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Highlights

- First report of *optrA*-mediated linezolid-resistant clinical isolates in Argentina.
- *optrA* gene was detected in multidrug-resistant *Enterococcus faecalis* isolates.
- *E. faecalis* isolates carrying three *optrA* gene variants and belonged to different lineages, highlighting the high diversity among them.
- Most of the isolates carried the *optrA* gene in plasmids.
- Emphasis on the importance of initiate the antimicrobial surveillance of *Enterococcus* spp. using the One Health strategy.

Emergence of *optrA*-mediated linezolid resistance in clinical isolates of *Enterococcus faecalis* from Argentina

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Key words: enterococci; *Enterococcus faecalis*; linezolid resistance; *optrA*; *optrA* variants; clonal spread

Abstract

Objectives: The aim of this study was to characterize the first fourteen *optrA*-carrying linezolid resistant *E. faecalis* clinical isolates recovered in seven Argentinian hospitals between 2016 and 2021. The epidemiology of *optrA*-carrying isolates and the *optrA* genetic context were determined.

Methods: The isolates were phenotypically and genotypically characterized. Susceptibility to 13 antimicrobial agents was performed, clonal relationship was assessed by pulsed field gel electrophoresis (PFGE) and multilocus sequence typing (MLST). Data provided by the whole-genome sequencing was used for identification of sequence types, antimicrobial resistance genes, *optrA* variants, phylogenetic tree, and mobile genetic elements responsible to the dissemination of these strains.

Results: All the *optrA*-carrying *E. faecalis* isolates were multidrug-resistant and harbored several antimicrobial resistance genes. They carried three *optrA* variants and belonged to different lineages, however, three of them belonged to the hyperepidemic CC16. Mobile genetic elements were detected in all the isolates. The analysis of the *optrA* flanking region suggests the plasmidic localization in most of the isolates.

Conclusions: To the best of our knowledge, this is the first report of *optrA*-mediated linezolid resistance in Argentina. The emergence and dissemination of the *optrA* genes in clinical *E. faecalis* isolates are of concern and highlights the importance of initiating the antimicrobial surveillance of *Enterococcus* spp. under a One Health strategy.

1. Introduction

Enterococcus faecalis and *Enterococcus faecium* can be harmless colonizers of the human intestinal tract, but on the other hand, they are also one of the most important bacterial genera related to hospital-associated infections worldwide (1). Due to its ability to cause nosocomial infections as well as the prevailing resistance to different antimicrobial agents, *Enterococcus* spp., especially *E. faecalis* and *E. faecium*, have become a particular clinical concern.

Enterococci not only contains intrinsic resistance mechanisms to several antimicrobial agents, but also has the capacity to acquire mobile genetic elements carrying antimicrobial resistance genes, which limits the therapeutic options (1).

Linezolid is the first class of oxazolidinones, a fully synthetic antibiotic that targeted at the large (50S) subunit of bacterial ribosomes and inhibits protein synthesis (2,3).

Due to their effectiveness against a wide range of Gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus* (VRE), linezolid and daptomycin are considered the "last line of defense" against Gram-positive multidrug-resistant bacteria after vancomycin (3).

Oxazolidinones are currently prescribed for severe infections caused by the aforementioned pathogens, including community-acquired pneumonia, nosocomial pneumonia, bloodstream infections, skin and soft tissue infections involving multidrug-resistant isolates, or when treatment has failed (4).

Although the overall prevalence of linezolid resistance among enterococcal clinical isolates has remained low (<1%) (3,5), the number of linezolid-resistant enterococci isolates has increased during recent years worldwide (6,7). Linezolid resistance mainly resulted from mutations in the central loop of the domain V of the 23S rRNA gene, especially G2576T, and/or in genes encoding for L3, L4 and L22 ribosomal proteins

