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1	Original Research
2	Linezolid resistance genes in enterococci isolated from sediment and
3	zooplankton in two Italian coastal areas
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30 Abstract

Linezolid is a last resort antibiotic for the treatment of severe infections caused by multi-resistant Gram-positives; although linezolid resistance remains uncommon, the number of linezolid-resistant enterococci has increased during recent years due to worldwide spread of acquired resistance genes (*cfr, optrA* and *poxtA*) in clinical, animal and environmental setting.

In this study we investigated the occurrence of linezolid-resistant enterococci in 36 marine samples from two coastal areas in Italy. Isolates grown on florfenicol-37 supplemented Slanetz-Bartley were investigated for their carriage of optrA, poxtA 38 and cfr genes: optrA was found in one E. faecalis, poxtA in three E. faecium and 39 two E. hirae and cfr was not found. Two of the three poxtA-carrying E. faecium 40 and the two E. hirae showed related PFGE profiles. Two E. faecium belonged to 41 the new ST1710, which clustered in the clonal complex CC94, encompassing 42 nosocomial strains. S1-PFGE/hybridization assays showed a double (chromosome 43 and plasmid) location of *poxtA* and plasmid location of *optrA*. WGS revealed that 44 poxtA was contained in a Tn6657-like element carried by two plasmids (pEfm-EF3 45 and pEh-GE2) of similar size, found in different species, and that *poxtA* were 46 flanked by two copies of IS1216 in both plasmids. In mating experiments all but 47 one (E. faecalis EN3) strains were able to transfer the poxtA gene to E. faecium 48 64/3. 49

50 The occurrence of linezolid resistance genes in enterococci from marine samples is 51 of great concern and highlights the need to improve practices aimed at limiting the 52 transmission of linezolid resistant strains to humans from the environmental 53 reservoirs.

54

55 **Importance**

Linezolid is one of the few antimicrobials available to treat severe infections due 56 to drug-resistant Gram-positive bacteria, thus the emergence of linezolid-resistant 57 58 enterococci carrying transferable resistance determinants is of great concern for public health. Linezolid resistance genes (cfr, optrA and poxtA), often plasmid 59 located, can be transmitted via horizontal gene transfer and have the potential to 60 61 spread globally. This study highlights the first detection of enterococci carrying linezolid resistance genes from sediment and zooplankton samples in two coastal 62 urban areas in Italy. The presence of clinically relevant resistant bacteria, such as 63 64 linezolid-resistant enterococci, in marine environment could reflect their spillover from human and/or animal reservoirs and could indicate that also coastal seawaters 65 could represent a source of these resistance genes. 66

67

69 **Introduction**

The anthropogenic release of antibiotics into environment, due to their
intensive use in human and veterinary medicine and in agriculture, has raised
global public health concerns.

The complex microbial community of aquatic environment also include transient bacteria from different sources, such as hospital, domestic and animal breeding effluents (1, 2). Antibiotic pollution imposes a selective pressure on bacterial populations which can facilitate the development and spread of antibiotic resistances through horizontal gene transfer (HGT). The evidence for horizontal dissemination of antibiotic resistances between environmental bacteria and human pathogens demonstrates the importance of environmental resistomes.

Enterococci are members of gut microbiota of humans and animals. They are released in large amounts into the environment with feces and therefore can be found in different niches including soil, foods of animal origin, vegetables, and water. Fecal indicator *Enterococcus* spp. has been well established for routine monitoring of water quality, and this principle has been extended to foods (3). More recently, enterococci have been also proposed for monitoring antibiotic resistance in food animals (4).

Although regarded as commensals, *Enterococcus* spp. are the leading causes of
nosocomial infections worldwide (5). Acquired resistances are growing and
considerably limit the therapeutic options and oxazolidinones are among the few
available last-resort antibiotics recommended to treat severe infections caused by
VRE and MDR enterococci (6).

