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Case report

# Recurrent joint infection caused by a multidrug-resistant capnophilic *Escherichia coli* ST131 O25H4 strain

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ABSTRACT

We report a case of a native knee septic arthritis and subsequent osteomyelitis due to a $CO_2$-dependent (capnophilic) multidrug-resistant *E. coli* ST131 O25:H4 strain. Capnophilic phenotype made microbiology investigation challenging; susceptibility testing could not be performed and the organism did not grow in the urine culture using standard method. The combination of unique virotype and capnophilia may have contributed to the aggressiveness of this organism and the initial unsuccessful carbapenem course, leading to recurrent infection.

## Introduction

*Escherichia coli (E. coli)* is a commensal organism of the gastrointestinal tracts of humans and many animals. Some pathogenic *E. coli* strains can cause gastrointestinal infections, as well as extraintestinal infections in the urinary tract, soft tissues, and other sites. *E. coli* ST 131 has recently emerged as the predominant clonal group of extraintestinal pathogenic *E. coli* (ExPEC) [1]. Important characteristics of *E. coli* ST131 are its possession of virulence factor genes that promote cell adherence and invasion, as well as multiple antimicrobial resistant genes for extended spectrum beta-lactamases and fluoroquinolone resistance mechanisms [1]. We report a unique strain of carbon dioxide-dependent ($CO_2$-dependent) *E. coli* ST131 O25:H4 [1,2] that caused native knee septic arthritis and subsequent osteomyelitis.

## Case report and microbiology investigation

A 69-year-old woman with a history of stroke and recurrent urinary tract infections presented on index admission for localized atraumatic right knee pain and swelling. She also had recent dysuria, with urinalysis revealing > 180 white blood cells and many bacteria. She was found to have a septic right knee joint requiring operative incision and drainage, with no evidence of osteomyelitis. Two right knee synovial fluid specimens and three tissue specimens were collected. Multiple strains of *E. coli* were isolated. All clinical specimens grew two *E. coli* isolates: a non-lactose fermenter and a lactose fermenter. VITEK2 susceptibility testing and Trek Sensititre broth microdilution testing failed on multiple attempts due to lack of growth. There was no growth of *E. coli* in urine or blood cultures. Her antibiotic treatment course consisted of 4 days of piperacillin-tazobactam followed by 3.5 weeks of ertapenem at appropriate dosage. She had resolution of her symptoms.

Three months after discharge from the index hospitalization, the patient again developed localized atraumatic right knee pain and swelling, with MRI evidence of septic joint and new osteomyelitis. Synovial fluid culture again yielded a non-lactose fermenter *E. coli*. She underwent another knee incision and drainage, with operative findings consistent with chronic osteomyelitis with significant bone loss; five of six cultures yielded two types of *E. coli* isolates, a lactose fermenter and non-lactose fermenter. Again, susceptibility testing failed due to lack of growth. The *E. coli* strains were judged to be $CO_2$-dependent since all routine cultures were incubated with 5 % $CO_2$, while standard susceptibility testing (VITEK2 and Trek Sensititre broth microdilution) was performed in ambient air, and the organisms failed to grow in ambient air. The isolates did grow anaerobically. Susceptibility data were

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obtained through modification of the standard disk-diffusion procedure by incubating the Mueller-Hinton agar supplemented with 5 % sheep blood incubation at 35 °C in 5 % $CO_2$, which promoted successful growth to identify susceptibilities. The organism was resistant to ampicillin-sulbactam, ampicillin, cefazolin, cefepime, ceftriaxone, cefuroxime, ciprofloxacin, gentamicin and piperacillin-tazobactam, intermediate/susceptible to amikacin, and susceptible to meropenem, ertapenem, and trimethoprim-sulfamethoxazole.

During this recurrent infection episode, the patient's blood and urine cultures had no bacterial growth. The patient required a second operative debridement, and then was treated with six weeks of meropenem with plans for a tail of suppressive trimethoprim-sulfamethoxazole. The trimethoprim-sulfamethoxazole was stopped early due to intolerance. She subsequently had no further symptom recurrence during three-months of follow up.

Whole genome sequencing was performed on five isolates from both her index and recurrent infections using the Illumina iSeq 100 platform and Illumina DNA library preparation kit. Data analysis included Multi-Locus Sequence Typing (MLST) determination using MLST 2.0.4. SeroTypeFinder was utilized for serotyping. Virulence genes were identified using VirulenceFinder and antimicrobial resistance (AMR) genes were identified for the strain and potential resistance markers.

All five strains tested, including the original index isolate, were identified as an *E. coli* ST131 O25:H4 serotype, which was virotype E-like with the presence of the virulence genes *sat, papGII, hlyA, neuC-K* [3]**.** In addition, *asIA, chuA, fimH, fyuA, gad, hra, iha, irp2, iss, iucC, iutA, kpsE, kpsmII-K5, nlpI, ompT, papA-F43, papC, shiB, sitA, terC, yehA, yehB, yehC, yehD, yfc* were also identified [4,5]. Antibiotic resistance markers affecting fluoroquinolones, aminoglycoside and beta-lactams were identified (Table 1).

