



Letter to the Editor

***optrA*-mediated linezolid resistance in an *Enterococcus faecalis* isolate recovered from a wild raptor (*Falco peregrinus peregrinus*), central Italy**

Editor by: Stefania Stefani



Sir,

The emergence of clinical linezolid-resistant enterococci (LRE) has been described in several countries, and LRE isolates harbouring a *cfr/cfr*-like, *optrA*- and *poxtA*-encoding plasmid were reported from animals, mostly from Asian countries and from livestock animals [1].

This is the first report of *optrA*-containing *E. faecalis* in a wild raptor.

The adult female Peregrine falcon (*Falco peregrinus* subspecies *peregrinus*) (id WU476), a medium-sized bird of prey, was found in Spoleto, central Italy, in June 2021. The animal presented with a perforating wound of the right wing, probably as a consequence of a gunshot. The falcon was collected by a wildlife rehabilitation center and then admitted to a veterinary hospital, where it was fed with frozen chicks and orally treated with doxycycline at 25 mg/Kg for fifteen days. After two weeks without sign of healing, the animal was humanely euthanized.

Lung, kidney, brain, and swabs from the skin lesion and from the humeral-radial joint underwent culture using a blood agar plate (5%) and were incubated at 37°C for 24 h. From every sample, pure colonies were identified as *E. faecalis* by MALDI-TOF MS (Bruker Daltonics, Bremen, Germany) and confirmed as belonging to the same clone by Smal-PFGE assay.

The strain, named *E. faecalis* 30488, exhibited resistance to linezolid, chloramphenicol, florfenicol, tetracycline, and erythromycin, and susceptibility to tedizolid and vancomycin using broth microdilution assays.

E. faecalis 30488 was screened by polymerase chain reaction for the presence of the *cfr/cfr*-like, *optrA*, and *poxtA* genes (Supplementary Table S1). The strain carried only the *optrA* gene and Sanger sequencing showed that was identical to the wild type sequence.

Conjugation and transformation assays, using 10 mg/L florfenicol for selection, failed to demonstrate the transferability of the *optrA* gene to *E. faecalis* JH2-2 recipient.

S1-PFGE analysis revealed a plasmid of ~75 Kb. However, hybridization experiments displayed a chromosomal location of the *optrA* gene.

E. faecalis 30488 was subjected to whole genome sequencing (WGS) using an Illumina MiSeq platform (MicrobesNG, Birmingham, UK) with a 2 × 250-bp paired-end approach. De novo assembly of WGS data was performed using Unicycler v0.4.0 (<https://github.com/rrwick/Unicycler>). In silico analysis of resistome, virulome, and sequence type was carried out using tools available at the Center for Genomic Epidemiology (<http://www.genomicepidemiology.org/>). The WGS data of the *E. faecalis* 30488

is available under the BioProject ID PRJNA850894 (accession no: **JAMYXD000000000**).

Resistome and virulome analysis revealed the presence of 12 resistance genes and several virulence factors. WGS analysis assigned the *E. faecalis* 30488 strain to sequence type (ST) 476, which has previously been associated with human and animal enterococci [2,3].

The WGS of the *E. faecalis* 30488 strain was compared with publicly available *optrA*-harbouring *E. faecalis* ST476 genomes: four clinical isolates and six isolates from swine and chicken faeces or chicken meat (Supplementary Table S2). The phylogenetic relatedness of sequenced isolates was evaluated with CSI Phylogeny 1.4 (<https://cge.food.dtu.dk/services/CSIPhylogeny/>), using default parameters, to infer phylogenetic trees from assembled WGS data. The *E. faecalis* V583 (GenBank accession no. **NC 004668.1**) susceptible to linezolid was used as reference genome, and all genomic relatedness is shown in Fig. 1 and in Supplementary Table S2. From phylogenetic analysis, the *E. faecalis* 30488 strain is closely related to the *E. faecalis* S338 strain (differing by 27 SNPs) previously isolated from swine farms in central Italy [3]. However, the cgMLST analysis assigns two different complex types to the raptor and pig isolates, since they differ by 18 alleles (Supplementary Fig. S1)

The strain exhibited 108–166 separating SNPs from *E. faecalis* isolates of clinical origin (Table S2).

Genome analysis showed that the *optrA* gene – linked to *fexA* and adjacent to a complete Tn554 element containing *erm(A)* and *spc* genes – was carried by a chromosomal Tn6674-like transposon (12.039 bp) (GenBank accession no. **ON456383**) inserted in *radC* (encoding a DNA repair protein). The Tn6674-like transposon was 99% identical (coverage 93%) to the Tn6674 (GenBank accession no. **MK737778**) first reported in the porcine *E. faecalis* E1731 strain from China (Supplementary Fig. S2).

Several authors have described an increased risk of acquiring antibiotic-resistant bacteria (ARB) for animals living close to anthropogenic sources [4,5]. Raptors have access to different ecological niches and are at the top of the food chain: here, the falcon might have acquired the linezolid-resistant *E. faecalis* from the environment, as it was found in an area characterized by human settlements and livestock farms. In addition, we cannot exclude that the falcon acquired the infection from the chicks used as feed during the period of hospitalization, as other authors have hypothesized this route for the transmission of ARB to raptors in captivity. These hypotheses could not be supported by phylogenetic analysis, which assigned our isolate to a newly described clonal type (Supplementary Fig. S1) [4,5].