(8,9). The 23S rRNA gene is found in multiple copies in the *Enterococcus* genome and there is a relationship between the mutated copy number in 23S rRNA and the level of linezolid resistance of the isolates (10,11). Transferable resistance determinants have also emerged, such as *cfr* (**c**hloramphenicol and **f**lorfenicol **r**esistance) (12), *optrA* (**o**xazolidinone **p**henicol **t**ransferable **r**esistance) (13) and *poxtA* (**p**henicols, **ox**azolidinones and **t**etracyclines resistance) genes (14,15) and are being increasingly reported in different enterococcal species and across different settings, being *optrA* the main transferable mechanism responsible for linezolid-resistant enterococci in human isolates (16). The *cfr* gene encodes a 23S rRNA methyltransferase that confers resistance to oxazolidinones, phenicols, lincosamides, pleuromutilins and streptogramins A (12), while the *poxtA* and *optrA* genes encode ATP-binding cassette (ABC)-F type transporters that confer resistance to oxazolidinones and phenicols through a ribosome protection mechanism (17).

The *optrA* gene was initially described among animal and human *E. faecalis* and *E. faecium* isolates from China (13), and nowadays *optrA*-carrying linezolid resistance is detected in hospitals worldwide (17). The *optrA* genes confers transferable resistance to linezolid and is often transmitted together with phenicol-exporting genes such as *fexA*, *fexB* and genes that confer resistance to macrolide-lincosamide-streptogramin B such as *ermA* and *ermB* (18). The *optrA* gene has been found in several and diverse genetic platforms among different Gram-positive species (enterococci, staphylococci, and streptococci), and numerous variants of the gene have been described (16). Among enterococci, mostly *E. faecalis*, *optrA* has been commonly described as adjacent to *fexA* (conferring resistance to phenicols), and surrounded by different ISs, located on plasmids or chromosomal platforms (16).

Little is known about the genetic diversity and resistance mechanisms of linezolid resistance in Argentina. In this study, we aimed to characterize the first linezolid-resistant *E. faecalis* clinical isolates using phenotypic and genotypic approaches.

2. Materials and Methods

2.1. Bacterial strains and identification

A total of 14 *E. faecalis* strains were referred to Antimicrobial Agents Division, INEI-ANLIS "Dr. Carlos G. Malbrán", National and Regional Reference Laboratory on Antimicrobial Resistance, Buenos Aires, Argentina, to confirm the linezolid resistance phenotype and further characterization. Strains were isolated from different clinical samples (one per patient) in seven hospitals located in Buenos Aires City and Buenos Aires Province, from 2016 to 2021 (Table 1). Species identification was performed using matrix-assisted laser desorption ionization-time of flight mass spectrometry MALDI-TOF (*Brucker Daltonics, Bremen, Germany).*

2.2. Antimicrobial susceptibility testing

Antimicrobial susceptibility was tested by disk diffusion method to the following antibiotics (disk content in brackets): penicillin (10 units), ampicillin (10 µg), tetracycline (30 µg), minocycline (30 µg), ciprofloxacin (5 µg), chloramphenicol (30 µg), erythromycin (15 µg), vancomycin (30 µg), teicoplanin (30 µg), tigecycline (15 µg), gentamicin (120 µg), streptomycin (300 µg), and linezolid (30 µg). All antimicrobial susceptibility tests were carried out and interpreted according to the CLSI guidelines (19), except tigecycline which was interpreted according to FDA breakpoints (https://www.fda.gov/drugs/development-resources/tigecycline-injection-products). Linezolid susceptibility was also evaluated by E-test (bioMérieux, France), Vitek-2 Compact® (bioMérieux, France) and Phoenix (BD, US). Isolates were considered as

multidrug-resistant (MDR) when they exhibited resistance to three or more different classes of antimicrobial agents (20).

2.3. Gene detection by PCR

Detection of the *optrA* gene was performed by PCR using primers A-F (5'-AGGTGGTCAGCGAACTAA-3') and A-R (5'-ATCAACTGTTCCCATTCA-3') that amplified an internal segment of 1,395 bp (13).