Oxazolidinones – linezolid and tedizolid – bind in the V domain of the 23S rRNA
of the 50S ribosomal subunit and inhibit protein synthesis (7). Besides the

94 mutations in 23S rRNA and/or in L3, L4, and L22 ribosomal proteins (6, 8),

95 linezolid resistance can develop following acquisition of the resistance genes *cfr*

96 and its variants, *optrA* and *poxtA*. Cfr and Cfr-like methylases confer resistance to

97 five classes of antimicrobial agents including phenicols, lincosamides,

98 oxazolidinones, pleuromutilines and streptogramin A (PhLOPS_A phenotype) by a

99 post-transcriptional methylation of the 23S rRNA (9, 10-13). The ABC-F proteins

100 OptrA and PoxtA leads to a decreased susceptibility to phenicols, oxazolidinones

101 (including tedizolid) and tetracyclines (PoxtA protein only) by a ribosomal

102 protection mechanism (14-16).

103 In enterococci, linezolid resistance genes are often carried by mobile genetic

elements and are easily transferred between bacteria by HGT (14, 17-20).

105 Enterococci spread in many natural habitats and, besides the occurrence of

linezolid-resistant enterococci in hospitals, their detection in other reservoirs is ofspecial concern (21).

The purpose of this study was to investigate the occurrence of linezolid resistance
genes in enterococci isolated from marine samples collected at two coastal urban
areas in Italy.

111 To our knowledge, this is the first report of linezolid resistance genes in

112 enterococci from the marine environment.

114 **Results and discussion**

115

Detection of oxazolidinone resistance genes in florfenicol-resistant enterococci and antimicrobial susceptibility profiles

Out of 77 total samples (seawater=33, sediment=33 and zooplankton=11) only ten 118 119 sediment and one zooplankton samples from six sampling sites (Figure 1) were positive for the presence of florfenicol-resistant enterococci. Thirty-five isolates 120 121 were found positive for poxtA or optrA, however only six different pulsotypes (one by site) were detected by SmaI-PFGE assay. The six isolates - 1 Enterococcus 122 faecalis, 3 E. faecium and 2 Enterococcus hirae - were then characterized (Table 123 124 1). The optrA gene was only detected in the E. faecalis isolate, poxtA was identified in the 3 E. faecium and the 2 E. hirae, whereas cfr was not found (Table 125

126 1).

The *poxtA* gene, first described in a MRSA from a patient with cystic fibrosis (15), 127 was shortly after reported in enterococci isolated from many different non-human 128 sources, e.g. pigs and chicken (22-24), retail meat and food-producing animals 129 (25), as well as from air samples of swine farm (26). Through metagenomic 130 approach, this gene was recently detected in livestock manures (27), and even in 131 132 microbiome of drinking water in environmental and clinical settings (28). The wide spread of poxtA in non-human enterococci, mainly E. faecium species, 133 suggested that selection of this gene could occurred in the animal setting owing to 134 135 extensive use of phenicols and doxycycline in veterinary medicine (29). poxtAcarrying strains can then reach water bodies, including coastal waters, through 136 manure contamination and runoff from husbandry and agriculture activities. On the 137

other hand, *poxtA* has also been increasingly reported on clinical isolates (30, 31),
confirming its diffusion also in human settings.

The six enterococcal isolates were all resistant to florfenicol (MIC range, 32-128 mg/L), chloramphenicol (MIC range, 16-128 mg/L), and tetracycline (MIC range, 128->128 mg/L)) and either susceptible or resistant to linezolid (MIC range, 2-8 mg/L) and tedizolid (MIC range, 2-4 mg/L). All tested strains were susceptible to vancomycin (MIC range, 0.5-1 mg/L) (Table 1).

145

146 **Typing assays**

Enterococcal isolates belonged to 3 different SmaI-PFGE types (A to C), and two
subtypes (A1 and C1) (Table 1). *E. faecium* EF3 and ES2 were found to be closely
related (C and C1, respectively), as well as *E. hirae* GE5 (from marine sediment)

and *E. hirae* GE2 (from zooplankton) (A1 and A, respectively).

151 E. faecalis EN3 belonged to ST585 which has been associated with human

152 enterococci (32-36). E. faecium EF3 and ES2 belonged to the same ST (ST1710),

while *E. faecium* TF3 to the ST1711. Although both STs have never been described

before, ST1710 clustered in the clonal complex CC94, encompassing human

155 intestinal enterococci, recovered from both community and hospitalized hosts (37).

