

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

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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*.¹ Although most of gram-positive cocci remain susceptible to linezolid, resistant isolates of *Enterococci* have been reported worldwide. The main resistance mechanisms of *Enterococcus spp.* to linezolid include the mutation of 23S rRNA gene,² acquired resistance genes such as *cfr*, *cfr(B)*, *optrA* or *poxtA*,^{3,4} and mutation of ribosomal proteins coding genes like *rplC*, *rplD*, and *rplV*.⁵ The *cfr* and *cfr(B)* genes encode a rRNA methyltransferase causing resistance of oxazolidinones, chloramphenicols, tetracycline, lincomycins, pleuro-mutilin, and streptogramin A and decreasing sensitivity of macrolide.^{3,4,6-9} *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.¹⁰⁻¹³ 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, isepamicin was used for the treatment of infection. Bacterial identification was conducted by MALDI-TOF (VITEK MS, BioMérieux). Antimicrobial susceptibility testing was performed by broth microdilution according to CLSI guideline.¹⁴ *E. faecalis* strain EF02 was resistant to linezolid (MIC =8 mg/L), nitrofurantoin (MIC =64 mg/L), tetracycline (MIC =64 mg/L), erythromycin (MIC =64 mg/L), chloramphenicol (MIC =64 mg/L) and levofloxacin (MIC =32 mg/L). However, this strain was susceptible to vancomycin (MIC =1 mg/L), teicoplanin (MIC =0.5 mg/L), and ampicillin (MIC =2 mg/L). PCR detection and

Table 1 MICs for *E. Faecalis* EF02, Transformants, and the Recipient Strains ($\mu\text{g}/\text{ML}$)

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

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 transformants 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

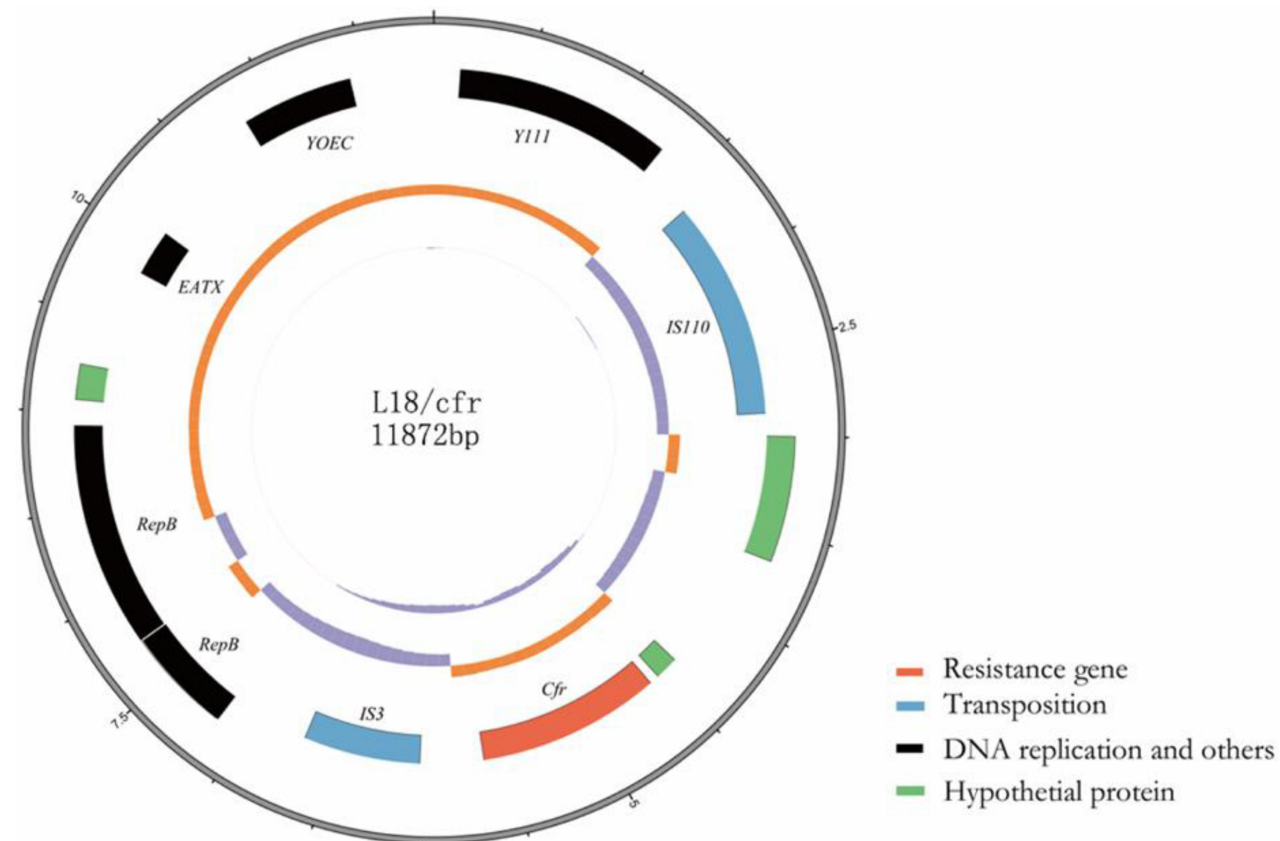


Figure 1 Circular representation of the L18/*cfr* plasmid. Moving from inside to outside in the plasmid circular map, slots 1–3 (slot 1, GC skew; slot 2, GC content; slot 3, open reading frames: Y-family DNA polymerase Y111, IS110 family transposase, *cfr*, IS3 family transposase, two replication protein RepB, antitoxin epsilon EATX, Probable integrase YOEC.).