2.4. Clonal relatedness

The clonal relatedness of *E. faecalis* isolates was determined by pulsed-field gel electrophoresis (PFGE) of total DNA restricted with the enzyme SmaI (New England Biolabs, Beverly, MA, USA) as previously described (21). DNA fragments were separated in 0.8% agarose using a CHEF DR III System (Bio-RadTM, Hercules, CA, USA) under the following conditions: switch time, 5 to 35s and running time 26 h; temperature 7°C, angle 120° and voltage 6 V/cm. Separated DNA fragments were stained with ethidium bromide and visualized with a UV transilluminator. Banding patterns were analysed by visual inspection and interpreted according to Tenover criteria (22).

2.5. Genome sequencing

All isolates were submitted to whole-genome sequencing (WGS). Genomic DNA was extracted with QIAamp1 DNA Mini Kit (*Qiagen, Valencia, CA, USA*) according to manufacturer instructions. Sequencing was performed using the Nextera XT DNA Sample Prep Kit (Illumina, San Diego, CA, USA) and the extracted DNA was sequenced using Illumina's MiSeq instrument at the "Unidad Operativa, Centro

Nacional de Genómica y Bioinformática. ANLIS "Dr. Carlos G. Malbrán" to generate 250-bp paired-end reads.

Assembly, annotation and analysis of genomes were done through the PATRIC software (https://www.patricbrc.org). Detection of resistance genes was carried out by ResFinder 4.1 (23) and PATRIC using the available CARD (*Comprehensive Antimicrobial Resistance Database*) and NDARO (National Database of Antibiotic Resistant Organisms) databases, and the gene content were compared with the phenotype presented by the isolates. LRE-Finder of the *Center for Genomic Epidemiology* (http://www.genomicepidemiology.org/) was used to detect 23S rRNA mutations and *optrA*, *cfr*, *cfr(B)* and *poxtA* genes encoding linezolid resistance in enterococci from whole-genome sequences (24).

MLST was peformed by MLST 2.0 (25) available at the Center for Genomic Epidemiology (http://www.genomicepidemiology.org/). Sequence types (STs) were assigned using the pubMLST *E. faecalis* multi-locus sequence typing schemes (26). The obtained ST was analyzed using PHULOViZ and the goeBURST algorithm to identify clonal complexes (27).

CGE PlasmidFinder 2.1 (available at https://cge.food.dtu.dk/services/PlasmidFinder/, accessed on 10 June 2023) was used to type plasmid replicons (28).

All genomes were mapped against the *E. faecalis* V583 (ATCC 700802) reference strain (GenBank accession number **AE016830**) to infer a phylogeny based on the concatenated alignment of high- quality single nucleotide polymorphisms (SNPs) using CSI Phylogeny (29) available on the website for the Center for Genomic Epidemiology http://www.genomicepidemiology.org/ (accessed on 12 July 2023) by using default parameters. Microreact was used to visualize the phylogenetic tree and metadata.

The genomic sequences of the *E. faecalis* isolates identified in this study were deposited in DDBJ/ENA/GenBank as BioProject **PRJNA1007381**.

2.6. OptrA variants and genetic context of the optrA gene

The aminoacid sequences of the OptrA protein of the all genomes were compared against the original OptrA WT protein (GenBank KP399637). A detailed investigation of the *optrA*-containing contigs obtained by de-novo assembly was performed using the *compare region viewer* tool (*PATRIC*). Additionally, the genetic context of the *optrA* gene was compared *in silico* with data previously published in the NCBI using BLASTn (30) and *Artemis Comparison Tool ACT* (www.sanger.ac.uk/science/tools) (31).

3. Results and Discussion

Linezolid is one of the last resort antimicrobial agents in the treatment of serious infections caused by Gram-positive pathogens. Resistance to linezolid represents a major public health problem, so it is very important to determine the associated resistance mechanisms. Currently, the most important factor associated with the establishment of nosocomial resistance to linezolid is the previous exposure to linezolid and prolonged treatments, eventually caused nosocomial outbreaks (5).

We describe the first *optrA* mediated linezolid resistant *E. faecalis* human clinical isolates in Argentina.

