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Letter to the Editor

Identification of a multiresistant mosaic plasmid carrying a new segment of IS*1216E*-flanked *optrA* with integrated Tn*551*-*ermB* element in linezolid-resistant *Enterococcus faecalis* human isolate

Sir,

Linezolid, the prototype of oxazolidinones, is recognized as an important therapeutic option for infections caused by drug-resistant Gram-positive pathogens including MRSA and VRE. Linezolid inhibits initiation of protein synthesis by binding to domain V of the 23S rRNA in the 50S ribosomal subunit. Since the first description of linezolid resistance among MRSA and VRE clinical isolates in 2001, linezolid-resistant staphylococci and enterococci have been increasingly reported throughout the world. Linezolid resistance in those isolates is attributed majorly to specific nucleotide substitutions in the domain V region of the 23S rRNA gene, and less frequently to amino acid substitutions in the L3, L4, and L22 50S ribosomal proteins encoded by altered *rplC*, *rplD* and *rplW* genes, respectively [1]. In addition, transferable plasmid-mediated linezolid-resistance genes *cfr*, *optrA*, and more recently *poxtA* have been identified in enterococci from food animals and humans [2]. We report here a multiresistant plasmid carrying the *optrA* from a linezolid-resistant *Enterococcus faecalis* clinical isolate.

E. faecalis isolate (strain S7316) was recovered in 2019 from a bile sample of a patient in his 70 s suffering from cholangitis. MICs were determined by broth microdilution method, in accordance with CLSI 2019 guidelines. The MICs of linezolid, chloramphenicol, erythromycin and minocycline were 8, 64, >4 and >8 mg/L, respectively, but the S7316 was susceptible to penicillins, glycopeptides, and levofloxacin. Detection of *optrA* gene was conducted by PCR. WGS analysis was performed using illumina HiSeq and *de novo* assembled by A5-miseq pipeline. The assembled genome was subjected to annotation (DFAST) and characterization (Center for Genomic Epidemiology). In-depth exploration of virulence genes was performed manually on WGS sequence data.

The genome sequences of *E. faecalis* S7316 were assembled into 41 contigs with a total length of 2,811,450 bp. Genome annotation identified probable 2614 protein-coding genes including antimicrobial resistance genes *optrA*, *fexA*, *ermB*, *tetL*, *tetM*, *dfrG*, and *lsaA*. No substitutions, deletions, and insertions were found in the nucleotide and amino acid alignments in both 23S rRNAs and L3, L4 and L22 ribosomal proteins. The *optrA* was located on a plasmid pS7316optrA (GenBank LC499744) with the size of 68,368 bp, GC content of 35.2 % harbouring probable 75 protein-coding genes (Fig. 1A). The pS7316optrA, which encodes RepA_N protein also carried other resistance genes including *fexA* located in close proximity upstream of the *optrA*, *ermB*, *tetL* and *tetM*. The complete sequence of the plasmid exhibited no significant structural homology to other plasmids available in the GenBank database (accessed February 2020) except for several regions. Namely, an 18,713-bp region containing Tn*551*-*ermB*, *optrA* and *fexA* shared 99.2% nucleotide sequence identity with that of the *optrA*-positive plasmid pN60443F-2 in an *E. faecalis* from cattle in the USA (GenBank CP028725). A 19,595-bp region containing Tn*6247*-like-associated *tetL* and *tetM* shared 99.9% nucleotide sequence identity with that of the plasmid p6742_2 in an *E. faecalis* from human in Poland (GenBank KY513281). A 4,642-bp region containing Tn*551*-*ermB*, and a 15,815-bp region containing *pcfD* to *pcfJ*, *zntA*, *mazF*, *mazE* had high nucleotide sequence homology (99%) with the corresponding regions in the plasmid pN60443F-1 of an *E. faecalis* from cattle in the USA (GenBank CP028726). The pS7316optrA carried *prgZ* and *pcfD* to *pcfJ* genes but lacked *prgX* and *prgQ*, which are associated with RepA_N pheromone-responsive pCF10 plasmid and needed for the regulation of pheromone-inducible conjugation. *E. faecalis* S7316 belonging to a rare sequence type ST634 had several virulence genes, including adhesins and biofilm-associated genes (*ace*, *srtA*, *srtC*, *fsrA*, *fsrB*, *fsrC*, *gelE*, *sprE*, *ebpABC* and *efaAfs*), survival genes (*tpx* and *elrABCDE*), hyaluronidase genes (*hylA* and *hylB*), and sex pheromone-associated genes (*cad*, *camE*, *cCF10* and *cOB1*).

The pS7316optrA had a unique feature in that its entire sequence showed no significant matches to the DNA sequences deposited in the GenBank database. Our analyses revealed that the plasmid displayed a mosaic structure probably evolved from pCF10, with containing several regions composed of mobile genetic elements and antimicrobial resistance genes *optrA*, *fexA*, *ermB*, *tetL* and *tetM* of different plasmid origins. The presence of ΔIS*1216E*-*fexA*-*optrA* with the integration of Tn*551*-*ermB* element, which is unique among plasmids harbouring *optrA*, *fexA* and *ermB* (Fig. 1B), is likely to be of particular concern because the IS*1216E* has been implicated in the wide spread of *optrA* among enterococcal plasmids and streptococcal chromosomes [3,4]. Tn*6247* containing *tetL* and *tetM* has only been identified in *E. faecium* of swine origin in China. Macrolides, tetracyclines and florphenicol are frequently used in farm animals, while oxazolidinones are not. Thus, the pS7316optrA carrying the resistance genes to those antimicrobials simultaneously, including *optrA* that confers cross-resistance to phenicols and oxazolidinones might be associated with livestock sources to which phenicols have been used for a longtime as veterinary drugs and feed additives. The emergence of such multiresistant *optrA*-positive mosaic plasmid in *E. faecalis* human clinical isolate would pose a future health risk in Japan where only one study has reported *optrA* [5].