156 The proximity of our sampling sites to the hospital and urban areas, could suggest

157 the spread in the environment of human strains carrying linezolid resistance genes.

158

159 Location of oxazolidinones resistance genes and detection of circular forms

160 In *E. faecalis* EN3, *optrA* gene was located on two plasmids of ~20 kb and ~140 kb

161 plasmids, while in the three *poxtA*-carrying *E*. *faecium* isolates hybridisation

162 occurred on both chromosome and plasmids. The *poxtA* gene was located on

163 plasmids of different sizes: ~30 kb in *E. faecium* EF3, ~15 and ~30 kb in *E.*

faecium ES2 and ~30, ~50 and ~80 kb in *E. faecium* TF3. In the closely related *poxtA*-carrying *E. hirae* GE5 and *E. hirae* GE2 only a plasmid localization of *poxtA* gene was detected. In both isolates the *poxtA* probe hybridized on two
plasmids of ~25 and ~100 kb in size (Table 1).

Inverse PCR experiments and sequencing showed that no circular form of *optrA*genetic context was detectable. Conversely, minicircles were obtained from all the *poxtA* genetic contexts.

Since the *optrA* is located on plasmids of different size (~20 kb and ~140 kb), and
WGS revealed a single *optrA* genetic context with no evidences of circularisation,
it is reasonable to assume that recombination events between plasmids occurred
(38).

As regards *poxtA*, its location on plasmids of different sizes and even on the
chromosome, suggests an intracellular mobility of the *poxtA*-carrying element due
to IS-mediated recombination events.

178

179 Transferability of oxazolidinones resistance genes

Five of six isolates successfully transferred the linezolid resistance genes in intra-180 and interspecific mating experiments with frequencies ranging from 6.5 x 10^{-1} to 3 181 x 10^{-6} CFU per recipient. MICs and genotypes for both donors and selected 182 transconjugants, and transfer frequencies are indicated in Table 2. The higher 183 frequencies were observed in intraspecific transfer of *poxtA* from *E*. *faecium* ES2 184 and E. faecium TF3 donors to E. faecium 64/3 recipient. Conversely, E. hirae GE2 185 and GE5 successfully transferred *poxtA* to the *E*. faecium recipient. 186 In both E. faecium and E. hirae transconjugants, poxtA gene was located on 187 plasmids of ~30 kb and ~25 kb, respectively and on the chromosome (Table 2). 188

Despite several attempts E. faecalis EN3 was not able to transfer optrA gene to E.
faecium 64/3 recipient. The interspecific transfer of the resistance genes from E.
hirae to E. faecium is worrisome since the former species is more common in
animals where phenicols and tetracyclines are widely used and therefore could be a
reservoir of linezolid resistance genes for more pathogenic species such as E.
faecium.

195

196 WGS analysis

All six test strains were subjected to WGS analysis. The maps of the plasmids areshown in Figures 2-4.

Bioinformatics analysis of the draft genome of E. faecalis EN3, coupled with PCR 199 mapping and Sanger sequencing experiments, revealed that the optrA gene was part 200 201 of a 16,500 bp plasmid, named pEfs-EN3 (G+C content, 33.0%) (accession no. MT683614). According to the nomenclature of optrA variants reported by Morroni 202 et al. (39). E. faecalis EN3 showed the optrA DP variant which has been described 203 in different E. faecalis clones from human and pigs (40). The optrA genetic 204 environment (6,810 bp), bounded by two IS1216 insertion sequences arranged in 205 the same orientation, also contained the *fexA* gene located 687 bp upstream optrA 206 (Figure 2). A similar organization has been previously described in plasmids of E. 207 faecalis isolates from dogs in China (41). The repA, parA and prgN genes (orf8, 208 orf10, and orf11, respectively) responsible for the plasmid replication and 209 partitioning were also detected. The plasmid pEfs-EN3 belonged to the RepA_N 210 family and showed a rep_9 -type, which are both typical features of E. faecalis sex 211 pheromone-responsive plasmids (42). Interestingly, pheromone-responsive 212 conjugative optrA-carrying plasmids have been identified in E. faecalis of swine 213 origin (43). 214