**Table 1**
Virulence genes and antibiotic resistance genes identified.

| **Virulence genes** | |
|---|---|
| **Virulence Gene** | **Description** |
| aslA | Arylsulfatase-like gene |
| chuA | Outer membrane hemin receptor, enables use of iron |
| cnf1 | Cytotoxic necrotizing factor 1, involved in cell necrosis |
| fimH | Type 1 fimbriae, role in extraintestinal colonization and biofilm formation |
| fyuA | Siderophore receptor |
| gad | Glutamate decarboxylase |
| hlyA | Hemolysin A |
| hra | High-resistance agglutinin |
| iha | Adherence protein, to iron regulating genes |
| irp2 | High molecular weight protein 2 non-ribosomal peptide synthetase, siderophore synthesis |
| iss | Increased Serum Survival, confers protection against phagocytosis and facilitates colonization |
| iucC | Aerobactin synthetase, for acquiring host iron |
| iutA | Ferric Aerobactin receptor |
| kpsE | Capsule polysaccharide export inner-membrane protein |
| kpsMII_ | Polysialic acid transport protein; Group 2 capsule, protection against phagocytosis |
| nlpI | Lipoprotein NlpI precursor |
| ompT | Outer membrane protease (protein protease 7), Evades host immunity |
| papA_F43 | Major pilin subunit F43 |
| papC | O major pilin subunit F43, facilitates colonization by stimulating T lymphocyte cytokine production |
| sat | Serine protease autotransporters of Enterobacteriaceae (SPATE), influences on cell vacuolization |
| shiB | Homologs of the Shigella flexneri SHI-2 pathogenicity island gene shiA |
| sitA | Iron transport protein |
| terC | Tellurium ion resistance protein |
| yehA | Outer membrane lipoprotein, YHD fimbriael cluster |
| yehB | Usher, YHD fimbriael cluster |
| yehC | Chaperone, YHD fimbriael cluster |
| yehD | Major pilin subunit, YHD fimbriael cluster |
| yfcV | Fimbrial protein |
| | |
| **Antimicrobial Resistance genes** | |
| **Resistance Gene** | **Description** |
| aac(6′)-Ib-cr, | Fluoroquinolone resistance |
| aac(3)-IIa | Aminoglycoside resistance |
| blaOXA-1 | Beta-lactam resistance |
| blaCTX-M-15 | Beta-lactam resistance |
| sitABCD | Disinfectant resistance |

## Discussion

The index infection most likely originated from the urinary tract given the patient's symptoms and strongly positive urinalysis. Since our standard procedure for urine culture does not require incubation with elevated $CO_2$, the capnophilic *E. coli* would not have been detected. Given this apparent origin, we suspect that her urinary tract infection led to transient bacteremia that seeded her knee joint. We postulate that if the patient had continuous bacteremia at the time of admission with the capnophilic *E. coli*, it could have been detected since we have demonstrated the organism can grow in liquid blood culture media. The lack of organism growth under standard conditions contributed to the challenges in clinical management. We recommend that for patients who have clinically apparent infection without identified organisms, or in cases with unexpected poor growth for susceptibility testing or culture, incubation with $CO_2$ should be considered.

Although enterobacteriales comprise up to 10 % of native joint infections, we did not find any other reports of capnophilic *E. coli* isolates causing septic arthritis [6]. There are scarce reports of cases of capnophilic *E. coli* causing extraintestinal bacteremia, urinary tract infection, and empyema [7–10]. However, the incidence of capnophilic bacterial infections is likely underappreciated given their poor growth under typical conditions, as they do not survive on solid media culture in ambient air [11].

Susceptibility testing is crucial for providing guidance for successful antimicrobial therapy. In this case, due to the special atmosphere requirement of this capnophilic *E. coli*, standard susceptibility testing could not be performed. We performed disc diffusion test in the presence of 5 % $CO_2$. Matsumoto et al. has demonstrated in a report of $CO_2$ dependent *E. coli* from blood culture, 5 % $CO_2$ will not affect the results of antimicrobial susceptibility test [12]. In addition, we have identified the antibiotic resistance genes (Table 1), and they correlate well with our susceptibility test results.

The antibiotic resistance genes we identified using whole genome sequencing have been described in prior ST131 strains, but their presence is variable, and this particular combination of antibiotic resistance has not been reported. In this case, despite ultimately confirming susceptibility to ertapenem, this patient had apparent relapse and progression of infection after a 3.5-week ertapenem treatment course. Possible reasons for initial treatment failure included inadequate antibiotic duration and lack of source control. Additionally, slow *in vivo* growth of the capnophilic organism could be a contributing factor, as there is a known genetic mutation associated with the capnophilic phenotype, *can gene* deletion, that has been associated with slower growth than wild-type *E. coli* [11,13]. The optimal treatment course for capnophilic organisms is not known but in light of initial treatment failure of this patient's septic joint, a 6-week treatment course should be considered in invasive infections.

ST131 virotypes have not previously been described as capnophilic. In this case, this unique combination of capnophilia and virulence factors may have contributed to unsuccessful carbapenem therapy and recurrent infection with this $CO_2$-dependent multidrug-resistant *E. coli* ST131 O25:H4.

## Ethical approval

NA.

## Consent

NA.

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## Author statement

All authors have seen and approved the manuscript, and they contribute significantly to this work. I confirm that this work is original and has not been published elsewhere, nor is it currently under consideration for publication elsewhere.

## CRediT authorship contribution statement

**Sara Geffert:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Data curation, Conceptualization. **Swapna Charla:** Investigation. **Charissa Chou:** Investigation. **Ece Dilber Gamsiz Uzun:** Investigation. **Deborah Sawyer:** Investigation. **Rozeen Badeel:** Investigation. **Tiffany Chargualaf:** Investigation. **Nouf K Almaghlouth:** Writing – review & editing, Validation. **Tao Hong:** Writing – review & editing, Validation, Supervision, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Eileen Katherine Nenninger:** Writing – original draft, Project administration, Investigation, Data curation, Conceptualization.

## Declaration of interest

None.

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