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Letter to the Editor

**Presence of tet(X4)-positive Citrobacter freundii in a cancer patient with chemotherapy-induced persistent diarrhoea**

*Sir,*

The clinical potential of tigecycline as a last-line antibiotic has been challenged by the wide dissemination of plasmid-mediated high-level tigecycline resistance gene, *tet(X)* and its orthologous [1,2], among which, *tet(X)* is predominantly reported in *Escherichia coli* isolated from food-producing animals [3]. Herein, we first identified *tet(X4)*-positive *Citrobacter freundii* from a cancer patient who underwent chemotherapy and developed persistent diarrhoea.

A 64-year-old male patient diagnosed with small cell lung cancer (SCLC) on 20 June 2018 (Day 0) was admitted to the Respiratory Department of a local hospital (Day 0) for his third cycle of chemotherapy. The patient was a rice farmer who had smoked 40 cigarettes per day for 30 years. Prior to his hospitalization, routine blood tests revealed that his white blood cell count (2.05 × 10^9/L) and absolute neutrophil count (0.24 × 10^9/L) were below normal, and he was treated with combination therapy using rhG-CSF and ceftazidime. On the day after his chemotherapy (Day 4), the patient experienced chemotherapy-induced nausea, vomiting and repeated diarrhoea. He was then treated with smectite, loperamide hydrochloride and aprepitant. From Day 8 to Day 10, he suffered from severe and persistent diarrhoea. No bacteria commonly associated with diarrhoea were isolated from his watery faecal samples. However, a stool culture on Day 11 flagged positive for tigecycline-resistant *C. freundii* (isolate 3863). The patient was further treated with medilac-S, pinaverium bromide, and nutrient and saline injection. He gradually recovered from diarrhoea from Day 13 and was discharged on Day 15. No antibiotic treatment was provided during his 16-day hospitalization.

Antibiotic susceptibility testing (AST) of *C. freundii* isolate 3863 identified by 16S rRNA gene-based sequencing was performed using the VITEK-2 platform. The isolate was resistant to tigecycline (MIC > 8 µg/mL) and tetracyclines (MIC<sub>doxycycline</sub> > 16 µg/mL; MIC<sub>tetracycline</sub> > 16 µg/mL) according to EUCAST ([http://www.eucast.org/clinical_breakpoints/](http://www.eucast.org/clinical_breakpoints/)), but remained susceptible to other antibiotics based on CLSI [4] (Table 1). Whole genome sequencing was performed with both the Illumina and Oxford nanopore sequencing platforms. Hybrid genome assembly with Unicycler v0.3.0 indicated that *C. freundii* 3863 contained a 4 947 352 bp chromosome and 51 531 bp plasmid designated as pCF3863_tetX [5]. The complete genome sequence of this isolate has been deposited in the NCBI database under BioProject accession number PRJNA650177.

The 51.5 kb pCF3863_tetX was an IncN plasmid encoding 68 ORFs, including tigecycline resistance gene *tet(X)*, and showed 99.97% nucleotide sequence identity to the 93.5 kb p13P484A-4 (CP019284) in *E. coli* of pig origin at 77% coverage (Fig. 1). *tet(X)* was associated with the genetic arrangement *hp-ahb-tet (X4)-ISVsa3* bracketed by IS26 in pCF3863_tetX, suggesting that the fragment carrying *tet(X)* was acquired by a prototype of pCF3863_tetX via horizontal gene transfer between different bacteria mediated by IS26. The conjugation assay used a rifampin-resistant *E. coli* EC600 as the recipient. Transconjugants were selected on agar plates containing 600 µg/mL rifampin and 1 µg/mL tigecycline. pCF3863_tetX was transferable to *E. coli* EC600 with the MICs of the tigecycline and tetracycline for transconjugant *E. coli* J3863 increased by ≥4-fold compared with the recipient *E. coli* EC600 (Table 1).

To the best of our knowledge, this is the first report of *C. freundii* carrying the tigecycline resistance determinant, *tet(X4).* The *C. freundii* isolate was from an immunocompromised cancer patient who underwent chemotherapy and then developed persistent diarrhoea. No tetracycline or tigecycline treatment was applied during the patient’s hospitalization, thus the *tet(X4)*-positive *C. freundii* could have been acquired from an environmental source related to his daily agricultural work and colonized in the GI tract of the patient. *tet(X)* was located on an IncN-type conjugatively transferable plasmid pCF3863_tetX. The mobility of the *tet(X4)*-carrying plasmid could further expand the host range and pose a threat to public health.

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**Competing interests**

None declared.

**Ethical approval**

This study was approved by the ethics committee of the Second Affiliated Hospital of Zhejiang University with reference No. 2019-074.

**References**


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Table 1
Antimicrobial susceptibility of C. freundii related strains.

<table>
<thead>
<tr>
<th>Strains</th>
<th>species</th>
<th>Description</th>
<th>MIC of antimicrobials (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>J3863</td>
<td>C. freundii</td>
<td>Donor</td>
<td>IPM 0.5 MEM 0.25 CAZ 0.12 FEP 4 TZP 8 SFP 2 AMK 0.25 CIP 16 LEV 16 DO 1 MNO 8 TGC 8 CT 0.5</td>
</tr>
<tr>
<td>J3863</td>
<td>E. coli</td>
<td>Transconjugant</td>
<td>0.5 0.25 0.5 0.12 4 8 2 0.25 0.5 16 8 2 0.5</td>
</tr>
<tr>
<td>EC600</td>
<td>E. coli</td>
<td>Recipient</td>
<td>≤0.25 0.25 0.5 ≤0.12 ≤4 8 2 0.25 0.12 1 2 ≤0.5 ≤0.5</td>
</tr>
</tbody>
</table>

IPM = imipenem; MEM = meropenem; CAZ = ceftazidime; FEP = cefepime; TZP = piperacillin/tazobactam; SFP = cefoperazone/sulbactam; AMK = amikacin; CIP = ciprofloxacin; LEV = levofloxacin; DO = doxycycline; MNO = minocycline; TGC = tigecycline; CT = colistin.

Fig. 1. Circular map of plasmid pCF3863_tetX. GC skew and GC content are indicated from the inside out. Pink, blue, yellow and green arrows represent the antimicrobial resistance genes, mobile elements, conjugative elements and other ORFs, respectively. Names of representative genes are labelled alongside the arrows.