Since the hybridization assays suggested that a *poxtA*-carrying plasmid of ~30 kb 215 216 was shared by E. faecium isolates, we decided to proceed with its assembly. In E. faecium EF3, poxtA gene was located on a 27,703-bp plasmid designed pEfm-217 EF3 (G+C content, 35.0%) (accession no. MT683615). The genetic context of 218 poxtA (4,003 bp), flanked by two IS1216 in the same orientation, was in turn 219 inserted in a Tn6657-like transposon also containing fexB as originally described 220 in the MRSA strain AOUC-0915 (accession no. MF095097) (20). Upstream the 221 Tn6657-like transposon a tetracycline resistance region containing tet(L) and 222 tet(M) genes arranged in tandem was found; downstream the Tn6657-like four 223 224 genes (orf28-orf31) involved in plasmid partitioning and replication were detected (Figure 3). pEfm-EF3 exhibited 99% DNA identity (cover 100%) with regions of 225 pC25-1 and pC27-2, two broad-host-range Inc18 plasmids from a CC17 E. faecium 226 227 of pig origin from China (accession numbers MH784601 and MH784602, respectively) (44). 228

In E. faecium ES2 and E. faecium TF3, poxtA-carrying plasmids identical to pEfmEF3 were found. It is noteworthy that the three E. faecium isolates have been
collected from different sampling sites (Table 1). Furthermore, the closely related
E. faecium EF3 and E. faecium ES2 belonged to ST1710, while E. faecium TF3
was assigned to ST1711 suggesting a spread of pEfm-EF3 by HGT may occur
among isolates with different backgrounds.

WGS analysis of *E. hirae* GE2 revealed that the *poxtA* gene was located on a

236 24,793-bp plasmid, named pEh-GE2 (G+C content, 38.0%) (accession no.

237 MT683616). BLASTN analysis displayed that in pEh-GE2 two regions exhibited a

high DNA identity with different genetic elements. The 12.8-kb region containing

the *poxtA* genetic context (*orf1* to *orf18*) showed high DNA identity (99%) with a

240 Tn6657-like transposon (20). As observed in pEfm-EN3, the *poxtA* genetic contest

241 was bracketed by IS1216 elements in the same orientation (Figure 4). The pEh-

GE2 region spanning from *orf19* to *orf31* (14.7 kb) and carrying the Tn916

243 conjugation region (including the *rep* gene) showed 99% DNA identity with

plasmid 3 of *E. faecium* E4457 (accession no. LR135260) (Figure 4).

245 WGS analysis of *E. hirae* GE5 displayed a *poxtA*-carrying plasmid with a complete

synteny to the pEh-GE2, despite the two strains come from different sampling sites

and samples (sediment and zooplankton, respectively) (Table 1).

248 Interestingly, the *poxtA*-carrying plasmids of *E. hirae* and *E. faecium* isolates

shared only the Tn6657-like region (cover 55%, DNA identity 99%) suggesting the

widespread of this element in enterococci. The pEh-GE2 resulted to belong to the

Rep_trans family which includes small size plasmids largely spread among
enterococcal populations (42).

253 Hybridization analysis also showed the presence of an *optrA*-carrying plasmid

(~140 kb) in *E. faecalis* EN3 and a *poxtA* plasmid (~100 kb) in *E. hirae* GE2 and
GE5 isolates that were not assembled.

WGS analysis also ruled out the presence of cfr(B), cfr(C), cfr(D) and cfr(E)

genes. No mutations were detected in the genes encoding the 23S rRNA orribosomal proteins.

259

260 **Conclusions**

The emergence of linezolid-resistant enterococci due to transferable resistance determinants is a matter of concern worldwide. This is – to the best of our knowledge – the first detection of enterococci carrying linezolid resistance genes in marine sediment and zooplankton. The evidence that also the coastal seawaters could serve as a reservoir of oxazolidinones resistance genes is of great concern for public health. Further surveillance and control efforts are needed to counteract the spread of linezolid-resistant bacteria in human and animal settings to prevent
the formation of environmental reservoirs of resistance genes transmissible to
humans via different routes including bathing, aquaculture and seafood
consumption.