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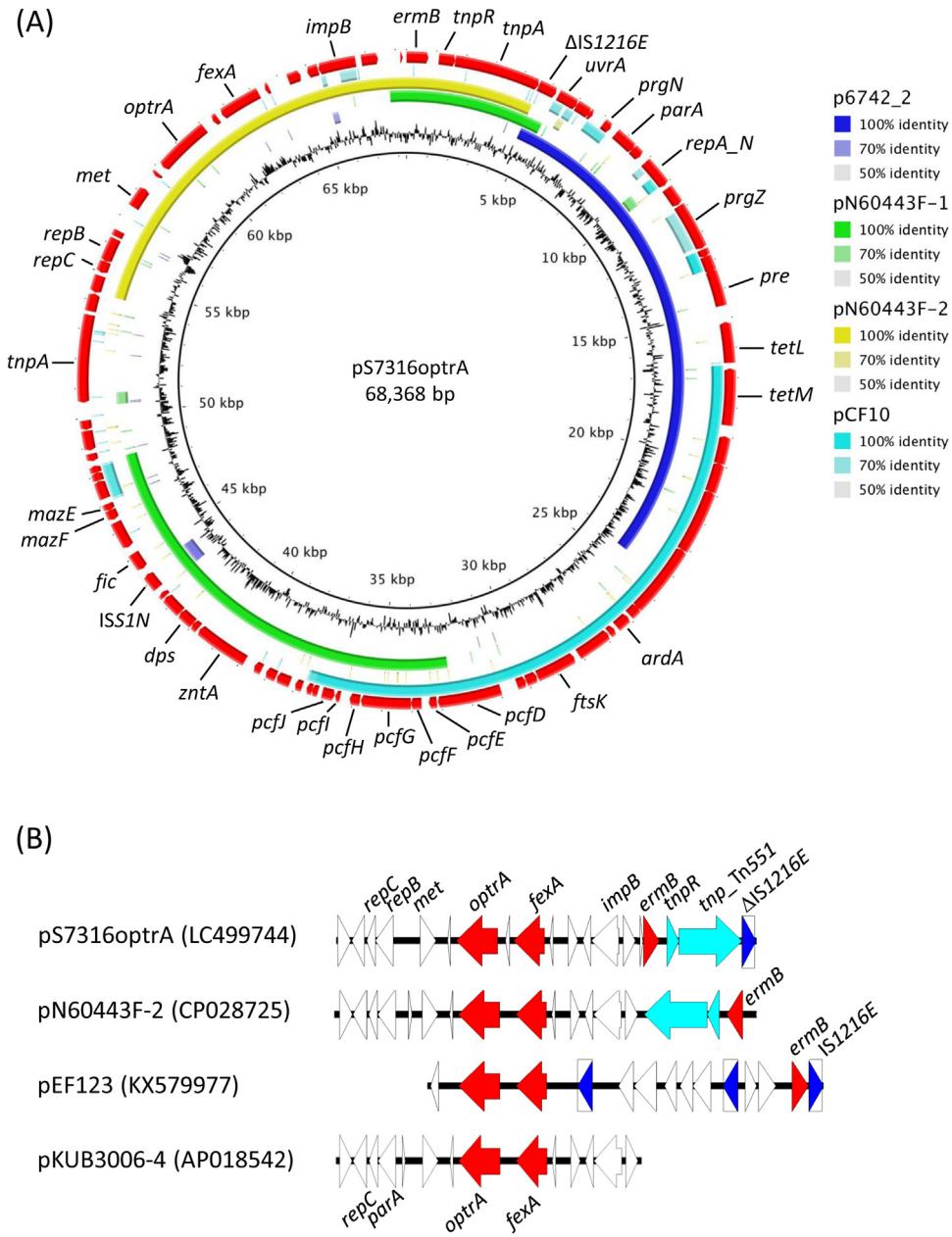

Fig. 1. Genetic representation of the *optrA*-carrying pS7316optrA. (A) Circular comparison between plasmid pS7316optrA (GenBank accession no. LC499744) and other similar plasmids. The predicted coding sequences of pS7316optrA (outermost, red), with shared regions with p6742_2 (purple), pN60443F-1 (green), pN60443F-2 (yellow), and pCF10 (sky blue) are depicted by BRIG. (B) Schematic presentation and comparison of the genetic environment of *optrA*-flanking regions in the pS7316optrA and other two plasmids pN60443F-2 and pEF123 harbouring *optrA*, *fexA* and *ermB*, and pKUB3006-4, first reported *optrA*-positive plasmid in Japan.

Conflict of interest statement

None declared.

Ethical approval

Not required.

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