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Molecular Epidemiology and Horizontal Transfer Mechanism of *optrA*-Carrying Linezolid-Resistant *Enterococcus faecalis*

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Abstract

The aim of this work was to provide a theoretical and scientific basis for the treatment, prevention, and control of clinical drug-resistant bacterial infections by studying the molecular epidemiology and horizontal transfer mechanism of *optrA*-carrying linezolid-resistant *Enterococcus faecalis* strains (LREfs) that were clinically isolated in a tertiary hospital in Kunming, China. Non-repetitive LREfs retained in a tertiary A hospital in Kunming, China. The strains were identified by Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). The transferability and horizontal transfer mechanism of *optrA* gene were analyzed using polymerase chain reaction (PCR), whole-genome sequencing (WGS), and conjugation experiments. A total of 39 LREfs strains were collected, and all of them were multi-drug resistant. There were 30 LREfs strains (76.9%) carrying the *optrA* gene, The *cfr*, *poxtA* genes and mutations in the 23S rRNA gene were not detected. The conjugation experiments showed that only three of 10 randomly selected *optrA*-carrying LREfs were successfully conjugated with JH2-2. Further analysis of one successfully conjugated strain revealed that the *optrA* gene, located in the donor bacterium, formed the *IS1216E-erm(A)-optrA-fexA-IS1216E* transferable fragment under the mediation of the mobile genetic element (MGE) *IS1216E*, which was then transferred to the recipient bacterium via horizontal plasmid transfer. Carrying the *optrA* gene is the primary resistance mechanism of LREfs strains. The *optrA* gene could carry the *erm(A)* and *fexA* genes to co-transfer among *E. faecalis*. MGEs such as insertion sequence *IS1216E* play an important role in the horizontal transfer of the *optrA* gene.

K e y w o r d s: *Enterococcus faecalis*, horizontal transfer, linezolid, *optrA*, whole genome sequencing

Introduction

Enterococcus faecalis is a facultative anaerobic Gram-positive bacterium that is widely found in the natural environment and the gastrointestinal tract of humans and animals and is considered one of the common pathogens responsible for urinary tract infections, endocarditis and hospital-associated infections (Carvalhaes et al. 2020). The China Antimicrobial Surveillance Network (CHINET) predicted that *E. faecalis* would become the third most-common Gram-positive bacterium and the fifth most-common pathogen of health care-associated infections in the world by 2020 (Weiner et al. 2016; Weiner-Lastinger et al. 2020).

Linezolid, the first synthetic oxazolidinone antimicrobial drug approved by the Food and Drug Administration for clinical use in 2000, binds to the V domain of the 23S rRNA component in the bacterial 50S ribosomal subunit. As a result, it inhibits the initial assembly of ribosomes and protein synthesis in various Gram-positive bacteria, thereby achieving an antimicrobial effect (Egan et al. 2020). Because of its unique mechanism of action and broad antimicrobial spectrum, linezolid is considered a drug of last resort for the treatment of serious infections caused by multidrug-resistant Gram-positive bacteria, particularly those caused by vancomycin-resistant enterococci (VRE), methicillin-resistant *Staphylococcus aureus* (MRSA), and penicillin-resistant *Streptococcus pneumoniae* (PRSP) (Ma et al. 2021). However, since linezolid was introduced into clinical use, the presence of linezolid-resistant enterococci (LRE) has frequently been

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reported. It has become a major challenge for clinical care and public health.

To date, two mechanisms of *Enterococcus* resistance to linezolid have been identified. The first is through chromosomal mutations, including mutations in 23S rRNA binding sites (e.g., G2576U, G2447U, and G2504A) and mutations in genes coding for 50S ribosomal proteins (e.g., *rplC*, *rplD*, and *rplV* in L3, L4, and L22*)*. The second is through the acquisition of transferable oxazolidinone-resistance genes, including the *cfr* gene family, the *optrA* and *poxtA* genes, which can be transferred between enterococci and other Gram-positive bacteria. They are mediated by mobile genetic elements (MGEs), such as plasmids, transposons, integrons, and insertion sequences (IS) (Hu et al. 2022; Lei et al. 2021). The *optrA* gene encodes the ATP-binding cassette (ABC)-F protein, which generates cross-resistance to linezolid and florfenicol by protecting the bacterial ribosome from the antibiotic inhibition (Sharkey et al. 2016). Since the first discovery of the *optrA* gene in *E. faecalis* and *E. faecium* isolated from humans and food-borne animals in China in 2015 (Wang et al. 2015), the gene has been detected in the plasmid or chromosomal DNA of enterococci isolated from human and food animal sources (Sadowy et al. 2018) but has mainly been localized to plasmids. The *optrA* gene can exhibit different genetic characteristics depending on their locations. When the *optrA* gene is located on the chromosome, it is typically found on the Tn*6674* transposon, which belongs to the Tn*554* family (Li et al. 2019). When the gene is present on plasmids, it is usually located upstream or downstream of insertion sequence elements of the IS6 family or ISL3 family (Freitas et al. 2020).

Although the vast majority of Gram-positive bacteria are susceptible to linezolid and its resistance remains uncommon, different antimicrobial surveillance studies or programs in recent years have shown an increase in the number of LRE strains (Cui et al. 2016; Deshpande et al. 2018; Mendes et al. 2018). For example, according to the latest data from the German Antibiotic Resistance Surveillance System, the LRE detection rate increased from 0.6% in 2019 to 1.2% in 2021 (Bender et al. 2024). The increase in the LRE detection rate warrants marked attention. Horizontal transfer of the transferable *optrA* gene has been recognized as an important mechanism of *Enterococcus* resistance to linezolid; thus, analyzing the molecular characteristics and transmission mechanism of *optrA*-carrying LRE is of great value for epidemiological studies.

In this study, 30 strains of *optrA*-carrying LREfs collected from September 2018 to July 2022 in a tertiary hospital in Kunming were used to analyze the molecular characteristics and clonal transmission of LREfs, as well as the horizontal transfer mechanism of the *optrA* gene to lay a basis for the monitoring and control of LREs.

Experimental

Materials and Methods

Bacterial isolation and species identification. Non-repetitive *Enterococcus* strains were isolated in a tertiary hospital in Kunming from September 2018 through September 2022 and frozen in a −80°C freezer. Strains had been isolated from different sample types, such as urine, secretions, drainage fluid, pus, bile, and puncture fluid. *E. faecalis* was identified using a MALDI-TOF MS mass spectrometer (Microflex® LRF; Bruker Daltonics GmbH & Co., Germany). The JH2-2 strain (rifampicin-resistant) of *E. faecalis* was kindly provided by Prof. Tieli Zhou (Wenzhou Medical University, China).

Antimicrobial susceptibility testing. Antimicrobial susceptibility testing was performed on 39 strains identified by MALDI-TOF MS as *E. faecalis*. A VITEK® 2 compact automated microbiology analyzer (bioMériux, France) and accompanying AST-GP67 cards (bioMériux, France) were used to determine the susceptibility of *E. faecalis* strains towards 14 antibiotics, including linezolid, ampicillin, tetracycline, erythromycin, nitrofurantoin, vancomycin, clindamycin, gentamicin-high concentration, ciprofloxacin, levofloxacin, moxifloxacin, streptomycin-high concentration, and tigecycline. The susceptibility results were interpreted according to the recommended criteria in the Clinical and Laboratory Standards Institute M100-S32 document (CLSI 2022), the composition of the AST-GP67 card, drug names, and MIC ranges tested are shown in Table I.

Additional confirmation of all LREfs was performed by the disk-diffusion susceptibility test. The antibiotics used in the disk-diffusion susceptibility test were chloramphenicol, teicoplanin, and minocycline, all at a concentration of 30 μg. To determine the minimum inhibitory concentration (MIC) of linezolid against *E. faecalis*, the broth microdilution method was employed to verify its linezolid MIC value. According to the CLSI interpretation guidelines, *E. faecalis* with a linezolid MIC value of ≤ 2 μg/ml is considered as sensitive to linezolid and a MIC value of ≥ 8 μg/ml is considered resistant to linezolid. The reference strain of *E. faecalis* ATCC® 29212™, was used as the quality control strain.

