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Cooccurrence of Two *tet*(X) Variants in an *Empedobacter brevis* Strain of Shrimp Origin

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KEYWORDS tigecycline resistance, plasmids, *tet*(X) variants, nanopore sequencing

Emerging novel resistance mechanisms pose a great public health concern. Recently, two studies reported the emergence of plasmid-mediated tigecycline resistance genes *tet*(X3) and *tet*(X4) among *Enterobacteriaceae* and *Acinetobacter* (1, 2), which suggested that the efficacy of tigecycline as a last-resort drug to treat multidrug-resistant (MDR) severe infections would be impaired. Despite low prevalence of *tet*(X) in clinical strains, the widespread presence of *tet*(X) in various bacteria is a great concern (1, 3). Here, we genetically and functionally investigated an *Empedobacter brevis* strain, SE1-3, harboring one novel plasmid-mediated *tet*(X3.2) variant of shrimp origin.

One strain SE1-3 isolate conferring resistance to tigecycline from a shrimp sample in Yangzhou, China, was isolated in June 2019. With 16S rRNA sequencing, this strain was identified as *Empedobacter brevis*, which can be involved in nosocomial infections (4). PCR with primers targeting *tet*(X) was performed (1), and two different *tet*(X) variants were identified after sequencing. MICs against different antibiotics were detected using the broth microdilution method with *Escherichia coli* ATCC 25922 as the control (5). A conjugation assay was performed with *E. coli* J53 Ari^{Z} as the receipt strain but failed after three repeats. An electrotransformation assay failed to obtain a positive transformant for extracted plasmids with *E. coli* EC600 and DH5α as receipt strains after three repeats. Whole-genome sequencing (WGS) combining short-read Illumina and long-read Nanopore MinION platforms was performed, followed by hybrid *de novo* assembly, long-read analysis, and annotation (6–8). Strain SE1-3 harbored one chromosome (3,591,742 bp, 32.7% GC content) and three plasmids, pSE1-3-20kb (20,734 bp, 31.4% GC content), pSE1-3-14kb (14,090 bp, 31.8% GC content), and pSE1-3-9kb (9,780 bp, 30.1% GC content). The GC content of the chromosome and plasmids in SE1-3 was consistent with that of four deposited *Empedobacter brevis* genomes (31.1% to 32.8%) in the NCBI database. One novel *tet*(X) variant, designated *tet*(X3.2), showing 97.26% and 84.29% identity to *tet*(X3) and *tet*(X4), respectively, was identified in pSE1-3-9kb (Fig. 1a). Tet(X3.2) displayed the most similarity (97.94%) to Tet(X3) with eight amino acid substitutions (Fig. 1c). No other resistance genes, replicons, or insertion sequences were found in pSE1-3-9kb, and no similar structure was retrieved from the NCBI nonredundant (nr) database, suggesting that this was a novel *tet*(X)-mediated plasmid. The surrounding genetic environment was different from that of *tet*(X3) and *tet*(X4), indicating that *tet*(X3.2) has not acquired the ability to transfer, which was proved by unsuccessful conjugation and electrotransformation assays. The cooccurrence of two instances of *tet*(X2) in two similar plasmids, pSE1-3-20kb and pSE1-3-14kb, of the strain was observed (Fig. 1b). Online BLASTn search of *tet*(X2) indicated that it mainly existed in non-*Enterobacteriaceae* bacteria such as *Myroides odoratimimus*. However, the occurrence of *tet*(X2) in *Enterobacteriaceae* should be considered. The *tet*(X3.2)-bearing

Citation Li R, Liu Z, Peng K, Liu Y, Xiao X, Wang Z. 2019. Cooccurrence of two *tet*(X) variants in an *Empedobacter brevis* strain of shrimp origin. Antimicrob Agents Chemother 63:e01636-19. https://doi.org/10.1128/AAC.01636-19.

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Accepted manuscript posted online 30 September 2019
Published 21 November 2019