Wildlife centers could represent a possible source of ARB for animals and for their caretakers, who in turn can spread them to the wider community [5]. Indeed, the falcon could have released this bacterium in the environment if returned to the natural habitat, contributing to the environmental pollution by ARB [5]. The identification of *E. faecalis* harbouring the linezolid resistance

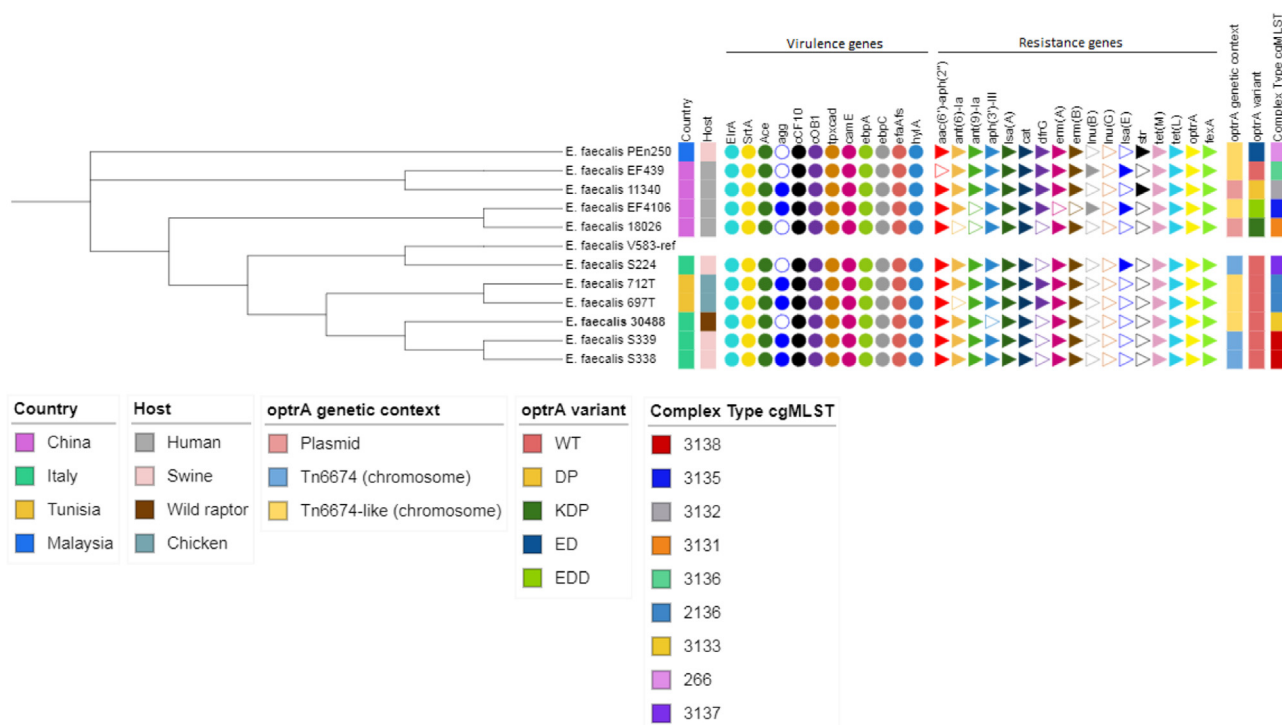


Fig. 1. Phylogenetic correlation between *E. faecalis* ST476 isolates resistant to linezolid and harbouring the *optrA* gene. The *E. faecalis* V583 strain is susceptible to linezolid and was used as a reference genome. The phylogenetic relatedness of sequenced isolates was evaluated with CSI Phylogeny 1.4 (<https://cge.food.dtu.dk/services/CSIPhylogeny/>) using default parameters. The tree was annotated using the iTOL interactive user interface (<https://itol.embl.de>). The colorful/colorless dots indicate the presence/absence of the virulence and resistance genes.

gene *optrA* in wild animals, especially in birds, is highly worrisome with regard to the dissemination of LRE among different ecological niches.

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Ethical approval

Not required

Declaration of competing interest

The authors declare no conflict of interest.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.jgar.2022.12.006](https://doi.org/10.1016/j.jgar.2022.12.006).

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Elisa Albini¹
Istituto Zooprofilattico Sperimentale dell’Umbria e delle Marche ‘Togo Rosati’, Perugia, Italy

Sonia N. Coccitto¹
Unit of Microbiology, Department of Biomedical Sciences and Public Health, Polytechnic University of Marche Medical School, Torrette di Ancona, Italy

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

Marco Gobbi, Francesca R. Massacci, Silvia Pavone, Chiara F. 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, Torrette di Ancona, Italy

*Corresponding author. Istituto Zooprofilattico Sperimentale dell’Umbria e delle Marche ‘Togo Rosati’, Via G. Salvemini 1, 06126, Perugia, Italy

E-mail address: c.magistrali@izsum.it (C.F. Magistrali)

¹ These authors equally contributed to this work.

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