Polymerase chain reaction. Polymerase chain reaction (PCR) was used to detect the *optrA* gene in 39 LREfs. Primers for PCR were synthesized by the Beijing Tsingke Biotech Co., Ltd. (China), after referring to the target gene sequences in GeneBank and reviewing the related literature. The primer sequences (5'–3') and sizes used were as follows: F: TTGTCCAAAGCCACCTTTGCAA, R: AACTCTACACCATCCTTATCAAAAAC, and the product size was 1,968 bp. All amplifications were carried out in a T100™ thermocycler (Bio-Rad, USA). The amplification conditions were as

Table I
The composition of the AST-GP67 card, drug names, and MIC ranges tested.

Antibiotics	Abbreviations	Measurement range (μg/ml)
1 Linezolid	LZD	0.5–8
2 Ampicillin	AMP	2–32
3 Penicillin	PEN	0.12–64
4 Erythromycin	ERY	0.25–8
5 Clindamycin	CLI	0.25–8
6 Tigecycline	TGC	0.12–2
7 Tetracycline	TET	1–16
8 Gentamicin – high concentration	GEH	S/R
9 Streptomycin – high concentration	STH	S/R
10 Ciprofloxacin	CIP	0.5–8
11 Levofloxacin	LVX	0.12–8
12 Moxifloxacin	MFX	0.25–8
13 Vancomycin	VAN	0.5
14 Nitrofurantoin	NIT	16–512

GEH – the high concentrations of gentamicin is 500 μg/ml,
STH – the high concentrations of streptomycin is 1,000 μg/ml
S – susceptible, R – resistant

follows: pre-denaturation at 98°C for 2 min; 35 cycles each consisting of denaturation (98°C for 10 s), annealing (63°C for 10 s), and extension (72°C for 2 min); and a final extension at 72°C for 5 min. The PCR-positive products underwent Sanger sequencing by the Beijing Qingke Biotech Co., Ltd. (China). The sequencing results were compared with the sequence of linezolid-sensitive wild-type *E. faecalis* strain ATCC® 29212™ (GenBank accession No. CP008816) via NCBI database (https://blast.ncbi.nlm.nih.gov).

Whole-genome sequencing and bioinformatics analysis. Genomic DNA was extracted from *optrA*-carrying LREfs using the TSINGKE Genomic DNA Extraction Kit (Beijing Qingke Biotechnology Co., Ltd., China). Its concentration and purity were determined. The whole-genome sequencing (WGS) was performed by Shanghai Meiji Biotech Co., Ltd. (China), using the Illumina® NovaSeq™ 6000 platform (Illumina, Inc., USA). Data filtering was conducted by Shanghai Meiji Biotech Co., Ltd., with sequencing results assembled using SPAdes Software (https://ablab.github.io/spades) and annotations of the assembled sequences via the RAST server (Aziz et al. 2008). The online tools ResFinder 4.1 (Bortolaia et al. 2020), LRE-Finder (Hasman et al. 2019), VirulenceFinder 2.0 (Joensen et al. 2014), and MLST 2.0 (Larsen et al. 2012) from the Center for Genome Epidemiology (https://cge.cbs.dtu.dk) were used to predict the resistance and virulence genes, mutations in 23SrRNA, and sequence types (STs) of the strains based on the Illumina WGS results. Phylogenetic trees were constructed using MEGA X software (Kumar et al. 2018), employing the ClustalW alignment method and the neighbor-joining (NJ) tree-building method, with 1,000 bootstraps. SNP analyses were performed using the Center for Genomic Epidemiology online site CSI Phylogeny 1.4 tool, using the default options and the A1 genome as the reference genome. The SNP distance matrix was obtained and an evolutionary tree visualization map was formed using the SNP comparison file (Kaas et al. 2014).

Conjugation experiments. Ten randomly selected *optrA*-carrying LREfs were used as donors and rifampicin-resistant *E. faecalis* JH2-2 as a recipient for filter mating and broth mating experiments. Transconjugants were screened on brain heart infusion agar containing forfenicol (16 mg/l) and rifampicin (30 mg/l). Conjugation frequency was determined by normalizing the colony formation units (CFUs) of transconjugants to those of recipients (Fioriti et al. 2021). Drug sensitivity testing of transconjugants were also performed using a VITEK® 2 Compact fully automated microbiological analyzer and an accompanying Gram-positive cocci AST-GP67 drug sensitivity card to determine the sensitivity of six antibiotics: linezolid, erythromycin, clindamycin, tetracycline, gentamicin-high concentration, and streptomycin-high concentration. The drug sensitivity results were interpreted according to the CLSI (2022). In addition, the susceptibility of *E. faecalis* to chloramphenicol was determined by a disk-diffusion susceptibility test with chloramphenicol at 30 μg. The broth microdilution method was used to detect the MIC of *E. faecalis* to linezolid, rifampicin, and florfenicol.

Analysis of the mechanism of horizontal transfer of the *optrA* gene. Randomly selected donor organism *E. faecalis* B2 and the resulting transc-onjugant *E. faecalis* TC-B2 from the successful conjugation experiment were sequenced using a long-read SMRT technology (Pacific Biosciences, USA). Data filtering i-ngwas was conducted by Shanghai Meiji Biotech Co., Ltd., with sequencing results assembled using SPAdes software and annotations performed on the assembled sequences via the RAST server. Easyfig was utilized for the comparative analysis of the linear structure of the plasmid genomes (Sullivan et al. 2011).

Results

Bacteria collection and identification. Thirty-nine strains identified as *E. faecalis* by MALDI-TOF MS and with MIC values ≥ 8 μg/ml against linezolid were collected from September 2018 to July 2022. Most of the 39 LREfs originated from the Urology Department

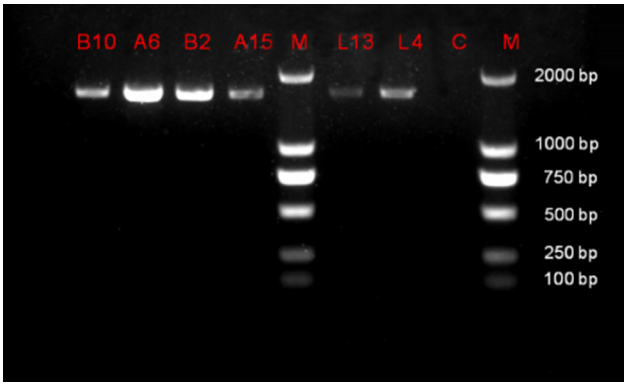

Fig. 1. Electrophoresis of polymerase chain reaction products of the *optrA* gene in the selected strains: M is a DNA Marker, C is a blank control; B10, A6, B2, A15, L13, L4 are strain numbers. Marker bands from top to bottom are in the order of 2,000 bp, 1,000 bp, 750 bp, 500 bp, 250 bp, 100 bp.

(44%, 17/39) and the Intensive Care Unit (21%, 8/39). Specimens included urine (57%, 22/39), drainage fluid (15%, 6/39), wound secretions (13%, 5/39), pus (5%, 2/39), blood (5%, 2/39), and bile (5%, 2/39).

LREfs resistance phenotypes. The drug susceptibility results for the 39 LREfs were as follows. The MIC values of linezolid against *E. faecalis* strains were 8 μg/ml, except for four strains with MICs of 16 μg/ml. No high-level resistance was found. All LREfs in this study were multi-resistant strains with the following resistance rates: linezolid (n = 39, 100%), tetracycline (n = 39, 100%), clindamycin (n = 39, 100%), minocycline (n = 37, 94.9%), and erythromycin (n = 38, 97.4%), Streptomycin-high concentration (n = 14, 35.9%), chloramphenicol (n = 34, 87.2%), gentamicin-high concentration (n = 28, 71.8%), ciprofloxacin (n = 22, 56.4%), levofloxacin (n = 22, 56.4%), and moxifloxacin (n = 21, 53.8%). Fewer strains were resistant to streptomycin (n = 14, 35.1%) and nitrofurantoin (n = 1, 2.6%). All isolates were susceptible to ampicillin, vancomycin, tigecycline, and teicoplanin.

