Complete Sequence of a *tet(X4)*-Harboring IncX1 Plasmid, pYY76-1-2, in *Escherichia coli* from a Cow Sample in China

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Recently, a novel plasmid-mediated tigecycline resistance mechanism, *tet(X4)*, has raised a global antimicrobial resistance concern (1, 2), but our understanding of the plasmid vectors associated with *tet(X4)* dissemination remains limited. Until now, the *tet(X4)* gene has only been identified on IncQ1, IncF, and IncHI1 plasmids in *Escherichia coli* strains from pig samples in China (1–3). Here, we report the first identification of *tet(X4)* on a highly transferable IncX1 plasmid from *E. coli* of cow origin (Fig. 1a).

During a routine antimicrobial resistance surveillance study, a *tet(X4)*-positive strain, YY76-1, was isolated from the feces of a cow using a MacConkey agar plate containing tigecycline (2 µg/ml) in May 2018 in Hainan, China, which was further identified as *E. coli* using 16S rRNA sequencing analysis as previously reported (1). Antimicrobial susceptibility testing was conducted using broth microdilution and interpreted according to the CLSI guidelines (4, 5) with the EUCAST breakpoint (http://www.eucast.org/clinical_breakpoints/) for tigecycline. The *tet(X4)*-positive *E. coli* YY76-1 was resistant to tigecycline, tetracycline, kanamycin, cefotaxime, ciprofloxacin, florfenicol, and sulfamethoxazole-trimethoprim and also exhibited high MICs to eravacycline (4 µg/ml) and omadacycline (16 µg/ml) (see Table S1 in the supplemental material).

Conjugation experiments showed that the tigecycline resistance could be successfully transferred from *E. coli* YY76-1 into the recipient *E. coli* C600 (streptomycin-resistant) by filter mating at a frequency of $(4.8 \pm 0.7) \times 10^{-5}$ per recipient cell (Fig. 1b). The resistance phenotypes for tetracycline, cefotaxime, and florfenicol were also cotransferred, as were those for eravacycline (2 µg/ml) and omadacycline (4 µg/ml) (Table S1). Additionally, the same phenotypes could be transferred with tigecycline resistance into the clinical KPC-2-producing sequence type 11 (ST11) *Klebsiella pneumoniae* 1332 (meropenem-resistant) and *Salmonella enterica* serovar Typhimurium ATCC 14028 (rifampin-resistant) at the frequency of $(2.0 \pm 0.4) \times 10^{-7}$ and $(1.7 \pm 0.5) \times 10^{-10}$, respectively (Fig. 1b; Table S1). After 14 days (approximately 140 generations) of passaging without antibiotic challenge, the plasmid was stable in both the parental strain YY76-1 and its transconjugants, with a >75% fraction of plasmid-containing populations (Fig. 1c).

Genomic DNA of *E. coli* YY76-1 was then completely sequenced using the combination of Nanopore GridION and Illumina HiSeq platforms (Nextomics, Wuhan, China), followed by assembling with Unicycler (6) and data mining via the Center for Genomic Epidemiology (CGE; <https://cge.cbs.dtu.dk/services/>). The results revealed that YY76-1 belonged to the sequence type 48 (ST48), which has been reported with the spread of NDM and MCR (7, 8). *E. coli* YY76-1 harbored one chromosome of 4,595,444 bp (cYY76-1) and two plasmids of 163,111 bp (IncFIB-like, pYY76-1-1) and 57,104 bp (IncX1, pYY76-

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A further blastn search of pYY76-1-2 against the NCBI database showed that it had >60% coverage and >99% identity to a number of IncX1 plasmids described from different *Enterobacteriaceae* species in human (KU254580), pig (CP035315), environmental (CP037995), and other sources. The sequence comparison conducted with Easyfig (9) indicated that they shared highly similar IncX1 plasmid backbones, including the regions for plasmid replication, maintenance, and transfer, but were diverse at the accessory regions with multiple resistance genes (e.g., *bla*_{TEM-176} and *floR*) (Fig. 1a). In addition, the *tet*(X4) gene in *E. coli* YY76-1 was identified between an IS26 element

upstream and a single copy of *ISCR2* downstream (Fig. 1a), which was different from the *tet(X4)*-harboring structure between two *ISCR2* described previously (1).

In summary, we report the first *tet(X4)*-harboring IncX1 plasmid in *E. coli* from cattle, highlighting the wide spread of *tet(X4)* genes in different animal sources. Worrisomely, our previous (1) and current studies suggest that this plasmid-mediated tigecycline resistance mechanism could be readily mobilized and stabilized in clinically important *Enterobacteriaceae* bacteria, posing a potential threat for the clinical usage of tigecycline (as well as eravacycline and omadacycline) as the last-resort antibiotic to treat multidrug-resistant bacterial infections.

Accession numbers. The genomic sequences of *E. coli* YY76-1 have been deposited in GenBank under the accession numbers CP040927 (cYY76-1), CP040928 (pYY76-1-1), and CP040929 (pYY76-1-2). The raw data are also deposited in the SRA (SRR10008514).

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at <https://doi.org/10.1128/AAC.01528-19>.

SUPPLEMENTAL FILE 1, PDF file, 0.3 MB.

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We declare no conflicts of interest.

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