TITLE: FIRST DETECTION OF OXAZOLIDINONE RESISTANCE DUE TO THE COEXISTENCE OF THE *cfr* AND *optrA* GENES IN BRAZIL.

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ABSTRACT:
Oxazolidinones are one of the most important last-line therapies for multidrug-resistant Gram-positive bacterial infections, including methicillin-resistant staphylococci (MRSA and MRCoNS) and vancomycin-resistant enterococci (VRE). Transferable resistance to oxazolidinones is related to the plasmid-borne or chromosomal multiresistance genes *cfr* and *optrA*. Here we report three *cfr*/*optrA*-carrying *Enterococcus faecalis* strains (ST29 and ST591) isolated from faeces of healthy nursery pigs from two unrelated piggeries located in DF, Brazil. The genetic environments of *cfr* and *optrA* were investigated. Whole genomic DNA was sequenced (Illumina Miseq), assembled (CLC 8.0.3) and annotated (NCBI's PGAP). The minimum inhibitory concentration (MIC) was determined by broth microdilution testing (CLSI). All *E. faecalis* strains exhibited linezolid MIC of 16 µg/ml. A 7.797 bp-segment was found to be carrier of the *cfr* gene, which was flanked upstream by the Tn*554*-related Δ*tnpB* gene. Further upstream of Δ*tnpB*, a *rep* gene was detected that was disrupted by the integration of an IS*1216*. Downstream of *cfr*, a recombinase *rec* gene, a gene coding for a hypothetical protein and a plasmid recombination/mobilization *pre*/*mob* gene were detected. Circles containing the IS*1216*-flanked segment, as predicted in silico analysis, were confirmed by PCR and Sanger sequencing. These *E. faecalis* strains co-carried a conserved *optrA*-carrying DNA segment of 3.453 bp, which was composed of genes coding for a hypothetical protein and an AraC family transcriptional regulator upstream of *optrA* (*araC*-hp-*optrA* array). The *araC*-hp-*optrA* array could also be circularized. These *cfr*/*optrA*-carrying DNA segments have been acquired horizontally from other bacteria, and the circles suggest that they were unstable, short-lived forms in these porcine *E. faecalis* isolates. The spread of the multiresistance genes *cfr* and *optrA* among various bacterial species has been a serious concern both in clinical settings and food-producing animal environments. However, little is known about how their spread inter- species/genera has been driven. It's very worrying that antimicrobial selective pressure may be selecting *cfr* and *optrA* in *E. faecalis* lineages, since these transferable resistance genes confer resistance to oxazolidinones, including the new tedizolid. Moreover, the spread of *cfr* and *optrA* has been driven by plasmids containing other important resistance determinants.

Keywords: oxazolidinones, transferable resistance, *Enterococcus faecalis*.

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