Detection of the *optrA* gene in LREfs. PCR results showed that 30 of 39 LREfs carried the *optrA* gene, accounting for 76.9% (30/39) of all strains. The electrophoretic profiles of the *optrA* gene of some strains are shown in Fig. 1. The sequencing results of the *optrA* gene-positive PCR products were compared using BLAST in the NCBI database, and were found to have high similarity with the sequence of the *optrA* gene carried by the conjugative plasmid pE349 (GenBank accession No. KP399637), with 99% identity.

Detection of drug-resistance genes. WGS was performed on 30 *optrA*-carrying LREfs, and drug-resistance genes and 23S rRNA mutations were detected based on the sequencing results, as detailed in Table II. The trimethoprim/sulfamethoxazole-resistance gene, dfrG, was detected in all strains, and the tetracycline-resistance gene, *tet(M)*, had a carriage rate of 93.3% (28/30), followed by the chloramphenicol-resistance gene *fexA* with a carriage rate of 80% (24/30), macrolide-resistance gene *erm(A)* with a carriage rate of 80% (24/30), *erm(B)* 76.7% (23/30), aminoglycoside-resistance gene *aac(6')-aph(2")* with a carriage rate of 60% (18/30), *ant(6')-la* 46.7% (14/30), and *aph(3')-III* with a carriage rate of 50% (15/30). The *cfr*, *cfr(B)*, and *poxtA* genes, as well as mutations in the V domain of the 23S rRNA gene were not detected in the LREfs.

Virulence gene analysis. Twenty different virulence genes were detected in 30 *optrA* gene-positive LREfs, as detailed in Table II. These virulence genes included biofilm-associated pilus genes (*ebpB*, *ebpA*, *ebpC*), sex pheromone genes (*ccf*, *cob*, *cad*), cytolysin genes (*cylA*, *cylL*, *cylM*), bacterial adhesin genes (*efaFs* and *ace*), thiol peroxidase gene (*tpx*), gelatinase gene (*gelE*), and hyaluronidases genes (*hylA*, *hylB*), and others. Of the 20 virulence genes, 10 (*elrA*, *srtA*, *ace*, *efaFs*, *ccf*, *cob*, *cad*, *camE*, *ebpA*, and *tpx*) were detected in the 30 *optrA* gene-positive LREfs.

Multilocus sequence typing. ST determination and phylogenetic tree construction of 30 *optrA* gene-positive LREfs were carried out using Multilocus Sequence Typing (MLST) v2.0, with the phylogenetic tree shown in Fig. 2. Twenty-one STs were identified, with ST16 being the predominant clonal lineage, accounting for 30.0% (8/30), followed by ST585, accounting for 10.0% (3/30). The other STs included: ST330, ST974, ST123, ST69, ST535, ST1287, ST619, ST207, ST632, ST816, ST968, ST300, ST179, ST660, ST631, ST506, ST902, and ST480.

The phylogenetic tree of 30 *optrA*-positive LREfs showed that A9, A8, A13, A15, A7, A1, B2, B13 belonging to the ST16 type and B12 belonging to the ST179 type were located on the same branch, and the alleles of the above nine *E. faecalis* strains were further analyzed. The results showed that the alleles of ST16-type strains were all: gdh(5), gyd(1), pstS(1), gki(3), aroE(7), xpt(7), yqiL(6); whereas, the allele type of ST179 was gdh(5), gyd(1), pstS(1), gki(3), aroE(7), xpt(1), yqiL(6), and there was only a single allele difference between the two strains. It might have led to the fact that the B12 strain (ST179) was located in the cluster of ST16 in the evolutionary tree.

SNP analysis. Evolutionary tree visualization plot and SNP distance matrix (Fig. S1) analysis revealed that A9, A8, A13, A15, A7, A1, B2, B12, and B13, which are all ST16-type, are closely related and located on the same branch. Among the five strains of LREfs collected in 2019: A9, A8, A13, A15, and A7, they were isolated from the same ward (urology department) within 4 months, a result that suggests that ST16-type strains of LREfs may be prevalent and transmitted on a small scale within the Urology Department of this hospital.

Table II
Characteristics of 30 *optrA*-positive linezolid-resistant *Enterococcus faecalis*.

