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SHORT REPORT

# 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 IS*110* transposase in upstream and an IS*3*-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 *poxt*A,[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, pleuromutilin, 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

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**Table 1** MICs for *E. Faecalis* EF02, Transformants, and the Recipient Strains (μg/ML)

| 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 |

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 electrotransformationmethod.[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 IS*110* transposase was located upstream of *cfr* and an IS*3*-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

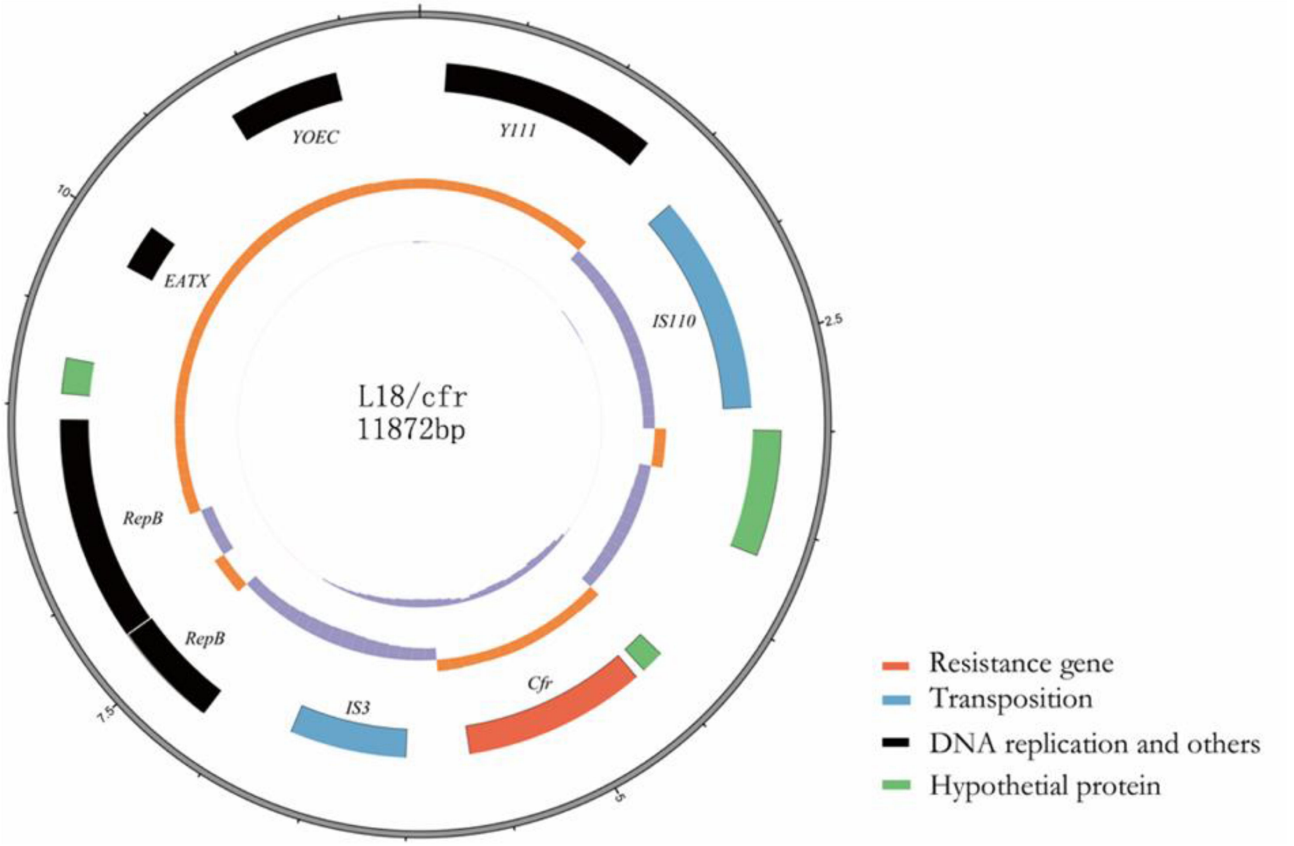


**Figure 1** Circular representation of the L18/*cfr* plasmid. Moving from inside to outside in the plasmid circular map, slots1–3 (slot 1, GC skew; slot 2, GC content; slot 3, open reading frames: Y-family DNA polymerase Y111, IS110 family transposase, *cfr*, IS3 family transposase, two replication protein RepB, antitoxin epsilon EATX, Probable integrase YOEC.).

