Journal of Global Antimicrobial Resistance 38 (2024) 245–246

Contents lists available at ScienceDirect

Journal of Global Antimicrobial Resistance

journal homepage: www.elsevier.com/locate/jgar

Letter to the Editor

Genetic elements harbouring oxazolidinone resistance genes detected in swine enterococci circulate in clinical isolates, Italy

Editor: S. Stefani

Sir,

Enterococci are common members of the gut microbiota of humans and animals, however, some species, particularly *Enterococcus faecium* and *Enterococcus faecalis*, are a common cause of severe nosocomial infections worldwide. The resistance to several antimicrobials makes these infections difficult to treat, especially those caused by vancomycin-resistant enterococci (VRE). Oxazolidinones (linezolid and tedizolid) are last-resort antibiotics effective against clinical infections due to MDR Gram-positive pathogens, including VRE [1].

Although the prevalence of oxazolidinone resistance among enterococcal clinical isolates remains uncommon, in recent years, an increasing spread of linezolid-resistant enterococci has been observed worldwide.

Linezolid resistance occurs through ribosomal mutations, but it can also develop following the acquisition of transferable genes: *cfr*, *cfr*(B), *cfr*(C), *cfr*(D), *cfr*(E), *optrA*, *poxtA* and *poxtA2* [1]. The *cfr* and *cfr*-like genes encode methyltransferases conferring resistance to phenicols, lincosamides, oxazolidinones, pleuromutilins and streptogramin A (PhLOPS$_A$ phenotype), whereas *optrA* and *poxtA*/*poxtA2* genes encode ABC-F proteins leading to a decreased susceptibility to phenicols and oxazolidinones (including tedizolid) [1].

Although oxazolidinones have been banned from use in food-producing animals, linezolid resistance genes circulate in human enterococci as well as in those of animal and environmental origin [1]. The antibiotics commonly administered in veterinary medicine might contribute to the spread of oxazolidinone resistance genes due to co-selection mechanisms.

Here we investigated four enterococci carrying linezolid resistance genes collected during a routine surveillance of antibiotic resistance in swine enterococci.

The strains were isolated from porcine manure collected on a farm located in central Italy, in July 2022. Isolation was performed as described by Brenciani et al. [2]; florfenicol-resistant enterococci were screened by PCR for the presence of linezolid resistance genes as previously reported [2].

Enterococcus thailandicus V1495 and V1402, *E. faecalis* V1461, and *Enterococcus avium* V1426 – identified by MALDI-TOF (Vitek-MS, bioMérieux), all carried the *optrA* gene; *E. thailandicus* V1495 and *E. avium* V1426 also harboured *poxtA* and *cfr* genes, respectively (Table S1).

Susceptibility tests were performed by reference broth microdilution and interpreted according to CLSI clinical breakpoints [3]. All isolates exhibited resistance to florfenicol (MIC range, 16–64 mg/L), chloramphenicol (MIC range, 16–64 mg/L), erythromycin (MIC range, 16->128 mg/L), and tetracycline (MIC range, 64–128 mg/L), and susceptibility to vancomycin (all MIC, 1 mg/L). *E. faecalis* V1461 and *E. avium* V1426 were intermediate to linezolid (MIC, 4 mg/L), whereas the two *E. thailandicus* strains were susceptible (MIC, 2 mg/L). The isolates showed resistance to tedizolid (MIC, 2 mg/L), except *E. thailandicus* V1402 (MIC, 0.5 mg/L) (Table S1).

Extracted DNAs were subjected to WGS using a hybrid process using both short-read Illumina MiSeq platform (MicrobesNG, Birmingham, UK) with a 2 × 250 approach and a long-read sequencing (MinION, Oxford Nanopore Technologies, Oxford, UK). Unicycler v. 0.4.8 software was used for the hybrid assembly of short and long reads (https://github.com/rrwick/Unicycler).

Bioinformatic data revealed that in *E. thailandicus* V1495 the *poxtA* gene was on a 38,209-bp plasmid 99.9% identical (99% coverage) to the 38.387-bp pEgFS4–2 plasmid previously detected in a porcine *Enterococcus gallinarum* collected in central Italy [4]. To the best of our knowledge, this is the first identification of the *poxtA* gene in this species. The *optrA* gene, chromosomally located, was on the Tn*7695* transposon identical (99% coverage) to the one previously identified on the *optrA*- and *poxtA*-carrying p249031-S plasmid (accession no. OP947907) from a bovine *E. faecium* recovered in Italy [5]. An identical chromosomal location of the *oprtA* gene was also detected in *E. thailandicus* V1402.

In *E. faecalis* V1461 – belonging to the lineage ST840 previously reported in *E. faecalis* from broilers in Poland [6] – the *optrA* gene was on a 112,928-bp plasmid, named pV1461-*optrA* (accession no. PP003004). This plasmid displayed high identity (99,9%) and coverage (90%) with the MDR p661-a plasmid (101,904 bp) from an *E. faecalis* clinical isolate from Switzerland (accession no. CP091228.1). Interestingly, the comparison between the two plasmids indicated the presence in pV1461-*optrA* of a 10,786-bp region missing in p661-a (Fig. 1). This region, inserted in p661-a between the *rep* and *tet*(L) genes, included: (*i*) the *optrA* gene, flanked by two IS*Efa15* with the same polarity, as previously observed in the MDR pE35048-oc plasmid (accession no. MF580438) from an *E. faecium* clinical isolate [7], and (*ii*) an ΔTn*558* transposon harbouring the *fexA* and *NADPH* genes. Compared to the wild-type Tn*558* (accession no. AJ715531.1), the ΔTn*558* was devoid of *tnpA, tnpB, tnpC* transposases. Inverse PCR experiments, using outward-directed primer pairs targeting the *optrA* and *fexA* genes (optrA-inv:GGTAGAAATGGATGTGGAAAAA; fexA-FW:CGCATCTGAGTAGGACATAGC), showed that the 10,786-bp region was able to excise in circular form.