271 Materials and methods

272

273 Sampling sites, sample processing and bacterial isolation

Sampling activities were carried out at 11 sites in two areas located on the 274 Western and Eastern coast of Italy (in Ligurian and Adriatic Sea), in a framework 275 of a research project aimed at the detection of antibiotic-resistant bacteria from 276 277 the marine environment (unpublished results). Sampling sites located in Ligurian Sea (n=3) were in front of the harbor and the hospital of Genoa city (GEN, GES, 278 GEF), whereas sampling sites in the Adriatic Sea (n=8) were in front of an urban 279 area close to the river Esino estuary and to an oil refinery (ESN, ESS, ESF), and in 280 front of the hospital (TN, TS, TF) and the harbor of Ancona city (PN, PS) (Figure 281 282 1).

At each site, seven samples (seawater n=3, sediment n=3, and zooplankton n=1)
were collected in July 2019.

All 77 samples were incubated overnight at 37°C in Azide broth (Oxoid,

Basingstoke, UK) for the selective enrichment of enterococci. Sediment (5g) 286 samples were immediately added to the enrichment broth whereas seawater and 287 zooplankton samples were processed as follows. Seawater (400 ml) were filtered 288 through 0,22 µm filter membranes (Merk Life Science, Milano, Italy) and filters 289 incubated in 30 ml Azide broth. Zooplankton (50 ml aliquots) organisms were 290 collected by dragging the water horizontally (~1m depth) with a 200 µm mesh 291 plankton net. Aliquots (50 ml) of the collected material were centrifuged 10 min at 292 15000xg; pellets were resuspended in 5 ml artificial sterile seawater and added to 293 40 ml of Azide broth. Each enrichment culture (100 µl) was spread on Slanetz 294

Bartley agar plates supplemented with florfenicol (10 mg/L) for the selection of
resistant enterococcal isolates.

297 From each selective agar plate eight presumptive resistant enterococcal colonies298 were randomly picked for further analysis.

299

300 Genotypic and phenotypic characterization

301 Selected florfenicol-resistant enterococci were screened by PCR for the presence

302 of cfr, optrA and poxtA genes using primer pairs previously described (22). The

303 PCR products were subjected to Sanger sequencing.

304 Isolates carrying linezolid resistance genes were identified by MALDI-TOF

305 (Vitek-MS, bioMérieux) and tested for their susceptibility to florfenicol,

306 chloramphenicol, linezolid, tetracycline and vancomycin (Sigma Aldrich, St.

307 Louis, MI) by standard broth microdilution assay, and to tedizolid using Etest

308 strips (Liofilchem, Roseto degli Abruzzi, Italy). Susceptibility tests were

interpreted according to clinical EUCAST (version 10.0, 2020.

310 http://www.eucast.org) or CLSI breakpoints

311 (https://clsi.org/standards/products/free-resources/access-our-free-resources/). E.

312 *faecalis* ATCC 29212 was used as quality control (EUCAST QC tables v 10.0,

313 2020. http://www.eucast.org).

314

315 SmaI-PFGE, S1-PFGE, southern blotting and hybridisation assays

316 Typing was performed by SmaI-PFGE as previously described (45).

317 Genomic DNA embedded in agarose gel plugs was digested with S1 nuclease

318 (Thermo Fisher Scientific, Milan, Italy) and chromosome and plasmids separated

by PFGE as previously described (46). After S1-PFGE, total DNA was blotted onto

positively charged nylon membranes (Ambion-Celbio, Milan, Italy) and hybridised
with biotin-labelled *cfr*, *optrA* and *poxtA* DNA probes as described elsewhere (47).