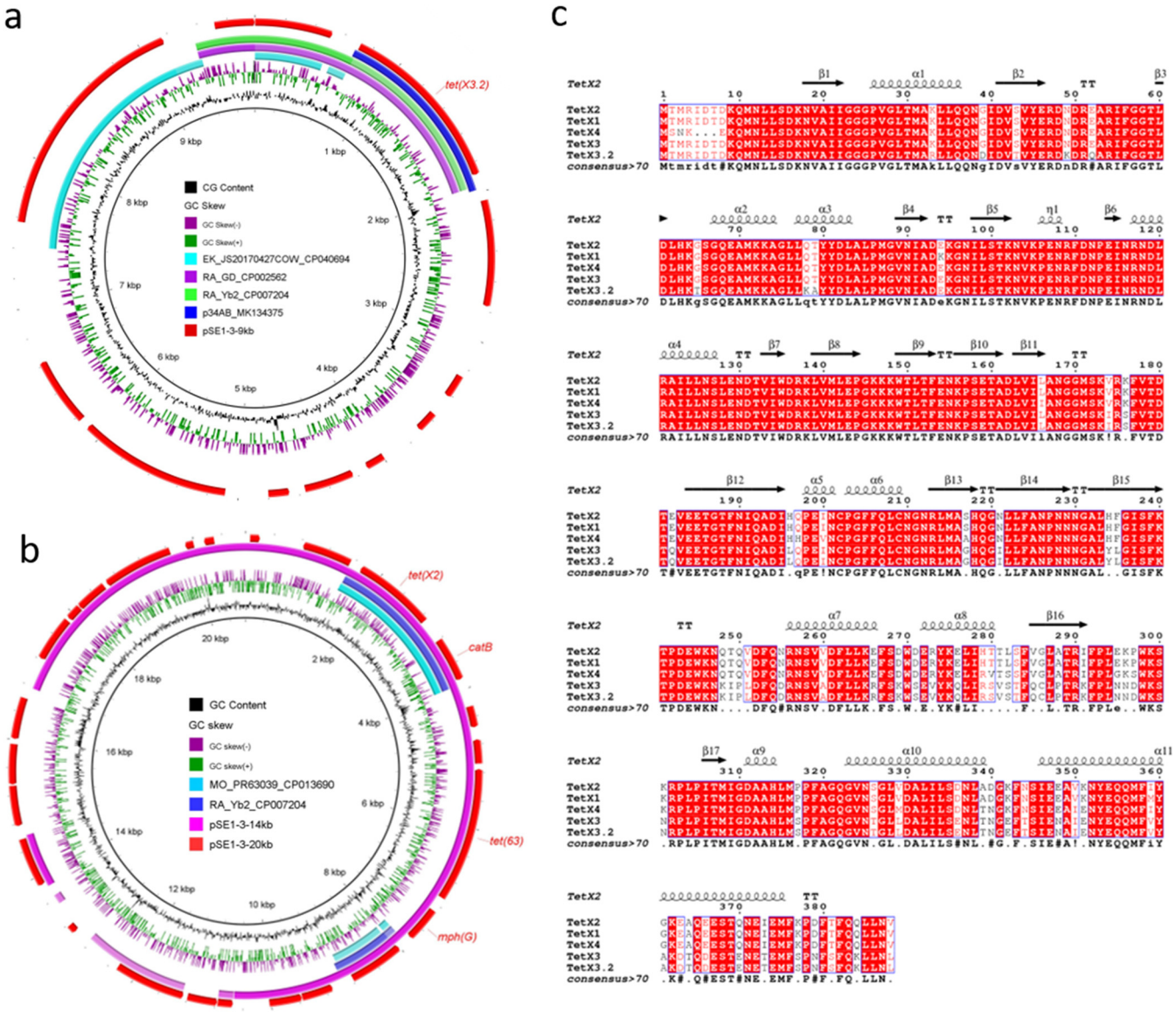

FIG 1 Circular comparison of *tet*(X)-bearing sequences and protein alignment of Tet(X) variants. (a) Comparison between *tet*(X3.2)-bearing plasmid pSE1-3-9kb, *tet*(X3)-bearing plasmid p34AB, and other similar sequences in the nr database. The outermost circle denotes the reference plasmid pSE1-3-9kb, with arrows showing the predicted coding sequences. (b) Comparison between tet(X2)-bearing plasmids pSE1-3-20kb, pSE1-3-14kb, and other similar sequences in the nr database. (c) Alignment of Tet(X3.2) reported in this study and other Tet(X) variants generated using ESPript 3.0 (9). A secondary structure based on the Tet(X2) protein (PDB number 4A6N) served as the structure reference.

plasmid pSE1-3-9kb was most similar to the chromosome of an *Elizabethkingia* species strain (CP040694, 99.90% identity at 26% coverage) and *Riemerella anatipestifer* (CP007204, 98.88% identity at 23% coverage) (Fig. 1a). This observation shed light on the origin of *tet*(X3.2).

To probe the function of *tet*(X3.2), the full length of *tet*(X3.2) in SE1-3 was amplified using PCR with primers tet(X)-F cgagctcATGACAATGCGAATAGATAC and tet(X)-R cccaagcttTACTGTTTATAGATTCAA, cloned into the pET28a vector, and transformed chemically into *E. coli* BL21, and the MICs were tested against different tetracycline antibiotics. Under IPTG (isopropyl-β-D-thiogalactopyranoside) induction, *E. coli* BL21 harboring the pET28a-tet(X3.2) conferred resistance to tetracycline (64 mg/liter), tigecycline (8 mg/liter), oxytetracycline (16 mg/liter), doxycycline (64 mg/liter), and minocycline (32 mg/liter) with at least 16-fold increased MICs compared with BL21 without the vector (see Table S1 in the Supplemental Material). This demonstrated that *tet*(X3.2) encoded similar resistance phenotypes as *tet*(X3) and *tet*(X4) (1, 2).

To conclude, a novel plasmid-mediated tigecycline resistance gene, *tet*(X3.2), identified from a food sample, was characterized. The potential risk of *tet*(X3.2) occurrence in *Enterobacteriaceae* warrants intense surveillance. Considering the wide distribution of *tet*(X) genes in different sources, other novel *tet*(X) variants conferring resistance to tigecycline should be investigated.

Data availability. The complete genome sequences of SE1-3 have been deposited in the NCBI database under BioProject accession number PRJNA563978 (CP043637.1, CP043636.1, CP043635.1, and CP043634.1).

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.01636-19.

SUPPLEMENTAL FILE 1, PDF file, 0.2 MB.

ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China (grants 31872523 and 31871899), the Natural Science Foundation of Jiangsu Province (grant BK20180900), and the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD).

We declare no conflicts of interest.

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