Finally, in *E. avium* V1426 *cfr* and *optrA* genes were co-located on a 39,043-bp plasmid 99% identical (100% coverage) to the above-mentioned pE35048-oc [7].

Conjugation assays, using florfenicol, rifampicin and fusidic acid (all, 10 mg/L) for selection, failed to demonstrate the transferability of linezolid resistance genes to *E. faecium* 64/3.

https://doi.org/10.1016/j.jgar.2024.06.016

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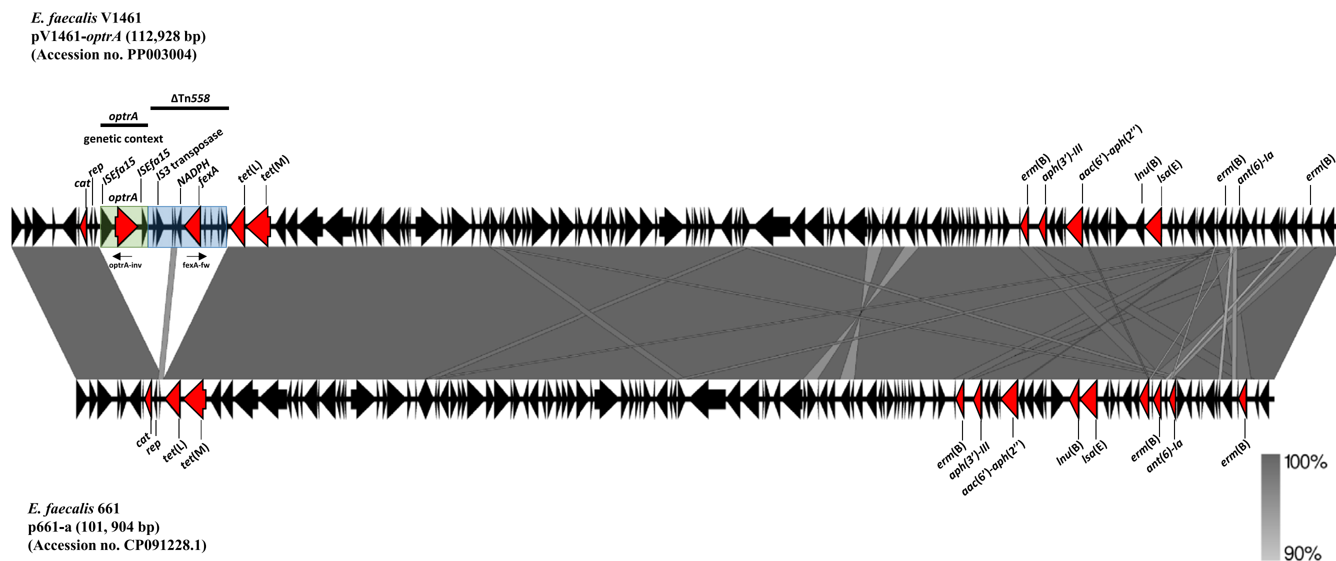

Fig. 1. Linear map of the pV1461-*optrA* from *E. faecium* V1461 in comparison with p661-a from *E. faecalis* 661 using Easyfig tool (https://mjsull.github.io/Easyfig/) and BLASTN tool. (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The positions and transcriptional direction of the ORFs are represented with arrows. Some antibiotic resistance determinants (in red) and relevant genes described in this study are shown.
tet(M) and *tet*(L) (resistance to tetracyclines), *erm*(B) (resistance to macrolides, lincosamides, and group B streptogramins), *aph*(3′)-III and *ant*(6)-Ia and *aac*(6′)-aph(2′′) (resistance to aminoglycosides), *lsa*(A) and *lsa*(E) (resistance to lincosamides, pleuromutilins and group A streptogramins), *cat* (resistance to chloramphenicol), and *lnu*(B) (resistance to lincosamides).

Our findings confirm the occurrence of oxazolidinone resistance genes among porcine enterococci of different species, including *E. faecalis*, and highlight how indistinguishable plasmids and transposons circulate in both porcine and clinical isolates. This sharing of linezolid resistance genetic elements between human and animal enterococci is of particular concern and emphasize how the administration of antibiotics in food-producing animals (e.g., phenicols, tetracyclines, lincosamides, aminoglycosides) results in the co-selection of the oxazolidinone resistance posing a significant risk to human health.

Declaration of Competing Interests

None declared.

Funding

This work was supported by the Italian Ministero della Salute (grant number:RC 004/2021 IZSUM).

Ethical approval

Not required.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jgar.2024.06.016.

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Sonia Nina Coccitto
Unit of Microbiology, Department of Biomedical Sciences and Public Health, Polytechnic University of Marche Medical School, Ancona, Italy

Marzia Cinthi
Unit of Microbiology, Department of Life and Environmental Sciences, Polytechnic University of Marche, Ancona, Italy

Francesca Romana Massacci, Elisa Albini, Chiara Francesca Magistrali
Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche 'Togo Rosati', Perugia, Italy

Andrea Brenciani*
Unit of Microbiology, Department of Biomedical Sciences and Public Health, Polytechnic University of Marche Medical School, Ancona, Italy

Eleonora Giovanetti
Unit of Microbiology, Department of Life and Environmental Sciences, Polytechnic University of Marche, Ancona, Italy

*Corresponding author.
E-mail addresses: a.brenciani@staff.univpm.it, a.brenciani@univpm.it (A. Brenciani)
Revised 18 June 2024