323 Detection of circular forms

324 To investigate the excision of genetic contexts carrying linezolid resistance genes,

325 PCR assays were performed using outward-directed primer pairs targeting the

326 linezolid resistance genes: (i) poxtAdiv-FW GACGAGCCGACCAACCACCT and

327 poxtAdiv-RV TTCAGGCGGACAAAAATCCAA; (ii) optrAdiv-FW

328 GAAAAATAACACAGTAAAAGGC and optrAdiv-RV

329 TTTTTCCACATCCATTTCTACC.

330 Briefly, 5 μ l of genomic DNA was added in a final volume of 25 μ l of mastermix

containing 0.2 μ M of each primer, 500 mM dNTP mix, 7 mM MgCl₂, and 2 U

332 Dream Taq DNA polymerase (ThermoFisher Scientific, Waltham, MA, USA). PCR

conditions were as follows: 94 °C for 3 min; 30 cycles of 94 °C for 1 min, 58 °C

for 1 min, and 72 °C for 5 min; and 72 °C for 5 min. PCR was performed in a

GeneAmp PCR System 9700 (Applied Biosystems System 9700 GeneAmp PCR

336 Thermal Cycler). PCR products were resolved by electrophoresis on 1.0% agarose337 gel.

The *cfr-*, *poxtA*-carrying *S. aureus* AOUC-0915 (48) and the *cfr-*, *optrA*-carrying *E. faecium* E35048 (49) isolates were used as positive controls in PCR experiments.

341

342 Conjugation experiments

Conjugal transfer was performed on a membrane filter as described previously
(47). In mating experiments, all isolates carrying linezolid resistance genes were
used as donors, and *Enterococcus faecium* 64/3 was used as a recipient (50).

Transconjugants were selected on brain heart infusion agar (Oxoid, Basingstoke,
UK) containing florfenicol (10 mg/L), fusidic acid (25 mg/L) and rifampicin (25
mg/L), grown colonies were tested for the presence of linezolid resistance genes
by PCR and for their susceptibility to florfenicol and linezolid.

SmaI-PFGE was carried out and patterns analysed to confirm the genetic
background of transconjugants. Conjugation frequencies were expressed as ratio of
cell number (CFU/ml) of transconjugants to recipient.

353

354 WGS and sequence analysis

355 Genomic DNA was extracted using a commercial kit (Sigma-Aldrich, St Louis,

356 MO, USA). Next-generation sequencing (NGS) was carried out using the Illumina

357 MiSeq platform (MicrobesNG, Birmingham, UK) by using a 2 x 250 paired end

approach. De novo assembly was performed with SPAdes v 3.11.1

359 (http://cab.spbu.ru/software/spades/), and ORFs (minimum length, 50 amino acids)

360 were annotated with the RAST Annotation server (http://rast.nmpdr.org) and ORF

361 Finder (https://www.ncbi.nlm.nih.gov/orffinder). The quality of the final contigs

362 was improved with Burrows–Wheeler Aligner. The gaps between the plasmid

363 contigs were closed by PCR mapping using primers targeting unique DNA regions

and Sanger sequencing of the resulting amplicons, after purification with a

365 GenElute PCR Cleanup kit (Sigma-Aldrich).

The presence of mutations in genes encoding all copies of the 23S rRNA and ribosomal proteins L3 and L4 were investigated by WGS analysis, comparing the sequences to those from linezolid-susceptible *E. faecalis* ATCC 29212 (accession no: ALOD01000000). The nucleotide sequences were compared with sequences in the GenBank database using BLASTN (http://blast.ncbi.nlm.nih.gov/blast). The ST was determined through the Center for Genomic Epidemiology

- 372 (https://cge.cbs.dtu.dk/services/MLST/) and MLST database
- 373 (https://pubmlst.org/general.shtml).
- 374

375 Data availability

- 376 The whole genomes of six isolates are available under the BioProject ID
- 377 PRJNA679166. The sequence of plasmids characterized in this study were
- submitted to GenBank and assigned to accession numbers: MT683614, MT683615
- and MT683616.

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386 **References**

40:25-39.

387	1.	Grenni P, Ancona V, Barra Caracciolo A. 2018. Ecological ef	fects of
388		antibiotics on natural ecosystems: A review. Microchem J 130	6:25-39.