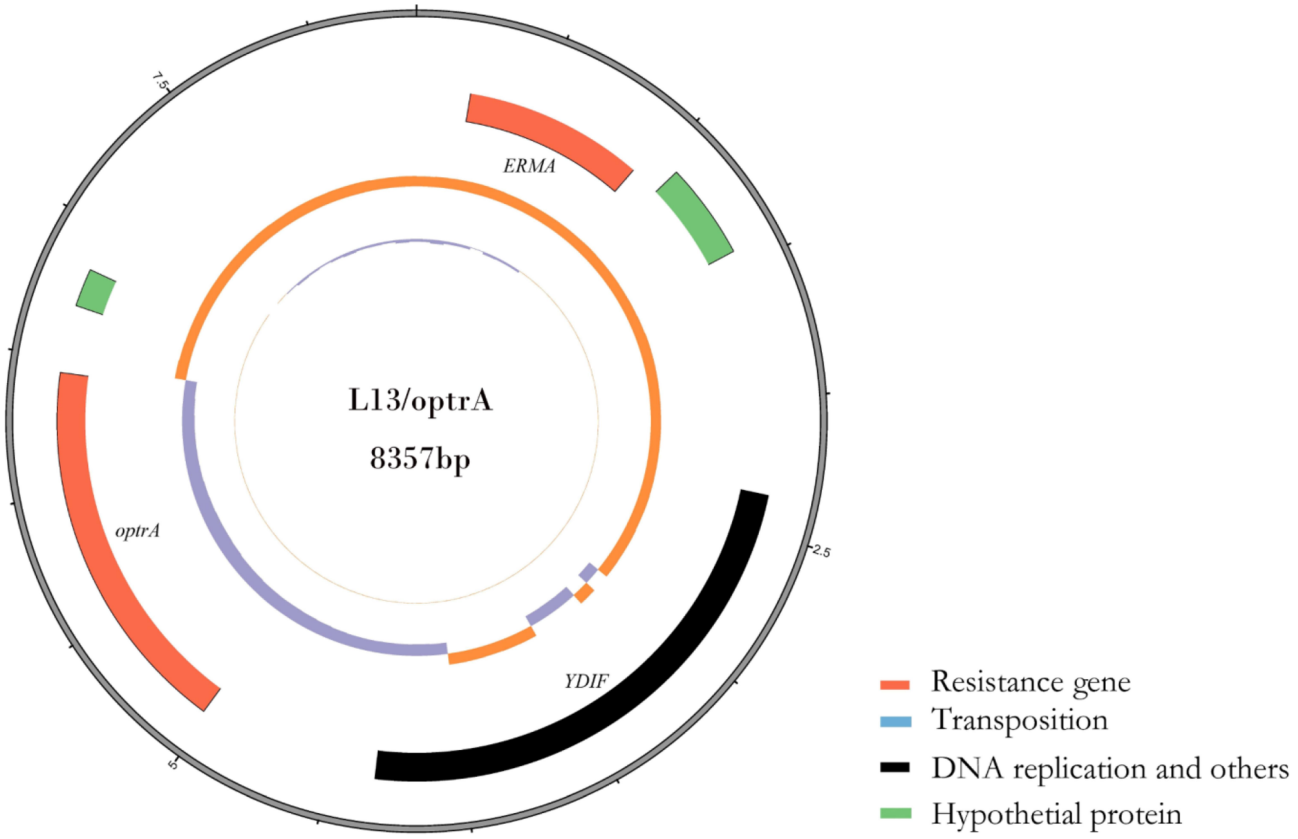


**Figure 2** Circular representation of the L13/*optrA* plasmid. Moving from inside to outside in the plasmid circular map, slots1–3 (slot 1, GC skew; slot 2, GC content; slot 3, open reading frames: 23S rRNA adenine(2058)-N(6)-methyltransferase ERMA, Putative ATP-binding protein YDIF, *optrA*).

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 *optr*A.[9,16] However, no conjugatant 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.

