ORIGINAL ARTICLE

First detection of an *optrA*-positive, linezolid-resistant ST16 *Enterococcus faecalis* from human in Greece

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Abstract

Until now, in Greece, the resistance of enterococci to linezolid was associated with mutations of domain V of 23S ribosomal RNA (G2576T). Here we report the first linezolid-resistant *optrA*-positive *Enterococcus faecalis* sequence type (ST) 16 isolated from a patient with a urinary tract infection (UTI). No travels overseas, contact with food-producing animals or previous treatment with linezolid were reported. Plasmid analysis suggested the chromosomal location of *optrA* gene. Additionally, whole genome sequencing data revealed the association of *optrA* with transposon Tn*554* and the coexistence with *fexA, spc* and *ermA*-like resistance genes. A similar genetic structure has been previously identified in an ST767 *E. faecalis* from Taiwan.

Keywords: E. feacalis, linezolid-resistance, optrA gene

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Introduction

Linezolid has great potency against Gram-positive cocci [1]. However, shortly after its introduction into clinical practice in 2002, linezolid-resistant enterococci emerged [2,3]. Resistance in enterococci can be either mediated by mutations in the 23S ribosomal RNA (rRNA) genes or in ribosomal proteins L3, L4 and L22 encoding genes [4]. In addition, transferable resistance determinants, such as *cfr, cfrB, cfrC, optrA* and the recently identified *poxtA,* have been detected as newer mechanisms responsible for decreased susceptibility to linezolid and/or tedizolid [5–7]. In Greece, however, previous studies have shown that all linezolid-resistant enterococci, which belonged to international epidemic clones sequence type (ST) 16, ST17, ST203 and ST65 for *Enterococcus faecium* and ST28 for *Enterococcus faecalis,* carried the G2576T mutation [2].

Therefore, the aim of the present study was to describe for the first time the detection of a linezolid-resistant *optrA*-positive *E. faecalis* recovered from a hospital in Greece.

Materials and methods

E. faecalis isolate (Efl-952) was isolated in 2018 from a urine sample of a patient in the University Hospital of Larissa, a tertiary-care hospital located in central Greece. Identification to the species level and susceptibility testing were performed by the VITEK2 automated system (bioMérieux, Marcy l'Etoile, France). MIC values for tedizolid and chloramphenicol were determined by Etest (Liofilchem, Roseto degli Abruzzi, Italy; and bioMérieux respectively). Susceptibility results were interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (http://www.eucast.org/clinical_breakpoints/) or Clinical and Laboratory Standards Institute (CLSI) (https://clsi.org/standards/products/microbiology/documents/m100/) when EUCAST breakpoints were not available.

The isolate was first tested for mutations of 23S rRNA *and* L3, L4 and L22 encoding genes; in addition, it was examined for the presence of *cfr, cfrB, cfrC, optrA* and *poxtA* genes. Molecular

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typing of Efl-952 was based on multilocus sequence typing (http://pubmlst.org/efaecalis/).

Conjugal transfer of *optrA* gene from the clinical strain was carried out in mixed broth cultures using a rifampin-resistant *E. faecalis* laboratory strain as a recipient. Transconjugants were selected on Müller-Hinton agar plates supplemented with rifampin (150 mg/L) and linezolid (4 mg/L).

To define the genetic units of the *optrA* gene, the plasmid content of Efl-952 was analysed by pulsed-field gel electrophoresis of total DNA digested with S1 nuclease (Promega, Madison, WI, USA) [8]. After pulsed-field gel electrophoresis, the DNA was transferred to a BrightStar-Plus positively charged nylon membrane (Applied Biosystems, Foster City, CA, USA) and hybridized with digoxigenin-labeled *optrA* probe.

To define the regions flanking the *optrA* resistance gene, genomic DNA of *E. faecalis* Efl-952 was extracted using a DNA-Sorb-B Kit (Sacace Biotechnologies Srl, Como, Italy). Whole genome sequencing (WGS) was performed by CeMIA (Larissa, Greece), using the Ion Torrent platform Ion PGM. Reads were then assembled using the de Bruijn graph–based *de novo* assembler SPAdes v.3.9.1 [9]. For sequence analysis, the BLAST algorithm (http://www.ncbi.nlm.nih.gov/BLAST) was utilized.