Isolate	Ward	Type of specimen	Isolation time	ST	LZD MIC (ug/ml)	Resistance genes	Virulence genes
1 A1	urology	blood	2018/10/19	16	8	*aac(6')-aph(2")*, *str*, *erm(A)*, *erm(B)*, *lsa(A)*, *fexA*, *cat*, *tet(M)*, *dfrG*	*elrA*, *srtA*, *ace*, *agg*, *ccf*, *cob*, *cad*, *camE*, *ebpA*, *ebpC*, *tpx*, *efaAfs*, *hylA*, *cylA*, *cylL*, *cylM*
2 A2	urology	urine	2018/11/02	585	8	*aac(6')-aph(2")*, *erm(A)*, *erm(B)*, *fexA*, *tet(M)*, *dfrG*	*elrA*, *srtA*, *ace*, *agg*, *ccf*, *cob*, *cad*, *camE*, *ebpA*, *ebpC*, *tpx*, *efaAfs*, *cylA*, *cylL*, *cylM*, *fsrB*, *gelE*
3 A3	urology	urine	2018/11/18	330	16	*aac(6')-aph(2")*, *aph(3')-III*, *Str*, *erm(A)*, *lnu(G)*, *fexA*, *cat*, *tet(M)*, *dfrG*	*elrA*, *srtA*, *ace*, *ccf*, *cob*, *cad*, *camE*, *ebpA*, *ebpC*, *tpx*, *efaAfs*, *fsrB*, *gelE*, *hylA*, *hylB*
4 A5	urology	urine	2019/01/12	974	8	*erm(A)*, *erm(B)*, *fexA*, *tet(M)*, *dfrG*	*elrA*, *srtA*, *ace*, *agg*, *ccf*, *cob*, *cad*, *camE*, *ebpA*, *ebpC*, *tpx*, *efaAfs*, *fsrB*, *gelE*, *hylA*, *hylB*, *cylA*, *cylL*, *cylM*
5 A6	ICU	drainage	2019/01/17	123	8	*aph(3')-III*, *erm(B)*, *cat*, *fexA*, *tet(L)*, *dfrG*	*elrA*, *srtA*, *ace*, *ccf*, *cob*, *cad*, *camE*, *ebpA*, *ebpC*, *tpx*, *efaAfs*, *fsrB*, *gelE*, *hylA*, *hylB*
6 A7	urology	urine	2019/03/13	16	8	*ant(6')-la*, *erm(A)*, *erm(B)*, *lsa(A)*, *fexA*, *cat*, *tet(M)*, *dfrG*	*elrA*, *srtA*, *ace*, *agg*, *ccf*, *cob*, *cad*, *camE*, *cylA*, *cylL*, *cylM*, *ebpA*, *ebpC*, *tpx*, *efaAfs*, *hylA*
7 A8	ICU	urine	2019/03/17	16	8	*aac(6')-aph(2")*, *ant(6')-la*, *erm(A)*, *erm(B)*, *lsa(E)*, *fexA*, *cat*, *tet(M)*	*elrA*, *srtA*, *ace*, *agg*, *ccf*, *cob*, *cad*, *camE*, *cylA*, *cylL*, *cylM*, *ebpA*, *ebpC*, *tpx*, *efaAfs*, *hylA*
8 A9	urology	urine	2019/04/09	16	8	*aac(6')-aph(2")*, *erm(B)*, *lsa(A)*, *fexA*, *tet(M)*, *dfrG*	*elrA*, *srtA*, *ace*, *ccf*, *cob*, *cad*, *camE*, *cylA*, *cylL*, *cylM*, *ebpA*, *ebpC*, *tpx*, *efaAfs*, *hylA*, *fsrB*, *gelE*
9 A10	urology	urine	2019/04/22	69	8	*aac(6')-aph(2")*, *aph(3')-III*, *erm(A)*, *erm(B)*, *lsa(A)*, *lsa(E)*, *fexA*, *cat(PC233)*, *tet(M)*, *dfrG*	*elrA*, *srtA*, *ace*, *agg*, *ccf*, *cob*, *cad*, *camE*, *cylA*, *cylL*, *cylM*, *ebpA*, *ebpC*, *tpx*, *efaAfs*, *hylB*, *fsrB*, *gelE*
10 A13	urology	urine	2019/07/01	16	8	*erm(A)*, *lsa(A)*, *fexA*, *tet(M)*	*elrA*, *srtA*, *ace*, *agg*, *ccf*, *cob*, *cad*, *camE*, *cylA*, *cylL*, *cylM*, *ebpA*, *ebpC*, *tpx*, *efaAfs*, *hylA*
11 A15	urology	urine	2019/07/23	16	8	*aac(6')-aph(2")*, *aph(3')-III*, *erm(A)*, *erm(B)*, *lsa(A)*, *fexA*, *tet(M)*, *dfrG*	*elrA*, *srtA*, *ace*, *agg*, *ccf*, *cob*, *cad*, *camE*, *ebpA*, *ebpC*, *tpx*, *efaAfs*, *hylA*, *cylA*, *cylL*, *cylM*
12 A20	general surgery	drainage	2019/12/20	902	8	*aac(6')-aph(2")*, *ant(6')-la*, *str*, *erm(A)*, *lsa(A)*, *cat*, *tet(M)*, *dfrG*	*elrA*, *srtA*, *ace*, *ccf*, *cob*, *cad*, *camE*, *ebpA*, *ebpC*, *tpx*, *efaAfs*, *hylA*, *hylB*
13 A22	hepatobiliary surgery	drainage	2020/07/07	535	16	*erm(B)*, *fexA*, *cat*, *tet(M)*	*elrA*, *srtA*, *ace*, *ccf*, *cob*, *cad*, *camE*, *ebpA*, *ebpC*, *tpx*, *gelE*, *efaAfs*, *hylB*, *hylA*, *fsrB*
14 A23	urology	urine	2020/07/29	1287	8	*aac(6')-aph(2")*, *ant(6')-la*, *erm(A)*, *erm(B)*, *lsa(A)*, *fexA*, *tet(M)*, *dfrG*	*srtA*, *ace*, *agg*, *ccf*, *cob*, *cad*, *camE*, *ebpA*, *ebpC*, *tpx*, *gelE*, *efaAfs*, *hylB*, *fsrB*
15 A26	ICU	drainage	2020/06/05	619	16	*aac(6')-aph(2")*, *ant(6')-la*, *aph(3')-III*, *erm(B)*, *lnu(G)*, *fexA*, *tet(M)*, *dfrG*	*elrA*, *srtA*, *ace*, *ccf*, *cob*, *cad*, *camE*, *ebpA*, *ebpB*, *tpx*, *gelE*, *efaAfs*, *hylB*, *hylA*, *fsrB*
16 L3	burn	wound secretion	2020/11/06	480	8	*aph(3')-III*, *ant(6')-la*, *erm(A)*, *erm(B)*, *fexA*, *tet(M)*, *dfrG*	*elrA*, *srtA*, *ace*, *ccf*, *cob*, *cad*, *camE*, *ebpA*, *ebpB*, *fsrB*, *tpx*, *efaAfs*, *gelE*, *hylA*, *hylB*
17 L4	hepatobiliary surgery	pus	2020/10/21	902	8	*ant(6')-la*, *erm(A)*, *lsa(E)*, *cat*, *fexA*, *tet(M)*, *dfrG*	*hylB*, *ace*, *efaAfs*, *elrA*, *srtA*, *ccf*, *cob*, *cad*, *camE*, *ebpA*, *ebpC*, *tpx*
18 L9	nephrology	urine	2020/12/01	585	8	*aac(6')-aph(2")*, *aph(3')-III*, *str*, *lsa(E)*, *fexA*, *cat*, *tet(M)*, *dfrG*	*elrA*, *srtA*, *ace*, *agg*, *ccf*, *cob*, *cad*, *camE*, *cylB*, *cylL*, *cylM*, *ebpA*, *ebpC*, *fsrB*, *tpx*, *efaAfs*, *gelE*

Table II
Continued.

Isolate	Ward	Type of specimen	Isolation time	ST	LZD MIC (ug/ml)	Resistance genes	Virulence genes
19 L13	urology	urine	2020/09/27	480	8	*aac(6')-aph(2")*, *ant(6')-la*, *aph(3')-III*, *lsa(E)*, *erm(A)*, *erm(B)*, *fexA*, *cat*, *tet(M)*, *dfrG*	*hylA*, *ace*, *efaAfs*, *elrA*, *srtA*, *ccf*, *cob*, *cad*, *camE*, *ebpA*, *ebpB*, *tpx*, *agg*
20 B2	urology	urine	2021/08/12	16	8	*aac(6')-aph(2")*, *ant(6')-la*, *aph(3')-III*, *lsa(A)*, *lsa(E)*, *lnu(B)*, *erm(A)*, *erm(B)*, *fexA*, *tet(L)*, *tet(M)*, *dfrG*	*elrA*, *srtA*, *ace*, *agg*, *ccf*, *cob*, *cad*, *camE*, , *ebpA*, *ebpC*, *tpx*, *efaAfs*, *hylA*, *cylA*, *cylL*, *cylM*
21 B3	dermato-venereology	wound secretion	2021/08/30	207	8	*aac(6')-aph(2")*, *aph(3')-III*, *erm(A)*, *erm(B)*, *lsa(A)*, *lsa(E)*, *fexA*, *tet(M)*, *dfrG*	*elrA*, *srtA*, *ace*, *agg*, *ccf*, *cob*, *cad*, *camE*, *ebpA*, *ebpC*, *tpx*, *efaAfs*, *hylB*, *fsrB*, *gelE*
22 B7	urology	urine	2021/10/09	632	8	*ant(6')-la*, *erm(A)*, *erm(B)*, *fexA*, *tet(M)*, *dfrG*	*elrA*, *srtA*, *ace*, *agg*, *ccf*, *cob*, *cad*, *camE*, *ebpA*, *ebpC*, *tpx*, *efaAfs*, *hylB*, *fsrB*, *gelE*
23 B9	urology	urine	2021/10/14	816	16	*cat(pC233)*, *fexA*, *tet(M)*	*elrA*, *srtA*, *ace*, *agg*, *ccf*, *cob*, *cad*, *camE*, *ebpA*, *ebpB*, *tpx*, *efaAfs*, *hylB*, *hylA*, *fsrB*, *gelE*, *cylA*, *cylL*, *cylM*
24 B10	ICU	bile	2021/10/23	968	8	*aac(6')-aph(2")*, *aph(3')-III*, *ant(6')-la*, *erm(A)*, *lsa(A)*, *lsa(E)*, *cat*, *fexA*, *tet(M)*, *dfrG*	*elrA*, *srtA*, *ace*, *ccf*, *cob*, *cad*, *camE*, *ebpA*, *ebpC*, *tpx*, *efaAfs*, *hylA*
25 B11	burn	wound secretion	2021/10/23	300	8	*ant(6')-la*, *erm(A)*, *erm(B)*, *lsa(A)*, *fexA*, *tet(M)*	*srtA*, *ace*, *agg*, *ccf*, *cob*, *cad*, *camE*, *ebpA*, *ebpC*, *tpx*, *efaAfs*, *hylA*, *hylB*, *fsrB*, *gelE*
26 B12	urology	urine	2021/11/06	179	8	*aac(6')-aph(2")*, *aph(3')-III*, *ant(6')-la*, *erm(A)*, *erm(B)*, *lsa(A)*, *lsa(E)*, *cat*, *fexA*, *tet(M)*	*elrA*, *srtA*, *ace*, *agg*, *ccf*, *cob*, *cad*, *camE*, *cylA*, *cylL*, *cylM*, *ebpA*, *ebpC*, *tpx*, *efaAfs*, *hylA*
27 B13	hematology	wound secretion	2021/11/07	16	8	*aac(6')-aph(2")*, *aph(3')-III*, *str*, *erm(A)*, *erm(B)*, *lsa(A)*, *lsa(E)*, *fexA*, *cat*, *tet(M)*, *dfrG*	*elrA*, *srtA*, *ace*, *ccf*, *cob*, *cad*, *camE*, *cylA*, *cylL*, *cylM*, *ebpA*, *ebpC*, *tpx*, *efaAfs*, *hylA*
28 B15	urology	drainage	2021/12/05	660	8	*ant(6')-la*, *aph(3')-III*, *erm(A)*, *erm(B)*, *fexA*, *tet(M)*, *dfrG*	*elrA*, *srtA*, *ace*, *ccf*, *cob*, *cad*, *camE*, *ebpA*, *ebpC*, *tpx*, *efaAfs*, *hylB*, *fsrB*, *gelE*
29 B18	TCM	pus	2021/08/13	631	8	*aph(3')-III*, *ant(6')-la*, *erm(A)*, *erm(B)*, *cat*, *fexA*, *tet(M)*	*elrA*, *srtA*, *ace*, *ccf*, *cob*, *cad*, *camE*, *cylA*, *cylL*, *cylM*, *ebpA*, *ebpB*, *ebpC*, *tpx*, *efaAfs*, *hylB*, *fsrB*, *gelE*
30 B19	gastro-enterology	urine	2021/12/03	585	8	*ant(6')-la*, *str*, *erm(A)*, *erm(B)*, *cat*, *fexA*, *tet(M)*, *dfrG*	*elrA*, *srtA*, *ace*, *agg*, *ccf*, *cob*, *cad*, *camE*, *cylA*, *cylL*, *cylM*, *ebpA*, *ebpC*, *tpx*, *efaAfs*, *fsrB*, *gelE*