All *E. faecalis* linezolid-resistant isolates were positive for the *optrA* genes (Table 1). By disk diffusion all the isolates displayed linezolid inhibition zones between 19 and 22 mm corresponding to CLSI intermediate or resistant categories with MICs ranging from 2 to 8 mg/L and (Table 1). However, is important to highlight that those *optrA*-carrying *E. faecalis* isolates with linezolid MIC 2 and 4 mg/l could be miss detected by EUCAST breakpoints (Susceptible ≤4 mg/L; Resistant >4 mg/L). Linezolid resistance caused by the *optrA* gene has been previously shown to have relatively low linezolid MICs (4–16 mg/L) (1), which is consistent with our findings.

Chromosomal point mutations in 23S rRNA or in genes encoding L3/L4/L22 ribosomal proteins, were not detected. In the same way, acquired *cfr* and *poxtA* genes were absent. In addition to linezolid, all the isolates were resistant to chloramphenicol, tetracycline, minocycline and erythromycin, and susceptible to ampicillin, penicillin, vancomycin, teicoplanin and tigecycline. High level resistance to at least one aminoglycoside was detected in seven isolates (50%). Five (35.7%) were resistant to fluoroquinolones and two presented intermediate resistance to ciprofloxacin. All the linezolid resistant *E. faecalis* isolates showed a multidrug resistance phenotype.

A large number of resistance genes, ranging from 6 to 13, have been identified in the genomes of the isolates studied. Acquired genes conferring resistance to oxazolidinones (*optrA*), phenicols (*fexA*), tetracycline and minocycline [*tet*(M), *tet*(L) and/or *tet*(O)], macrolides and lincosamides (*ermB, ermA, lsaA* and/or *lnuB*) were present in all the isolates. The seven isolates with high-level resistant to gentamicin and/or streptomycin carried at least one acquired aminoglycoside resistance genes including *aac(6')-aph(2'')*, *ant(6)-Ia, ant(9)-Ia, aph(3')-III* or *str*. Although genes related to resistance to trimethoprim (*dfrG*) were found in seven isolates, trimethoprim/sulfamethoxazole was not tested because it is not clinically active in enterococci. Amino acid replacements in GyrA S83I and ParC S80I responsible for resistance to ciprofloxacin were detected in the five phenotypically resistant strains. as previously described (32). Two isolates M8691 and M8757 had a single amino acid replacement in the ParC protein (V307I and Q482K, respectively) and both displayed intermediate resistance to ciprofloxacin. Complete correlation between phenotype and genotype was observed for all the antimicrobial agents tested.

The genome sequences were analyzed with PATRIC software and showed an average size of 2,907,680 bp, with an average of 2,810 genes annotated (range 2,632 to 3,042) (Table 2).

The *optrA* gene was initially identified in *E. faecalis* and *E. faecium* from humans, pigs, and chickens in China in 2015 (13), and nowadays *optrA*-carrying *E. faecalis* strains have been detected in humans from countries in all the continents (33). In Latin American region, clinical isolates of *E. faecalis* carrying *optrA* genes were sporadically reported in Ecuador, Guatemala, Mexico, Panama, and Colombia. (6,7,33,34,35).

The *optrA* nucleotide sequences of the *E. faecalis* genomes were compared against the *optrA* gene wild type (GenBank accession number **KP399637**). Three different variants were identified, *optrA*_2 or EDM variant (T526G; A1812G) in twelve isolates, *optrA*_6 or EDD variant (T526G; C1040T; G1170A) in one isolate, and *optrA*_5 in one isolate (Table 1, Figure 1). The *optrA* variants identified in this study, *optrA_2*, *optrA_5* and *optrA_6*, have been previously reported in humans and foods of animal origin (35).

