Emergence of Linezolid Resistant Enterococcus Faecalis Co-producing bla_{cfr} and bla_{optr}A in China

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Abstract

An Enterococcus Faecalis strain was isolated from patient in china and exhibited resistance to linezolid (8μg/ml). The strain was positive for bla_{cfr} and bla_{optr}A without mutation among bla_{optr} and rplV. Transferability of bla_{cfr} and bla_{optr}A were investigated by transformation. bla_{cfr} and bla_{optr}A were located on two different plasmids, and both could be transferred from the donor strain, making the MICs of linezolid and chloramphenicol against the recipient strain to increase. Plasmid DNA sequencing of the L18/bla_{cfr} plasmid suggested that an IS110 transposase was located upstream of bla_{cfr}. To the best of our knowledge, this is the first report of one linezolid-resistant Enterococcus Faecalis strain isolated from the patient, and co-produced bla_{cfr} and bla_{optr}A without mutation among bla_{optr} and rplV in China.

Keywords: Enterococcus Faecalis; blacfr; blaoptrA; Linezolid

Introduction

Linezolid is considered as a last resort drug in treatment of severe infections caused by multi-drug-resistant gram-positive pathogens, such as vancomycin-resistant Enterococcus spp. (VRE), methicillin-resistant Staphylococcus spp. and Streptococcus pneumoniae [1]. Although most gram-positive pathological bacteria remain susceptible to linezolid, resistant isolates of Enterococcus have been reported worldwide. The resistance mechanism of Enterococcus spp. to linezolid is in addition to the mutation of 23S rRNA gene, and the acquired resistance genes such as bla_{cfr}, bla_{optr}A, bla_{cfr} and bla_{optr}A. The bla_{cfr} and bla_{optr}A genes encode a rRNA methyltransferase cause oxazolidinones, chloramphenicol, tetracycline, lincomycins, pleuromutilin and streptavidin A resistance and macrolide sensitivity decreased [2-7]. Until now, the co-occurrence of bla_{cfr} or bla_{optr}A and bla_{optr}A along with bla_{optr}A has been reported on the same plasmid in Enterococcus faecalis, Staphylococcus sciuri or Enterococcus spp. from swine and farm environment [8-11]. Here, we firstly reported the emergence of linezolid-resistant Enterococcus faecalis clinical strain co-producing bla_{optr}A and bla_{optr}A which were located on different plasmids in China.

Case Report

Enterococcus faecalis strain EF02 was isolated from a 72-year-old patient in during hospitalization on November 19th, 2018, in China. Strain identification was conducted by VITEK MS (French Mérieux). The strains exhibited resistance to linezolid (8μg/ml) by broth microdilution. PCR detection and sequencing of 23S rRNA V region on the chromosome and the ribosomal protein coding genes bla_{apc}, bla_{prt}, bla_{optr}A and bla_{optr}A. Enterococcus faecalis strain EF02 was positive for bla_{optr}A without mutation among bla_{optr} and bla_{optr}A. The plasmids extracted from the donor strain Enterococcus faecalis EF02 were then guided into the recipient strain Enterococcus faecalis OG1RF by the electro-transformation method. The transformants were selected on brain heart infusion (BHI) plates containing...
3mg/L linezolid and 10mg/L chloramphenicol. Colonies that grew on these selective plates were further confirmed by antimicrobial susceptibility testing and PCR for the detection bla\textsubscript{ap} and bla\textsubscript{ap} genes. Transformants harboring the plasmid with bla\textsubscript{ap} and bla\textsubscript{ap} were successfully obtained, the linezolid and chloramphenicol MICs of transformants increased 4-8-fold, compared with the recipient strain (Table 1). PCR mapping according to plasmid DNA sequencing of the L18/bla\textsubscript{ap} plasmid suggested that an IS110 transposase was located upstream of bla\textsubscript{ap} and an IS3-like transposase was located downstream of bla\textsubscript{ap}. L18/bla\textsubscript{ap} plasmid contained eight open reading frames including Y111, IS110, bla\textsubscript{ap}, IS3, RepB, RepB, EATX, and YOEC (Figure 1). However, L13/bla\textsubscript{ap} plasmid, which contained three open reading frames including ERMA, YDIF and bla\textsubscript{ap} (Figure 2).

**Figure 1**: Plasmid DNA sequencing of the L18/cfr plasmid. Y-family DNA polymerase. Y111, IS110 family transposase, cfr, IS3 family transposase, two replication protein RepB, EATX, and YOEC.

**Figure 2**: Plasmid DNA sequencing of the L13/optrA plasmid. 23S rRNA adenine(2058)-N(6)-methyltransferase Erm(A), YDIF, optrA.

**Discussion**

bla\textsubscript{ap} and bla\textsubscript{ap} genes have been detected in various gram-positive bacteria, they can be transferred to recipient bacteria by transformation experiments and cause an increase in the MIC values of linezolid and chloramphenicol [3,5,12]. The co-producing of bla\textsubscript{ap} and bla\textsubscript{ap} located on one plasmid in a strain has been reported. Fan R [7] and Morrone G et al. [13]. reported Staphylococcus sciuri isolated from pig origin in Germany and Enterococcus faecium isolates in Italy carrying both bla\textsubscript{ap} and bla\textsubscript{optrA}. However, no conjugating or transformant was acquired to demonstrate the resistance mediated by bla\textsubscript{ap} or bla\textsubscript{optrA} [7,13]. Li [9] also reported co-producing bla\textsubscript{ap} and bla\textsubscript{optrA} of Staphylococcus sciuri and got the transformant with bla\textsubscript{optrA} [9]. Similarly, two Enterococcus faecium clinical isolates carrying bla\textsubscript{ap} and bla\textsubscript{optrA} were collected in Italy, in which bla\textsubscript{optrA} was transferred from donor to recipient, whereas bla\textsubscript{ap} was not transferrable [14]. In our study, we found bla\textsubscript{ap} and bla\textsubscript{optrA} genes carried by Enterococcus 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 Enterococcus faecalis co-producing bla\textsubscript{ap} and bla\textsubscript{optrA} in a clinical isolate in China.

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**Availability of Data and Materials**

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

**Contribution**

The study was planned and designed by ZZ, FH, DM, QC. QC, DY, PL, YG, DM, YL, XY collected the data and interpretation of the results. The manuscript was prepared by QC. All authors contributed to and commented on the manuscript. All authors read and approved the final manuscript.

**Ethics Approval and Consent to Participate**

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

**References**