Analysis of *optrA* gene transferability. Ten randomly selected *optrA* gene-carrying LREfs were conjugated with the *E. faecalis* JH2-2 strain in vitro. The *optrA* genes of three LREfs (*E. faecalis* B2, *E. faecalis* B3, and *E. faecalis* B10) was successfully transferred to *E. faecalis* JH2-2 by conjugation (with the corresponding transconjugant referred to as *E. faecalis* TC-B2, *E. faecalis* TC-B3, *E. faecalis* TC-B10). The drug-resistant phenotypes and genotypes of the strains before conjugation were basically the same as those after conjugation, while the transconjugants all showed the same ST as that of the recipient bacterium JH2-2, i.e., ST8. The remaining seven donor strains were not successfully conjugated, and we hypothesized that the *optrA* gene of these strains might be located on the chromosome or other non-conjugative genetic elements. The resistance phenotypes, genotypes, and STs of the three successfully produced transconjugants, the corresponding donor strains, and the recipient strain are shown in Table III. Three transconjugants exhibited resistance to rifampicin, linezolid, and florfenicol. The MIC values for linezolid were consistent with the donor bacteria at 8 μg/ml, which represents a four-fold increase compared to the recipient strain JH2-2 (from 2 μg/ml to 8 μg/ml). Similarly, the MIC values for florfenicol were consistent with the donor bacteria at 128 μg/ml, marking a 16-fold increase compared to JH2-2 (from 8 μg/ml to 128 μg/ml). Moreover, the three transconjugants

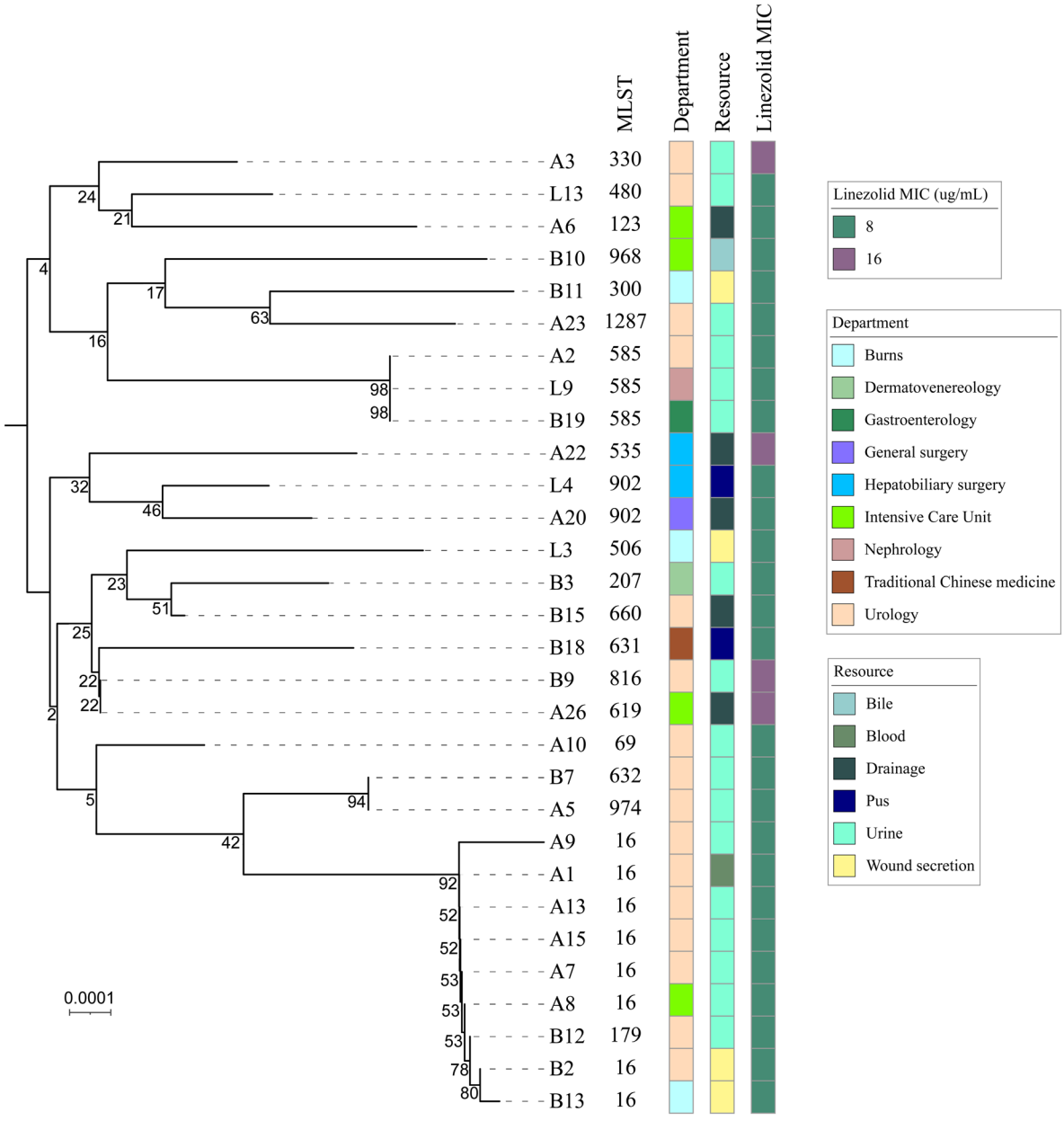

Fig. 2. Phylogenetic tree of 30 *optrA* gene-positive LREfs. The columns to the right side of the phylogenetic tree sequentially refer to strain names, sequence types, source departments, specimen types, and linezolid minimal inhibitory concentrations from left to right.

were all resistant to erythromycin, chloramphenicol, tetracycline, and clindamycin. In terms of resistance genotype, WGS results showed that the transconjugants all carried the *optrA* gene, along with other-resistance genes, such as *fexA*, *erm(A)*, *erm(B)*, *aac(6')-aph(2")*, and *aph(3')-III*. These results indicated that the *optrA* gene can be horizontally transferred among *E. faecalis*. While causing the transconjugants to develop resistance to linezolid, the *optrA* gene can also co-transfer other-resistance genes, such as *fexA*, *erm(A)*, *erm(B)*, *aac(6')-aph(2")*, and *aph(3')-III* to the recipient bacteria under specific mechanisms, leading to multidrug resistance of the transconjugant.