Among the 12 isolates with *optrA_2* variant, 11 showed the same gene arrangement in the *optrA* containing contig, while isolate M8732 showed differences downstream of the *optrA* gene (Figure 2A). In all the cases the region analyzed showed 99% of nucleotide identity with *E. faecalis* K198 plasmid pK198-1-A (GenBank accession number **CP116570.1**). The isolate M8726 with *optrA*_5 variant showed a different genetic context (Figure 2B). The *optrA* containing contig (59.17 Kb) also contains the genes *fexA, ermA, efrA, ant(9)-I* and genes encoding transposases A and B of Tn*554*. An NCBI BLASTn search with the *optrA* containing contig sequence as a query sequence showed that it displayed 100% nucleotide sequence identity (query cover, 100%) with *faecalis* strain JF3A-223 chromosome (GenBank accession number **CP102065.1**), suggesting its chromosomic location.

The *optrA* flanking region could not be visualized in the isolate M8644 with *optrA*_6 variant due to the short contig length (2.19 Kb). By BLASTn it shared 100% of nucleotide identity with *E. faecalis* L15 plasmid (GenBank accession number **CP042214.1**).

MGEFinder detect at least one mobile genetic element, such as transposons or insertion sequences (IS) in each isolate. Tn917, Tn6009 and Tn554 were detected in seven, four and two isolates, respectively. IS6, IS1380 and IS256 were detected in eight, four and two isolates, respectively.

Additionally, PlasmidFinder revealed a total of seven different plasmid associated replication genes (repUS43, rep9b, rep22, repUS12, repUS59, rep7a, rep11C), detected in all the isolates except M8726. *rep9b* belonging to the RepA_N family and *repUS43* were the most common replicon types occurring in thirteen (92.9%) and twelve (85.7%) isolates, respectively. Replicon type *repUS12* were found in four (28.6%) isolates. *rep22* and *rep7a* were found in two isolates each and *repUS59* and *rep11C* in one isolate each.

Twelve isolates belonged to *optrA_2* variant and carried *rep9b* variants of RepA_N plasmids which are narrow host range plasmids considered specific for *E. faecalis* (36). In six of them *rep9b* and *optrA* genes were found in the same contig. Likewise, analysis of the *optrA*-flanking regions suggests that all but one isolate could be plasmid-associated.

Limitations of bioinformatics regarding whole genome assemblies from short read data could underestimate the detection of mobile genetic elements involved in *optrA* dissemination. However, the diversity of plasmids found in *optrA*-carrying *E. faecalis* illustrate the diverse nature of mobile genetic elements that can be encountered and poses a particular threat to the spread of this resistance determinant (10).

Regarding the dissemination of *optrA*-carrying *E. faecalis* in Argentina, according to the PFGE profiles and MLST types, the collection studied showed a high diversity. By PFGE the isolates belong to 14 different pulsotypes A to N, and by MLST they belong to 13 sequence types: ST16, ST59, ST116, ST234, ST253, ST415, ST476, ST590 (n:2),

ST591, and ST1062, already included in PubMLST database plus three novel sequence types ST1246, ST1247 and ST1248 (Table 1).

Contrary to what happens in *E. faecium*, traditional molecular epidemiology methods are unable to differentiate *E faecalis* isolated from different sources/hosts in various clusters. As it was previously described, MLST studies revealed the presence of many STs in different hosts, including hospitalized patients, farm animals and companion animals (10). Recent studies explored the population structure of *E. faecalis* by using PopPUNK (Population Partitioning Using Nucleotide K-mers), which uses variable length *k*-mer comparisons to find genetic distances between isolates to find sets of isolates significantly similar in both their core and accessory genomes relative to the rest of the species (37). A recent work analyzed a large collection of *E. faecalis* recovered from a wide variety of sources and found that the three largest PopPUNK clusters overlapped with three major *E. faecalis* STs, such as ST6, ST16, and ST40. The latter two have been isolated from human and non-human sources, while ST6 has been described only associated with humans (38). Of the STs detected in the present study, three isolates belong to CC16 (ST16, ST591 and ST1246), which has been recovered from humans and farm animals and is considered a zoonotic lineage, involved in the spread of resistance to all antimicrobial agents used in animals (10). ST16 has been identified in *optrA*-carrying *E. faecalis* clinical isolates from Germany, China, Denmark, Greece, Spain, Scotland, and Thailand (3,7,39,40,41). Three isolates belonged to CC476 (ST116, ST234 and ST476), and ST116 carrying the *optrA* gene was previously described in clinical isolates from China and Ireland (7). Coincidentally, ST476 and ST16 were recently reported in Colombia in *optrA*-carrying *E. faecalis* clinical strains isolated between 2016 and 2018 (35). In agreement with our findings, in Colombia ST16 and ST476 were associated with *optrA*-2 and *optrA*-5 variants, respectively (35).