- 389 2. Karkman A, Do TT, Walsh F, Virta MPJ. 2018. Antibiotic-resistance genes
 390 in wastewater. Trends Microbiol 26:220-228.
- 391 3. Jay JM. 2005. Indicators of food microbial quality and safety, p 473-495. In
 392 Jay JM, Loessner MJ, Golden DA (ed). Modern food microbiology, 7th ed,
 393 New York: Springer Science and Business Media.
- 4. European Food Safety Authority (EFSA). 2008. Report from the task force
 on zoonoses data collection including guidance for harmonized monitoring
 and reporting of antimicrobial resistance in commensal *Escherichia coli* and
 Enterococcus spp. from food animals. EFSA journal 141:1-44.
- 398 5. Arias CA, Murray BE. 2012. The rise of the *Enterococcus*: beyond
 399 vancomycin resistance. Nat Rev Microbiol 10:266-278.
- 6. Bender J, Cattoir V, Hegstad K, Sadowy E, Coque TM, Westh H,
 Hammerum AM, Schaffer K, Burns K, Murchan S, Novais C, Freitas AR,
 Peixe L, Del Grosso M, Pantosti A, Werner G. 2018. Update on prevalence
 and mechanisms of resistance to linezolid, tigecycline and daptomycin in
 enterococci in Europe: towards a common nomenclature. Drug Resist Updat
- Wilson DN, Schluenzen F, Harms JM, Starosta AL, Connell SR, Fucini P.
 2008. The oxazolidinone antibiotics perturb the ribosomal peptidyltransferase center and effect tRNA positioning. Proc Natl Acad Sci USA
 105:13339-133344.

410	8. Long KS, Vester B. 2012. Resistance to linezolid caused by modifications
411	at its binding site on the ribosome. Antimicrob Agents Chemother 56:603-
412	612.

413 9. Long KS, Poehlsgaard J, Kehrenberg C, Schwarz S, Vester B. 2006. The Cfr
414 rRNA methyltransferase confers resistance to phenicols, lincosamides,

415 oxazolidinones, pleuromutilins, and streptogramin A antibiotics.

416 Antimicrob Agents Chemother 50:2500-2505.

417 10. Deshpande LM, Ashcraft DS, Kahn HP, Pankey G, Jones RN, Farrell DJ

418 Mendes RE. 2015. Detection of a new *cfr*-like gene, *cfr*(B), in *Enterococcus*

419 *faecium* isolates recovered from human specimens in the United States as

420 part of the SENTRY Antimicrobial Surveillance Program. Antimicrob
421 Agents Chemother 59:6256-6261.

- 11. Tang Y, Dai L, Sahin O, Wu Z, Liu M, Zhang Q. 2017. Emergence of a
 plasmid-borne multidrug resistance gene *cfr*(C) in foodborne pathogen *Campylobacter*. J Antimicrob Chemother 72:1581-1588.
- 425 12. Pang S, Boan P, Lee T, Gangatharan S, Tan SJ, Daley D, Lee YT, Coombs
 426 GW. 2020. Linezolid-resistant ST872 Enteroccocus faecium harbouring
 427 optrA and cfr(D) oxazolidinone resistance genes. Int J Antimicrob Agents

428 55:105831.

429 13. Stojković V, Ulate MF, Hidalgo-Villeda F, Aguilar E, Monge-Cascante E,
430 Pizarro-Guajardo M, Tsai K, Tzoc E, Camorlinga M, Paredes-Sabja D,

- 431 Quesada-Gómez C, Fujimori DG, Rodríguez C. 2019. *cfr*(B), *cfr*(C), and a
- 432 new *cfr*-like gene, cfr(E), in *Clostridium difficile* strains recovered across
- 433 Latin America. Antimicrob Agents Chemother 64:e01074-19.
- 434 14. Wang Y, Lv Y, Cai J, Schwarz S, Cui L, Hu Z, Zhang R, Li J, Zhao Q, He
- 435 T, Wang D, Wang Z, Shen Y, Li Y, Feßler AT, Wu C, Yu H, Deng X, Xia X,