Antibiotic resistance genes were identified using the ResFinder 3.1 tool (https://cge.cbs.dtu.dk/services/ResFinder/) with an identity threshold of >90% [10].

Results

E. faecalis Efl-952 was isolated from a patient admitted to the department of internal medicine with a urinary tract infection. The patient did not report either previous travels overseas or contact with food-producing animals; in addition, he had never received oxazolidinone therapy. According to susceptibility results, Efl-952 was susceptible to ampicillin, nitrofurantoin, teicoplanin, tigecycline and vancomycin, but it exhibited resistance to gentamicin (MIC = 256 mg/L), streptomycin (MIC = 1024 mg/L) and linezolid (MIC = 32 mg/L); the isolate also exhibited resistance to tedizolid (MIC = 1.5 mg/L) and chloramphenicol (MIC = 256 mg/L) according to CLSI criteria.

Molecular analysis revealed that Efl-952 belonged to the ST16 clone and carried the *optrA* gene; it was negative for mutations to 23S rRNA and to ribosomal proteins L3, L4 and L22 encoding genes, as well as for the presence of *cfr, cfrB, cfrC* and *poxtA* genes.

Efl-952 failed to transfer *optrA* gene by conjugation to *E. faecalis* laboratory strains. Moreover, plasmid analysis revealed that only the largest DNA band in the S1 profile of Efl-952 hybridized with *optrA* probe, suggesting its chromosomal location.

WGS data showed that isolate Efl-952 harboured an *optrA*-carrying fragment of 18,260bp, exhibiting 99% coverage and 99% identity to the respective sequence of *E. faecalis* strain 743142 [6]. *E. faecalis* strain 743142, which belonged to ST767, was previously characterized from Taiwan [6]. Upstream of *optrA,* the *fexA, spc* and *ermA*-like resistance genes conferring resistance to chloramphenicol, spectinomycin and MLS_B (macrolides, lincosamides and streptogramin B) antibiotics, respectively, were found. Similar to the isolate from Taiwan, the *optrA* gene was associated with transposon Tn*554*.

Additionally, analysis of WGS data by the ResFinder 3.1 tool revealed that isolate Efl-952 included additional genes for resistance to aminoglycosides (*aadD, aph*(3′)-*III, aac*(6′)-*aph*(2″)), fosfomycin (*fosD*), MLS_B antibiotics (*ermB, ermC, lnuA, lnuB, lsaA* and *lsaE*) and tetracyclines (*tetM*).

Discussion

The *optrA* gene encodes an ABC-F type protein that protects the bacterial ribosome from the antibiotic inhibition and confers resistance to oxazolidinones and phenicols [11]. The *optrA* gene in particular was discovered in enterococci of human and animal origin isolated in China between 2005 and 2014, where it was detected in both plasmids and chromosomes and different genetic environments [11,12]. Since then, *optrA*-positive enterococci have been reported worldwide [4]. Multiple variants of the gene, which are located on distinct plasmids and mobile genetic elements, have been described, demonstrating the plasticity of this resistance region [4,6]. WGS data of Efl-952 showed the coexistence of *optrA, spc, ermA*-like and *fexA* genes; the genetic environment was similar to that previously described in the chromosome of ST767 *E. faecalis* from Taiwan.

According to the epidemiologic data of our country, linezolid-resistant enterococci are rare and carry the known G2576T mutation [2]. Strains of *E. faecalis* ST16 that carry the *optrA* gene were recently identified in China, Denmark, Poland and Spain [11,13–15]. In contrast to ST16 isolates from China and Poland [11,13], the *optrA* gene in Efl-952 was localized in the chromosome and was associated with a different genetic environment, indicating the plurality of mechanisms that could be implicated in the transfer of resistance determinants.

In our hospital, when a linezolid-resistant *Enterococcus* is isolated, specific prevention measures are taken, including isolation of the patient in a single-bed room under strict infection control measures, surveillance faecal cultures are performed of samples from patients who are being treated in the same unit and intense cleaning of the environment. In addition, after the detection of Efl-952, all enterococci with a decreased susceptibility to linezolid are tested for the presence

of *optrA* gene. To our knowledge, this is the sole isolate carrier of this gene in Greece.

Conflict of Interest

None declared.

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