Other STs were found unrelated to globally common linages, with the exception of ST59 which was first reported in Spanish pigs in 2001 (42) and in Malaysia in 2014 (7). ST9 was also detected among *optrA*-positive isolates from human and animal sources in China (13,43) as well as in two retail chicken isolates collected in Colombia in 2010-2011 (34). Additionally, other STs were reported in *optrA*-carrying *E. faecalis* clinical strains from the Latin American region, ST103 in Panamá in 2011; ST86 in Ecuador in 2013 ST256 in Guatemala in 2016 and ST480 in Mexico in 2016 (7).

The *optrA* gene has further been detected in various countries in enterococci isolated from food animals, animal carcasses, animal food products and wastewater, highlighting the importance of this resistance gene in a One Health context (44).

Although *E. faecalis* remain susceptible to several agents, the dissemination of *optrA* compromises the clinical utility of the oxazolidinones and other antimicrobial agents whose resistance determinants are located in the same mobile genetic elements. Oxazolidinones were only approved for therapeutic use in humans, but were strictly forbidden in food-producing animals. In contrast, phenicols, such as chloramphenicol, plays only a minor role in human medicine, while florfenicol is widely and exclusively used for medicinal purposes in farm animals worldwide (33). Selective pressure through the use of a selective agent is sufficient to ensure that the bacteria do not lose the corresponding multidrug-resistant mobile genetic element (45). Indirect selection pressure induced by the use of non-oxazolidinone antibiotics could explain the presence of oxazolidinone resistance genes in bacterial isolates from humans and animals.

The emergence of *optrA*-carrying *E. faecalis* isolates in clinical settings could be attributed to the use of oxazolidinones, unlike what occurs in animals and foods where the co-selection by other antimicrobial agents such as chloramphenicol, florfenicol macrolides and tetracyclines could acquire a relevant role. However, because the oxazolidinone resistance genes circulate in bacteria of human, animal, environmental

and food origin, a One Health approach is needed to monitor the occurrence and spread of these genes among different hosts and environments.

In summary, we report for the first time the presence of the *optrA* genes among clinical isolates of *E. faecalis* resistant to linezolid in Argentina. The phenotypic and genotypic characterization showed that these isolates were MDR and belonged to different lineages, so continuous monitoring enterococci under One Health surveillance should be encouraged.

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Table 1. Characterization of clinical linezolid resistant *E. faecalis* isolates

Isolate	Hospital No.	Year	Type of sample	Linezolid MIC (mg/L)			OptrA variant	PFGE type	MLST	CC	Resistance phenotyope
				Vitek-2	Phoenix	E-test					
M8440	3	2016	Nephrostomy catheter	≥8	8	8	2	A	ST116	476	TET-MIN-CMP-ERY
M8644	2	2019	Blood	4	4	2	6	B	ST1062		TET-MIN-CMP-ERY-CIP-GEH-STH
M8653	5	2019	Skin and soft tissues	≥8	8	6	2	C	ST591	16	TET-MIN-CMP-ERY-CIP-GEH-STH
M8689	2	2019	Urine (catheter)	4	4	4	2	D	**ST1246**	16	TET-MIN-CMP-ERY-GEH
M8691	6	2019	Skin and soft tissues	4	4	4	2	E	ST234	476	TET-MIN-CMP-ERY-CIP (I)
M8713	4	2020	Urine	≥8	8	6	2	F	**ST1247**	21	TET-MIN-CMP-ERY
M8722	1	2020	Blood	4	8	4	2	G	ST59		TET-MIN-CMP-ERY
M8726	1	2020	Punction fluid	≥8	8	6	5	H	ST476	476	TET-MIN-CMP-ERY-CIP-GEH-STH
M8729	1	2020	Blood	4	8	4	2	I	ST253		TET-MIN-CMP-ERY
M8732	4	2021	Urine	4	8	4	2	J	ST590		TET-MIN-CMP-ERY-CIP-GEH-STH
M8733	1	2021	Punction fluid	≥8	8	8	2	M	ST16	16	TET-MIN-CMP-ERY-GEH-STH
M8734	1	2021	Abdominal abscess	4	8	4	2	K	**ST1248**		TET-MIN-CMP-ERY
M8738	1	2021	Abdominal fluid	4	4	4	2	L	ST590		TET-MIN-CMP-ERY-CIP-GEH-STH
M8757	7	2021	Blood	≥8	8	8	2	N	ST415		TET-MIN-CMP-ERY-CIP (I)