Analysis of the mechanism of horizontal transfer of the plasmid-borne *optrA* gene. To analyze the mechanism of horizontal transfer of the *optrA* gene among *E. faecalis* further, *E. faecalis* B2 and its transconjugant, *E. faecalis* TC-B2, were randomly selected for WGS. The results showed that *E. faecalis* B2 carried an *optrA* gene-containing plasmid, which was termed PB2-1. PB2-1 was 62,351bp long, with a GC content of 34.89%, containing 70 coding sequences (CDs). PB2-1 not only contained the *optrA* gene but also carried-resistance genes *fexA*, *tet(L)*, *erm(A)*, and *erm(B)*. Blast analysis showed that PB2-1 was highly homologous to the corresponding region of plasmid pS7316 of *E. faecalis* S7316 (GenBank accession No. LC499744) with a 99.92% identity. The transconjugant *E. faecalis* TC-B2 carried an *optrA* gene-containing plasmid named PTC-B2, which had a size of 105,222 bp,

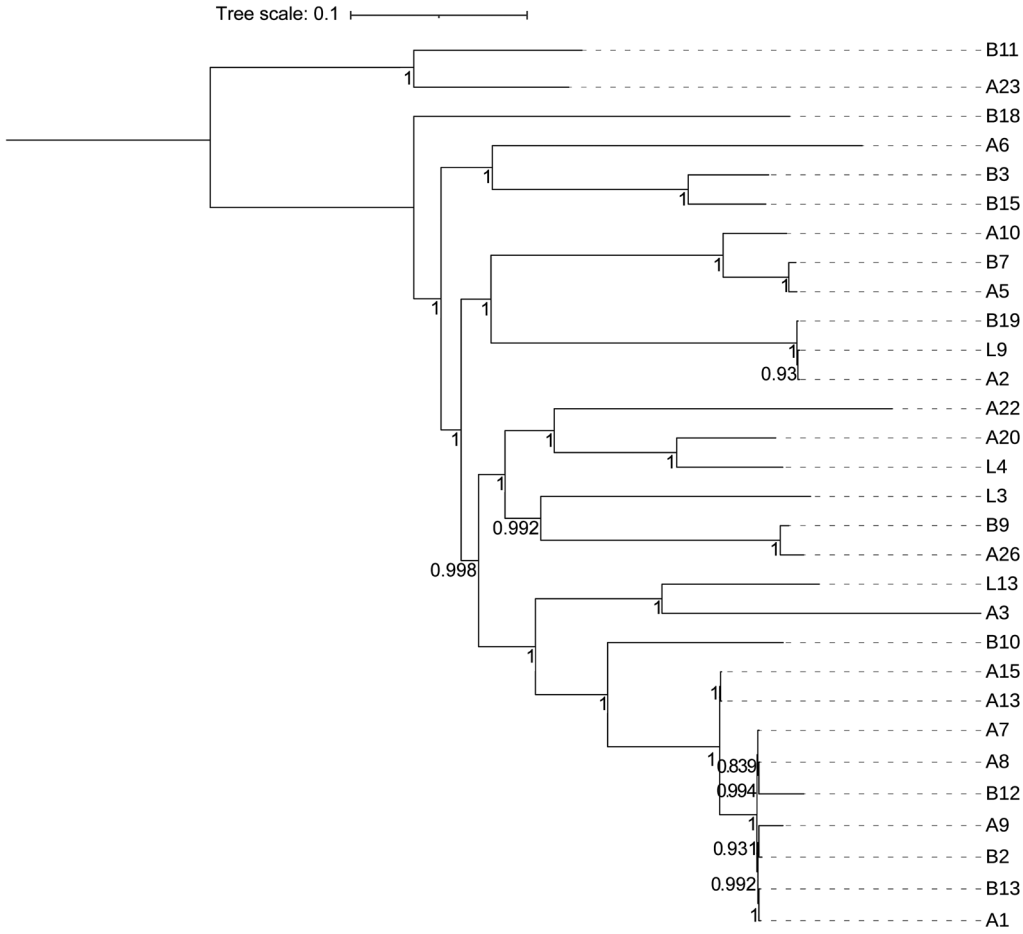

Fig. 3. Evolutionary tree constructed using SNP comparison files of 30 *optrA* gene-positive LREfs. A9, A8, A13, A15, A7, A1, B2, B12, B13 on same branch.

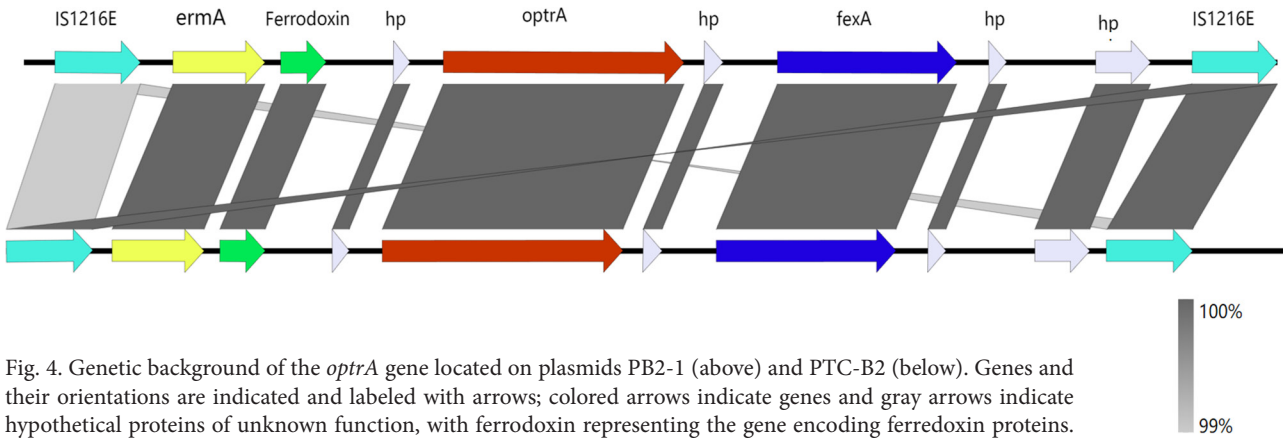

Fig. 4. Genetic background of the *optrA* gene located on plasmids PB2-1 (above) and PTC-B2 (below). Genes and their orientations are indicated and labeled with arrows; colored arrows indicate genes and gray arrows indicate hypothetical proteins of unknown function, with ferrodoxin representing the gene encoding ferredoxin proteins.

a GC content of 34.32%, and had 115 CDs. The-resistance genes carried on this plasmid were *optrA*, *erm(A)*, *erm(B)*, *lnu(B)*, *lsa(A)*, *lsa(E)*, *aac(6')-aph(2")*, and *aph(3')-III*. The *optrA* gene sequences on plasmids PB2-1 and PTC-B2 were identical, with a length of 1,914 bp, a 99.5% identity to the *optrA* gene in the Comprehensive Antibiotic Resistance Database (ARO: 3003746), and a 100% coverage. The plasmids PB2-1 and PTC-B2 shared a 7,771-kb-long genetic segment carrying the *optrA* gene, i.e., the *IS1216E-erm(A)-optrA-fexA-IS1216E* segment. *fexA* and *erm(A)* were located upstream and downstream of the *optrA* gene, respectively, with two ISs of the same size and orientation (*IS1216E*) on both sides of the segment. The presence of the same genetic environment around the *optrA*-carrying fragment in both PB2-1 and PTC-B2

Table III
Resistance phenotypes, genotypes and STs of recipient bacteria, donor bacteria and transconjugants.

