First Report Cfr and OptrA Co-harboring Linezolid-Resistant Enterococcus faecalis in China

This article was published in the following Dove Press journal: Infection and Drug Resistance

Abstract: A linezolid-resistant E. faecalis strain harboring optrA and cfr resistance genes were isolated from a patient in China, which had no mutations in rplC, rplD, rplV, and 23S rRNA gene. Transformation indicated that optrA and cfr were located on two different plasmids and both could be transferred to recipient strain, resulting in the increase of MICs of linezolid and chloramphenicol. Cfr, carried by an 11,872-bp plasmid, was enclosed with an IS110 transposase in upstream and an IS3-like transposase in downstream, while optrA was on an 8357-bp plasmid. As far as we know, this is the first report of an E. faecalis clinical strain co-harboring optrA and cfr in China.

Keywords: E. faecalis, linezolid, cfr, optrA

Linezolid is considered as a last resort drug for the treatment of severe infections caused by multidrug-resistant gram-positive pathogens including vancomycin-resistant Enterococcus spp. (VRE), methicillin-resistant Staphylococcus spp. and Streptococcus pneumoniae. Although most of gram-positive cocci remain susceptible to linezolid, resistant isolates of Enterococcus have been reported worldwide. The main resistance mechanisms of Enterococcus spp to linezolid include the mutation of 23S rRNA gene, acquiring resistance genes such as cfr, cfr(B), optrA or poxtA, and mutation of ribosomal proteins coding genes like rplC, rplD, and rplV. The cfr and cfr(B) genes encode a rRNA methyltransferase causing resistance of oxazolidinones, chloramphenicol, tetracycline, lincomycins, pleuromutilin, and streptogramin A and decreasing sensitivity of macrolide. Cfr or cfr(B) and optrA, poxtA along with optrA has been previously reported on the same plasmid in E. faecalis, Staphylococcus sciuri, or Enterococcus spp. from swine and farm environment. Here, we firstly reported the emergence of linezolid-resistant E. faecalis clinical strain with cfr and optrA in China.

E. faecalis strain EF02 was isolated from the midstream urine of a 72-year-old patient with diabetes during hospitalization in November, 2018. Prior to the isolation of E. faecalis strain EF02, 10 days of treatment with vancomycin (MIC =8 mg/L), linezolid (MIC =64 mg/L), and ampicillin (MIC =2 mg/L). PCR detection and
sequencing revealed that *E. faecalis* strain EF02 was positive for *cfr* and *optrA* without mutation among *rplC, rplD, rplV* and 23S rRNA gene. The plasmids extracted from the donor strain *E. faecalis* EF02 were then guided into the recipient strain *E. faecalis* OG1RF by the electrotransformation method.\(^3,4\) The transforms were selected on brain heart infusion agar containing 3 mg/L linezolid and 10 mg/L chloramphenicol. Colonies that grew on these selective plates were further confirmed by antimicrobial susceptibility testing and PCR for the detection *cfr* and *optrA* genes. Transformants harboring the plasmid with *optrA* and *cfr* were named L13/ *optrA* and L18/ *cfr*, respectively. Comparing with the recipient strain, the linezolid and chloramphenicol MICs of transformants increased 4–8 fold (Table 1).

PCR mapping according to plasmid DNA sequencing of the L18/ *cfr* plasmid suggested that an IS110 transposase was located upstream of *cfr* and an IS3-like transposase was located downstream of *cfr*. Mobile elements like these might lead to the transfer of resistance genes among plasmids. L18/ *cfr* plasmid contained eight open reading frames encoding *Y111*, *IS110*, *cfr*, *IS3*, RepB, RepB, EATX, and YOEC (Figure 1). However, L13/ *optrA* plasmid, which contained three open reading frames encoding ERMA, YDIF, and *optrA* (Figure 2). The result of multilocus sequence typing indicated that *E. faecalis* strain EF02 belonged to ST 330. The DNA sequencing of plasmid pEF-L18/ *cfr* and pEF-L13/ *optrA* has been submitted to the NCBI database (NCBI number: MT874923 and MT874924).

*cfr* and *optrA* genes have been reported in various gram-positive bacteria, they can be transferred to recipient bacteria by transformation experiments and cause an

| Antimicrobial Agents | E. faecalis EF02 | L13/ *optrA* | L18/ *cfr* | E. faecalis OG1RF |
|---------------------|-----------------|--------------|------------|------------------|
| Linezolid           | 8               | 8            | 8          | 1                |
| Tetracycline        | >64             | 0.25         | 0.25       | 0.25             |
| Erythromycin        | >64             | 4            | 8          | 2                |
| Chloramphenicol     | >64             | 8            | 8          | 2                |

**Figure 1** Circular representation of the L18/ *cfr* plasmid. Moving from inside to outside in the plasmid circular map, slots 1–3 (slot 1, GC skew; slot 2, GC content; slot 3, open reading frames: *Y* family DNA polymerase *Y111*, *IS110* family transposase, *cfr*; *IS3* family transposase, two replication protein RepB, antitoxin epsilon EATX, Probable integrase YOEC.).
increase in the MIC values of linezolid and chloramphenicol.\textsuperscript{3,4,15} The cfr and optrA located on one same plasmid in a strain have been reported. Fan R and Morroni G et al reported \textit{Staphylococcus sciuri} isolated from pig origin in Germany and \textit{E. faecium} isolates in Italy carrying both cfr and optrA.\textsuperscript{9,16} However, no conjugant or transformant was acquired to demonstrate the resistance mediated by cfr or optrA. Li et al also reported co-producing cfr and optrA of \textit{Staphylococcus sciuri}, and got the transformant with optrA.\textsuperscript{11} Similarly, two \textit{E. faecium} clinical isolates carrying cfr and optrA were collected in Italy, in which optrA was transferred from donor to recipient, whereas cfr was not transferrable.\textsuperscript{17}

In our study, we found cfr and optrA genes carried by \textit{E. faecalis} EF02 were located on two different plasmids, and both plasmids could be transferred from the donor strain to the recipient by transformation experiments, making an increase of MICs of linezolid and chloramphenicol. To our knowledge, this is the first report of linezolid-resistant \textit{E. faecalis} co-producing cfr and optrA in a clinical isolate in China.

**Abbreviations**

VRE, vancomycin-resistant \textit{Enterococcus} spp.

**Ethics Approval and Consent to Participate**

The study was approved by the Ethics Board of Fujian Medical University.

**Consent for Publication**

Not applicable.

**Funding**

This study was supported by the Science and Technology Project of Quanzhou (2019N030S) and China Antimicrobial Surveillance Network (WI207259). The funders had no role in study design, data collection and analysis, the decision to publish, or preparation of the manuscript.

**Disclosure**

The authors declare that they have no competing interests.
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