## References


1. Sadowy E. Linezolid resistance genes and genetic elements enhancing their dissemination in enterococci and streptococci. *Plasmid*. 2018;99:89–98.
2. Wong A, Reddy SP, Smyth DS, Aguero-Rosenfeld ME, Sakoulas G, Robinson DA. Polyphyletic emergence of linezolid-resistant staphylococci in the United States. *Antimicrob Agents Chemother*. 2010;54(2):742–748. doi:10.1128/AAC.00621-09
3. Liu Y, Wang Y, Schwarz S, et al. Transferable multiresistance plasmids carrying cfr in Enterococcus spp. from swine and farm environment. *Antimicrob Agents Chemother*. 2013;57:42–48. doi:10.1128/AAC.01605-12
4. Wang Y, Lv Y, Cai J, et al. A novel gene, optrA, that confers transferable resistance to oxazolidinones and phenicols and its presence in Enterococcus faecalis and Enterococcus faecium of human and animal origin. *J Antimicrob Chemother*. 2015;70:2182–2190. doi:10.1093/jac/dkv116
5. Mendes RE, Deshpande LM, Farrell DJ, Spanu T, Fadda G, Jones RN. Assessment of linezolid resistance mechanisms among Staphylococcus epidermidis causing bacteraemia in Rome, Italy. *J Antimicrob Chemother*. 2010;65:2329–2335. doi:10.1093/jac/dkq331
6. Schwarz S, Werckenthin C, Kehrenberg C. Identification of a plasmid-borne chloramphenicol-florfenicol resistance gene in Staphylococcus sciuri. *Antimicrob Agents Chemother*. 2000;44:2530–2533. doi:10.1128/AAC.44.9.2530-2533.2000
7. Vester B. The cfr and cfr-like multiple resistance genes. *Res Microbiol*. 2018;169:61–66. doi:10.1016/j.resmic.2017.12.003
8. Antonelli A, D'Andrea MM, Brenciani A, et al. Characterization of poxtA, a novel phenicol-oxazolidinone-tetracycline resistance gene from an MRSA of clinical origin. *J Antimicrob Chemother*. 2018;73:1763–1769. doi:10.1093/jac/dky088
9. Fan R, Li D, Fessler AT, Wu C, Schwarz S, Wang Y. Distribution of optrA and cfr in florfenicol-resistant Staphylococcus sciuri of pig origin. *Vet Microbiol*. 2017;210:43–48. doi:10.1016/j.vetmic.2017.07.030
10. Kuroda M, Sekizuka T, Matsui H, et al. Complete genome sequence and characterization of linezolid-resistant enterococcus faecalis clinical isolate KUB3006 carrying a cfr(b)-transposon on its chromosome and optrA-Plasmid. *Front Microbiol*. 2018;9:2576. doi:10.3389/fmicb.2018.02576
11. Li D, Wang Y, Schwarz S, Cai J, Shen J. Co-location of the oxazolidinone resistance genes optrA and cfr on a multiresistance plasmid from Staphylococcus sciuri. *J Antimicrob Chemother*. 2016;71:1474. doi:10.1093/jac/dkw040
12. Lazaris A, Coleman DC, Kearns AM, et al. Novel multiresistancecfr plasmids in linezolid-resistant methicillin-resistant Staphylococcus epidermidis and vancomycin-resistant Enterococcus faecium (VRE) from a hospital outbreak: co-location of cfr and optrA in VRE. *J Antimicrob Chemother*. 2017;72:3252–3257.
13. Hao W, Shan X, Li D, et al. Analysis of a poxtA- and optrA-co-carrying conjugative multiresistance plasmid from Enterococcus faecalis. *J Antimicrob Chemother*. 2019;74:1771–1775. doi:10.1093/jac/dkz109
14. Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing.,* 30th ed. Wayne, PA: CLSI supplement M100. Clinical and Laboratory Standards Institute; 2020.
15. Shen J, Wang Y, Schwarz S. Presence and dissemination of the multiresistance gene cfr in Gram-positive and Gram-negative bacteria. *J Antimicrob Chemother*. 2013;68:1697–1706. doi:10.1093/jac/dkt092
16. Morroni G, Brenciani A, Antonelli A, et al. Characterization of a multiresistance plasmid carrying the optra and cfr resistance genes from an enterococcus faecium clinical isolate. *Front Microbiol*. 2018;9:2189. doi:10.3389/fmicb.2018.02189
17. Brenciani A, Morroni G, Vincenzi C, et al. Detection in Italy of two clinical Enterococcus faecium isolates carrying both the oxazolidinone and phenicol resistance gene optrA and a silent multiresistance gene cfr. *J Antimicrob Chemother*. 2016;71:1118–1119. doi:10.1093/jac/dkv438