Strain	MIC (μg/ml)									ST	Conjugation frequency	Resistance genes
	RA	FFC	LZD	CHL	ERY	CLI	TET	GEH	STH			
Enterococcus faecalis JH2-2	128	8	2	S	≤0.25	≤ 6	≤0.12	<500 (S)	<1,000 (S)	8	–	–
Enterococcus faecalis B2	2	128	8	6 (R)	≥8 (R)	≥8 (R)	≥16 (R)	<500 (S)	≥1,000 (R)	16	–	*aac(6')-aph(2")*, *ant(6')-la*, *aph(3')-III*, *lsa(A)*, *lsa(E)*, *lnu(B)*, *erm(A)*, *erm(B)*, *optrA*, *fexA*, *tet(L)*, *tet(M)*, *dfrG*
Enterococcus faecalis TC-B2	128	128	8	6 (R)	≥8 (R)	≥8 (R)	≥16 (R)	≥500 (R)	≥1,000 (R)	8	1.2×10^{-4}	*aac(6')-aph(2")*, *ant(6')-la*, *aph(3')-III*, *lsa(A)*, *lsa(E)*, *lnu(B)*, *erm(A)*, *erm(B)*, *optrA*, *fexA*, *dfrG*
Enterococcus faecalis B3	2	128	8	6 (R)	≥8 (R)	≥8 (R)	≥17 (R)	≥500 (R)	<1,000 (S)	207	–	*aac(6')-aph(2")*, *ant(6')-la*, *aph(3')-III*, *erm(A)*, *erm(B)*, *lnu(B)*, *lsa(A)*, *lsa(E)*, *optrA*, *fexA*, *tet(L)*, *tet(M)*, *dfrG*
Enterococcus faecalis TC-B3	128	128	8	6 (R)	≥8 (R)	≥8 (R)	≥16 (S)	≥500 (S)	<1,000 (S)	8	2.0×10^{-4}	*aac(6')-aph(2")*, *ant(6')-la*, *aph(3')-III*, *lsa(A)*, *lsa(E)*, *lnu(B)*, *erm(A)*, *erm(B)*, *optrA*, *fexA*, *dfrG*
Enterococcus faecalis B10	0.5	128	8	7 (R)	≥8 (R)	≥8 (R)	≥16 (R)	≥500 (R)	≥1,000 (R)	968	–	*aac(6')-aph(2")*, *aph(3')-III*, *ant(6')-la*, *erm(A)*, *erm(B)*, *lsa(A)*, *lsa(E)*, *lnu(B)*, *cat*, *optrA*, *fexA*, *tet(L)*, *tet(M)*, *dfrG*
Enterococcus faecalis TC-B10	128	128	8	6 (R)	≥8 (R)	≥8 (R)	≥16 (R)	≥500 (R)	<1,000 (S)	8	1.2×10^{-5}	*aac(6')-aph(2")*, *aph(3')-III*, *ant(6')-la*, *erm(A)*, *erm(B)*, *lsa(A)*, *lsa(E)*, *lnu(B)*, *optrA*, *fexA*, *dfrG*

RA – rifampicin, FFC – florfenicol, CHL – chloramphenicol

suggests that plasmids carrying the *optrA* gene can undergo horizontal transfer among *E. faecalis* through conjugation, and the insertion sequence *IS1216E* may play an important role in the horizontal transfer of the *optrA* gene.

Table IV
Genomic characterization of *Enterococcus faecalis* B2 and *Enterococcus faecalis* TC-B2.

Genomic Features	*Enterococcus faecalis* B2	*Enterococcus faecalis* TC-B2
Genome size (bp)	3,171,404	2,989,374
Number of plasmids	2	1
Size range of plasmid(s) (bp)	62,351 (PB2-1)	105,222
GC content of plasmid(s) (%)	34.89 (PB2-1)	34.32
Coding genes of plasmid(s)	70 (PB2-1)	115
Location of the *optrA* gene	46108–48021	97914–99827

Discussion

Enterococci are not only naturally resistant to a wide range of antibiotics, including cephalosporins and sulfonamides, but also acquire additional resistance through plasmid and transposon transfer, leading to further spread in the hospital setting, and are thus considered to be a significant challenge in hospital infection control. *E. faecalis* is frequently isolated from patients with urinary tract infections and is one of the major pathogens in patients with urinary tract infections. In this study, 22 of the 39 isolated LREfs strains

primarily came from urine samples from patients in the Urology Department, with 14 strains testing positive for the *optrA* gene. This indicated a high incidence of LREfs among patients with urinary tract infections. The resistance mechanism to linezolid in *E. faecalis* may be due to the carriage of the *optrA gene*, which is consistent with previous research (Ma et al. 2021).

According to the drug-sensitivity results, the 39 LREfs all showed multidrug resistance, with high rates of resistance to tetracycline, clindamycin, minocycline, erythromycin, and chloramphenicol (all above 80%), and low rates of resistance to penicillin, ampicillin, furotoxin, vancomycin, tigecycline, and teicoplanin (all below 2.6%), which was in line with the results of a previous study (Deshpande et al. 2018). Typically, the *optrA* gene confers a relatively low MIC (4–16 mg/l) for linezolid (Zhang et al. 2018; Ruiz-Ripa et al. 2020), which is consistent with the results of our study. ResFinder analysis showed that the tetracycline-resistance gene, *tet(M)*, and macrolide-resistance gene, *erm(B)*, had high carriage rates among the 30 *optrA*-carrying LREfs in our study, which was similar to the detection rates in a Chinese survey across six provinces (Chen et al. 2019). The survey showed that most of the *optrA*-positive strains carried multiple resistance genes, such as tetracycline-resistance genes *tet*, *erm(A)*, *aac(6')-aph(2")*, *ant(6')-la*, and *aph(3')-III*. Tetracycline- and macrolide-type antibiotics are now less commonly used to treat human infections. However, they are still widely and repeatedly used in aquaculture, resulting in resistance to these antibiotics in poultry and livestock. Tetracycline- and macrolide-resistant genes can be acquired by humans through the food chain, leading to human-animal-environmental transmission of resistance genes. We should be alert to the spread of food-animal-borne drug-resistant bacteria and cut off the chain of transmission at the source.

The virulence genes in *E. faecalis* strains contribute to their acquisition of adaptive elements, thus providing an evolutionary advantage for their relative fitness in the hospital environment. In the present study, 20 virulence genes essential for *Enterococcus* pathogenicity were detected from the *optrA*-positive LREfs, of which ten virulence genes, including *ace*, *ebpA*, *efaFs*, and *ccf*, were expected to all the strains, similar to the results of a previous study (Ruiz-Ripa et al. 2020). Both genes *ace* and *efa* encode adhesins that promote adhesion between *E. faecalis* and the host and are closely related to *E. faecalis* biofilm formation (Haghi et al. 2019; Janjusevic et al. 2021). The cell wall of *E. faecalis* is thick and prone to biofilm formation, which not only contributes to resistance against antibiotics but also resists the bactericidal action of antibodies. This enables *E. faecalis* to evade the immune system, preventing its complete elimination and leading to recurrent infections.