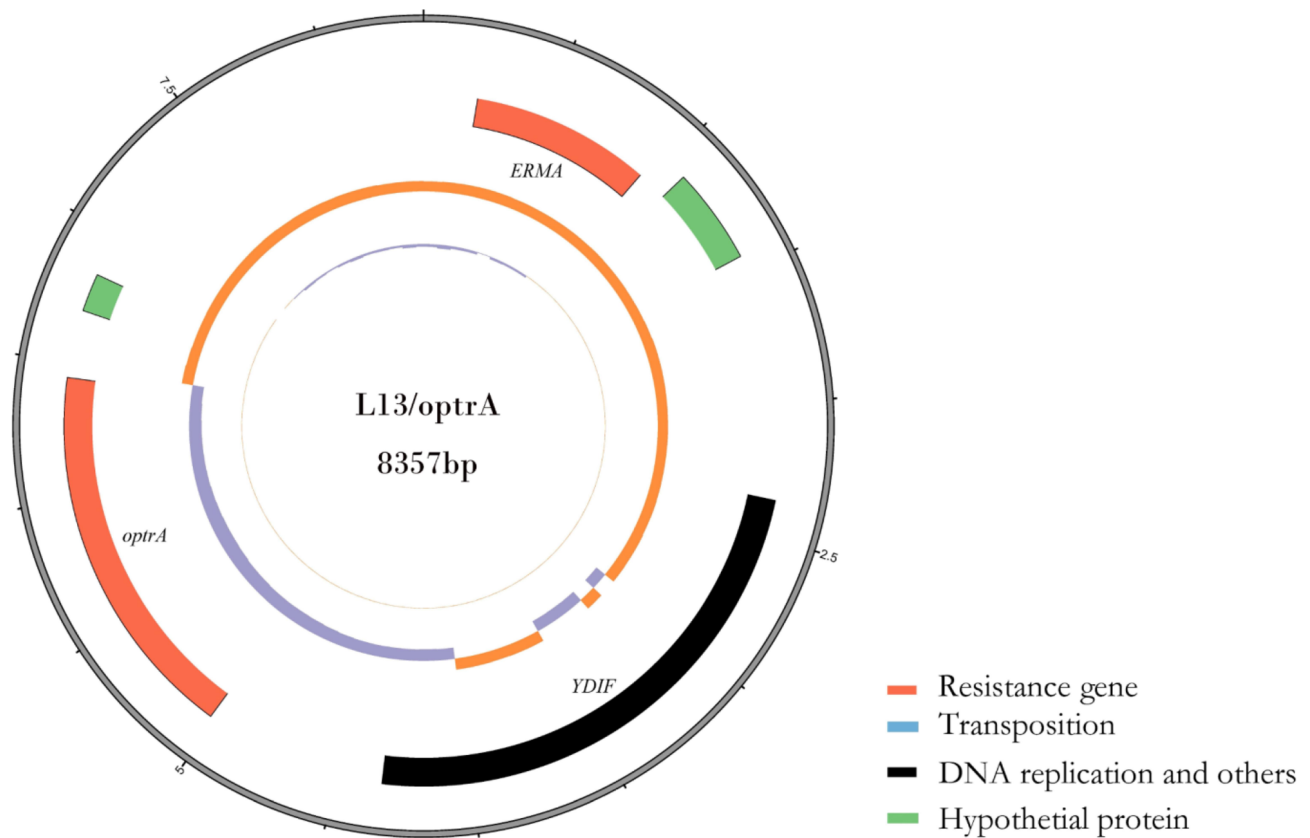


Figure 2 Circular representation of the L13/*optrA* plasmid. Moving from inside to outside in the plasmid circular map, slots 1–3 (slot 1, GC skew; slot 2, GC content; slot 3, open reading frames: 23S rRNA adenine(2058)-N(6)-methyltransferase ERMA, Putative ATP-binding protein YDIF, *optrA*).

increase in the MIC values of linezolid and chloramphenicol.^{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 *Staphylococcus sciuri* isolated from pig origin in Germany and *E. faecium* isolates in Italy carrying both *cfr* and *optrA*.^{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 *Staphylococcus sciuri*, and got the transformant with *optrA*.¹¹ Similarly, two *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.¹⁷

In our study, we found *cfr* and *optrA* genes carried by *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 *E. faecalis* co-producing *cfr* and *optrA* in a clinical isolate in China.

Abbreviations

VRE, vancomycin-resistant *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.

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Disclosure

The authors declare that they have no competing interests.

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