TET, tetracycline; MIN, minocycline; CMP, chloramphenicol; ERY, erythromycin; CIP, ciprofloxacin; GEH, gentamicin high level resistance; STH, streptomycin high level resistance; I, intermediate resistance; ST: sequence type. STs in bold were assigned in the present study.

Table 2. Genome and assembly characteristics of linezolid resistant *E. faecalis* isolates

Isolate ID	BioSample	Sequence length (bp)	No. of contigs	GC content (%)	N50 value (bp)	L50 value
M8440	SAMN37067326	2,832,500	34	37.48	414,094	2
M8644	SAMN37067327	2,994,458	90	37.37	254,096	4
M8653	SAMN37067328	2,925,122	81	37.39	308,366	3
M8689	SAMN37067329	2,972,765	52	37.39	254,149	4
M8691	SAMN37067330	2,791,608	19	37.55	1,408,784	1
M8713	SAMN37067331	2,927,286	48	37.54	385,792	3
M8722	SAMN37067332	2,908,134	63	37.36	216,891	4
M8726	SAMN37067333	2,937,597	135	37.44	303,549	4
M8729	SAMN37067334	2,794,891	38	37.51	380,734	3
M8732	SAMN37067335	3,043,490	107	37.35	170,542	6
M8733	SAMN37067336	2,916,188	65	37.44	254,136	4
M8734	SAMN37067337	2,811,238	32	37.54	280,373	4
M8738	SAMN37067338	3,071,573	123	37.30	168,233	6
M8757	SAMN37067339	2,780,672	38	37.53	271,325	3

N50 = smallest contig of the size-sorted contigs that make up at least 50% of the respective assembly

L50 = number of contigs that make up at least 50% of the respective total assembly length

Legends to figures:

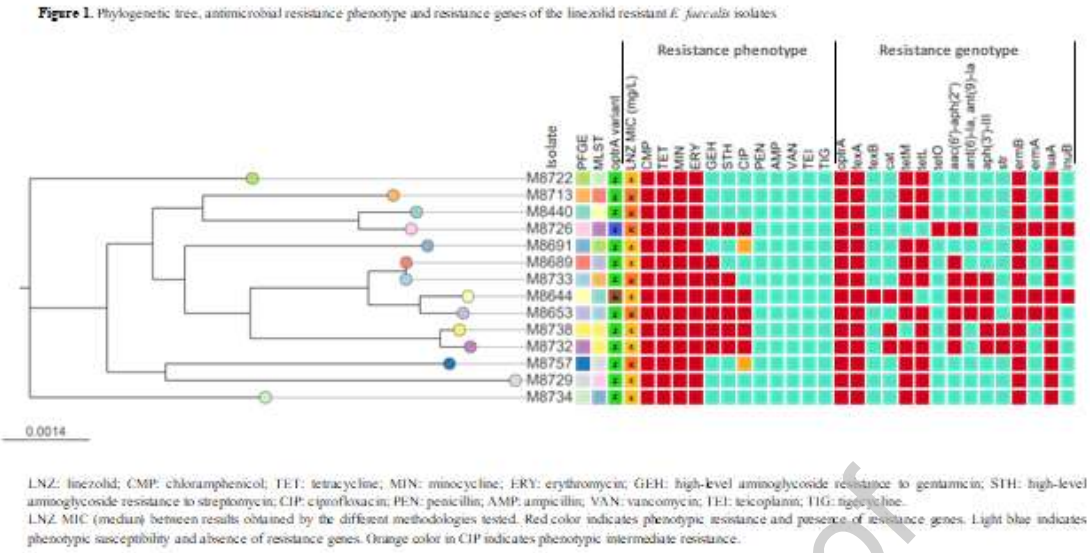
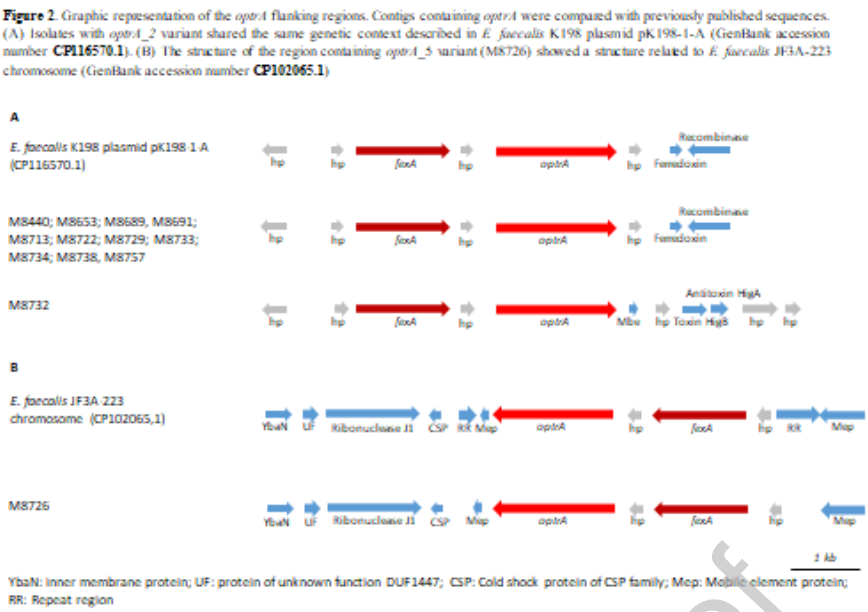


Figure 1. Phylogenetic tree, antimicrobial resistance phenotype and resistance genes of the linezolid-resistant *E. faecalis* isolates.

LNZ: linezolid; CMP: chloramphenicol; TET: tetracycline; MIN: minocycline; ERY: erythromycin; GEH: high-level aminoglycoside resistance to gentamicin; STH: high-level aminoglycoside resistance to streptomycin; CIP: ciprofloxacin; PEN: penicillin; AMP: ampicillin; VAN: vancomycin; TEI: teicoplanin; TIG: tigecycline.
LNZ MIC (median) between results obtained by the different methodologies tested. Red color indicates phenotypic resistance and presence of resistance genes. Light blue indicates phenotypic susceptibility and absence of resistance genes. Orange color in CIP indicates phenotypic intermediate resistance.



YbaN: inner membrane protein; UF: protein of unknown function DUF1447; CSP: Cold shock protein of CSP family; Mep: Mobile element protein; RR: Repeat region

Figure 2. Graphic representation of the *optrA* flanking regions. Contigs containing the *optrA* gene were compared with previously published sequences. (A) Isolates with *optrA_2* variant shared the same genetic context described in *E. faecalis* K198 plasmid pK198-1-A (GenBank accession number **CP116570.1**). (B) The structure of the region containing *optrA*_5 variant (M8726) showed a structure related to *E. faecalis* JF3A-223 chromosome (GenBank accession number **CP102065.1**).

YbaN: inner membrane protein; UF: protein of unknown function DUF1447; CSP: Cold shock protein of CSP family; Mep: Mobile element protein; RR: Repeat region.

The authors declare that they have no conflict of interests.