In this study, MLST analysis revealed a high genetic diversity of 30 *optrA*-positive LREfs with up to 21 STs, which have been observed in clinical isolates, animals, and environments worldwide. For example, ST16 of *optrA*-positive LREfs, the most common ST, has been found in clinical isolates from China, Denmark, and Spain (Ruiz-Ripa et al. 2020; Schwarz et al. 2021). ST902 of *optrA*-positive LREfs was found in clinical isolates from Bangladesh (Roy et al. 2020). Among the 21 STs in this study, ST16 (n = 8, 26.7%) was the representative type, consistent with the findings of previous reports (Hua et al. 2019; Zhou et al. 2019). Studies have shown that ST16 *E. faecalis* is better adapted to the hospital environment, acquires multidrug resistance, and has greater biofilm formation ability, leading to more excellent bacterial resistance under unfavorable environmental conditions (e.g., antibiotics and disinfectants), with mature biofilms of *E. faecalis* being able to tolerate antimicrobial agents even at concentrations of up to 100 to 1,000 times higher than MIC values (Farman et al. 2019; Ma et al. 2021). In addition, previous studies (Tamang et al. 2017; Freitas et al. 2020; Nüesch-Inderbinen et al. 2021) have identified ST16 strains of *optrA*-carrying *E. faecalis* from food-source animal feces and aquatic environments and found that strains from different sources even share the same resistance genes and virulence profiles. It highlighted the ability of such ST strains of *E. faecalis* to spread across the human-animal-environmental interface, which becomes a public health concern. A small-scale clonal transmission of ST16 LREfs in the Urology Department of this hospital should be taken seriously. In order to avoid further occurrence and spread of nosocomial infections, antimicrobial drugs should be used scientifically and rationally, and preventive and control measures should be taken.

Since its first discovery, the detection rate of the *optrA* gene in *Enterococcus* has gradually increased, and its prevalence has gained wide research attention in various countries. None of the 30 LREfs in this study had detectable mutations in the *cfr*, *cfr(B)*, *poxtA* genes or in V region of the 23s rRNA gene. The *optrA* gene was the only linezolid-resistance gene detected in our study, with a detection rate of 76.9%, and this result, along with previous studies, confirmed that carriage of the *optrA* gene is the primary resistance mechanism in LREfs of clinical origin (Hua et al. 2019; Sassi et al. 2019; Almeida et al. 2020). Previous reports have shown that the *optrA* gene can be widely distributed in samples of animal origin and in the environment, and there are domestic and international studies in which *optrA*-positive *Enterococcus* have been isolated from carnivorous animal food products, hospital environments, and municipal wastewater (Freitas et al. 2017; Park et al. 2020; Jung et al. 2021). Moreover, the *optrA* gene,

located on the plasmid, is similar to pE349, suggesting that the gene can be efficiently transmitted among humans, animals, and the environment. Therefore, the study of the transmission mechanism and genetic environment of the *optrA* gene is of great significance in guiding the use of clinical antimicrobial drugs and controlling the spread of drug-resistant bacteria.

The results of the conjugation experiments showed that only three of the 10 *E. faecalis* strains were successfully conjugated. The reasons for the conjugation failures are varied and may include the following aspects. First, the *optrA* gene of these seven strains may be located on the chromosome or a non-conjugative plasmid. Plasmids can be categorized as conjugative plasmids, mobilizable plasmids, and non-mobilizable plasmids based on the completeness required for plasmid conjugation (i.e., the presence of *oriT*, relaxase genes, T4-CP, and T4SS) as well as their transferability differences (Smillie et al. 2010). Among them, only the conjugative plasmids can carry out conjugative transfer autonomously and independently, while those located on non-conjugative plasmids cannot be transferred. Secondly, most LREfs are multidrug-resistant. The presence of other-resistance genes on plasmids can affect the host bacterium's growth metabolism and environmental adaptability, which is not conducive to the conjugative gene transfer between strains, resulting in a low conjugation rates (Young et al. 2019).

WGS analysis revealed that the *optrA* resistance genes located in PB2-1 and PTC-B2 share the same genetic environment, i.e., the *IS1216E-erm(A)-optrA-fexA-IS1216E* fragment with a length of 7,771 kb. Co-localization of the *fexA*, *erm(A)*, and *optrA* genes suggests that the emergence of LREfs may be due to selective pressure for the use of non-oxazolidinone antibiotics (such as macrolides, florfenicol) and other antibiotics. The *fexA* gene mediates resistance to amidol antibiotics (chloramphenicol, florfenicol), a veterinary antibiotic used to prevent and treat respiratory and gastrointestinal tract infections in animals. Many studies have found that *fexA* is upstream of almost all *optrA* genes located on plasmids. The widespread and repeated use of florfenicol in the treatment of livestock infections exerts selective pressure on environmental bacteria. It may be positively correlated with the development of linezolid-resistance genes (Wang et al. 2020). Two *IS1216E* ISs of the same orientation and size are present upstream and downstream of the *optrA* gene on the recipient bacterial plasmid PB2-1, where they can form an *IS1216E-fexA-optrA-erm(A)-IS1216E* transposition unit with *fexA and erm(A)*, and the two *IS1216E* sequences can be further combined to form a circular intermediate, allowing the integration of the *optrA* gene into the plasmid. This suggests that the insertion sequence *IS1216E* on the plasmid plays an important role in the horizontal transfer of the *optrA* gene. *IS1216E* is a subtype of IS1216 belonging to the IS6 family. It is widely found in multidrug-resistant Gram-positive bacteria, and it is the most common insertion sequence located in the regions flanking the *optrA* gene. Regardless of the presence or absence of additional genes, the *optrA* gene is usually enclosed by two *IS1216E* copies of the same orientation and size. When these *IS1216E* copies recombine, a translocatable unit is generated, which can be integrated into plasmids, integrative and conjugative elements (ICEs), or different chromosomal sites. If it is integrated into a conjugative plasmid or ICE, it may result in the transmission of the *optrA* gene between the same or different strains of bacteria (Shang et al. 2019; Schwarz et al. 2021). It is noteworthy that, in addition to facilitating the horizontal transfer of the plasmid-carried *optrA* gene, the IS6 family may also facilitate the exchange of other clinically important antibiotic-resistance genes, i.e., the transferrable genes include but are not limited to the *optrA* gene (Che et al. 2021; Liu et al. 2024). Therefore, regular monitoring of the dynamic mobility of antibiotic-resistance genes mediated by ISs, including the IS6 family, is essential to control the spread of antibiotic resistance.

This study also has some limitations. First, the number of LREfs included in the study was relatively small given that cases of linezolid-resistance in *E. faecalis* are still rare. Second, this study was a single-center study, and further multicenter studies are needed to analyze the epidemiological characteristics of LREfs containing the *optrA* gene. Notably, the development of antibiotic resistance in bacteria does not exist in isolation. It requires not only the detection and control of infections in patients, hospitals, and the environment but also the implementation of prevention and control measures in agriculture and aquaculture to block the spread of drug-resistant bacteria.

Conclusions

In this study, we analyzed the molecular characteristics and transmission mechanism of *optrA*-carrying LREfs isolated in a tertiary hospital in Kunming. The *optrA*-carrying LREfs were resistant to various antibiotics and carried multiple resistance genes and virulence genes. ST16 was the most prevalent ST among the *optrA*-carrying LREfs in this hospital. In addition, the study emphasized the transferability of the *optrA* gene among *E. faecalis* and the important role played by mobile genetic elements, such as the insertion sequence *IS1216E* on the plasmid in the horizontal transfer of the *optrA* gene. The emergence of multidrug-resistant *E. faecalis* poses a significant challenge for clinical

anti-infective therapy and potentially threatens public health. Therefore, the use of drugs in clinical practice should be rationalized according to the actual situation. Under the One Health concept, continuous surveillance of the *optrA* gene carried by *E. faecalis* should be conducted, and effective preventive and control measures should be formulated.

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Availability of data and material

All the DNA sequences determined in this research were deposited in the Gen Bank database under the BioProject ID PRJNA1084254.

Author contributions

Conceptualization: Peini Yang; methodology: Jiang Li, Guibo Song; software: Mei Lv, Pingan He; validation: Guibo Song, Peini Yang; formal analysis: Bin Shan, Xu Yang; writing – review and editing: Peini Yang and Xu Yang; supervision: Guibo Song; project administration: Xu Yang; funding acquisition: Xu Yang. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

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