translocation). Very few cases of this have been described in the literature and the few reported cases all occurred in immunocompromised patients (Brzozowski & Ross, 1997; Kang *et al.*, 2010; Sleiman *et al.*, 2007; Yoon *et al.*, 1998).

Recently, *E. coli* seems to have emerged as a serious problem among patients with haematological malignancies. In a series of six cases of *E. coli* pyomyositis, three patients required transfer to intensive care and two patients died. Molecular analysis revealed that five of the six isolates originated from the same *E. coli* lineage (ST131, phylogenetic group B2) (Vigil *et al.*, 2010). The ST of the two clinical cases reported here were different (ST357 and ST95). Clinical presentation was locally less severe: cellulitis without pyomyositis, one with a single location and the other with multiple locations. Nevertheless, the prognosis seems to be poor, with the death of one of the two patients. Non-neutropenic patients may also be affected; in fact, patient 1 was only treated with corticotherapy. Consequently, among patients with haematological malignancies, *E. coli* SSTI secondary to blood diffusion appears as a polymorphous syndrome with poor prognosis. While immunosuppression is likely to play a role in disease outcome, bacterial virulence may also have an impact on the pathogenesis.

Both *E. coli* strains belonged to phylogenetic group B2, which was the main group found in a prospective study aimed at characterizing the risk factors for *E. coli* bacteraemia (Lefort *et al.*, 2011). *E. coli* of the B2 group usually carry more of virulence-associated genes than those of other phylogenetic groups (Johnson *et al.*, 2002). The two isolates were of serogroups O1:K1 (patient 1) and O18:K1 (patient 2). Isolates belonging to these serogroups are frequently identified in urinary tract infections, bacteraemia and neonatal meningitis (Ananias & Yano, 2008; Blanco *et al.*, 1996; Croxen & Finlay, 2010). MLST showed that neither strain had any lineage. Up to now, only 12 strains have been recorded as ST357 in the MLST database at the Environmental Research Institute of University College Cork (http://mlst.ucc.ie/mlst/dbs/Ecoli/GetTableInfo_html). All are of human origin, except one, which is of avian origin. Half of them are known to be pathogenic and were isolated from urinary tract infections or bacteraemia. Eleven of these ST357 strains belonged to serological group K1. ST95 has been more frequently observed, with 144 recorded strains, of which 75% were of human origin. Fifty-six per cent of the human pathogens from this ST are known to be pathogenic; 52% of them were isolated from urinary infections, 29% from meningitis cases (mainly neonatal meningitis) and 16.5% from bacteraemia. ST95 complex has been reported...
to contain most of the related bacteria of serogroup O1, O2, and O18 which express the K1 polysaccharide (Mora et al., 2009; Wirth et al., 2006).

Molecular analysis revealed that both strains harboured virulence-factor genes commonly found in ExPEC. Both isolates possessed neuC. E. coli isolated from patient 1 possessed the ibeA gene, encoding IbeA invasive, which is involved in crossing the blood–brain barrier. Because of the limited number of strains studied, we are not able to determine whether these virulence-factor genes play a direct role in the pathogenesis of E. coli SSTI.

In summary, cellulitis due to E. coli seems to be attributed to the immunocompromised status of patients, induced by haematological malignancy and worsened by the immunosuppressive treatments, rather than to a highly virulent strain, although the role of some virulence factors remains to be determined.

In contrast to the E. coli pyomyositis cases reported by Vigil et al. (2010), the isolates in our cases did not belong to the virulent and multidrug-resistant E. coli lineage ST131, which has been identified as an emerging cause of fluoroquinolone-resistant and ESBL-positive extra-intestinal E. coli infection worldwide. Thus, the emergence of E. coli SSTIs cannot be explained by the dissemination of this clone alone, and other E. coli lineages may be involved.

Because of their potential for morbidity, Enterobacteriaceae, notably E. coli, should be considered in cases of cellulitis in patients with haematological malignancies. These patients frequently use the health system and as antimicrobial therapy is common in the field of haematology, initial antibacterial treatment could consist of broad-spectrum β-lactams.

References