436	Shen J. 2015. A novel gene, optrA, that confers transferable resistance to
437	oxazolidinones and phenicols and its presence in Enterococcus faecalis and
438	Enterococcus faecium of human and animal origin. J Antimicrob Chemother
439	70:2182-2190.
440	15. Antonelli A, D'Andrea MM, Brenciani A, Galeotti CL, Morroni G, Pollini S
441	Varaldo PE, Rossolini GM. 2018. Characterization of poxtA, a novel
442	phenicol-oxazolidinone-tetracycline resistance gene from an MRSA of
443	clinical origin. J Antimicrob Chemother 73:1763-1769.
444	16. Sharkey LKR, O'Neill AJ. 2018. Antibiotic resistance ABC-F proteins:
445	bringing target protection into the limelight. ACS Infect Dis 9:239-246.
446	17. Shen J, Wang Y, Schwarz S. Presence and dissemination of the
447	multiresistance gene cfr in Gram-positive and Gram-negative bacteria.
448	2013. J Antimicrob Chemother 68:1697-1706.
449	18. Lazaris A, Coleman DC, Kearns AM, Pichon B, Kinnevey PM, Earls MR,
450	Boyle B, O'Connell B, Brennan GI, Shore AC. 2017. Novel multiresistance
451	cfr plasmids in linezolid-resistant methicillin-resistant Staphylococcus
452	epidermidis and vancomycin-resistant Enterococcus faecium (VRE) from a
453	hospital outbreak: co-location of cfr and optrA in VRE. J Antimicrob
454	Chemother 72:3252-3257.
455	19. Morroni G, Brenciani A, Antonelli A, D'Andrea MM, Di Pilato V, Fioriti S,
456	Mingoia M, Vignaroli C, Cirioni O, Biavasco F, Varaldo PE, Rossolini GM,
457	Giovanetti E. 2018. Characterization of a multiresistance plasmid carrying
458	the optrA and cfr resistance genes from an Enterococcus faecium clinical
459	isolate. Front Microbiol 9:2189.
460	20. D'Andrea MM, Antonelli A, Brenciani A, Di Pilato V, Morroni G, Pollini S,
461	Fioriti S, Giovanetti E, Rossolini GM. 2019. Characterization of Tn6349, a

462	novel mosaic transposon carrying poxtA, cfr and other resistance
463	determinants, inserted in the chromosome of an ST5-MRSA-II strain of
464	clinical origin. J Antimicrob Chemother 74:2870-2875.
465	21. Hölzel CS, Tetens JL, Schwaiger K. 2018. Unraveling the role of vegetables
466	in spreading antimicrobial-resistant bacteria: a need for quantitative risk
467	assessment. Foodborne Pathog Dis 5:671-688.
468	22. Brenciani A, Fioriti S, Morroni G, Cucco L, Morelli A, Pezzotti G, Paniccià
469	M, Antonelli A, Magistrali CF, Rossolini GM, Giovanetti E. 2019.
470	Detection in Italy of a porcine Enterococcus faecium isolate carrying the
471	novel phenicol-oxazolidinone-tetracycline resistance gene poxtA. J
472	Antimicrob Chemother 74:817-818.
473	23. Lei CW, Kang ZZ, Wu SK, Chen YP, Kong LH, Wang HN. 2019. Detection
474	of the phenicol-oxazolidinone-tetracycline resistance gene poxtA in
475	Enterococcus faecium and Enterococcus faecalis of food-producing animal
476	origin in China. J Antimicrob Chemother 74:2459-2461.
477	24. Kang ZZ, Lei CW, Yao TG, Zhang Y, Wang YL, Ye XL, Wang XC, Gao YF,
478	Wang HN. 2019. Whole-genome sequencing of Enterococcus hirae CQP3-9,
479	a strain carrying the phenicol-oxazolidinone-tetracycline resistance gene
480	poxtA of swine origin in China. J Glob Antimicrob Resist 18:71-73.
481	25. Elghaieb H, Freitas AR, Abbassi MS, Novais C, Zouari M, Hassen A, Peixe
482	L. 2019. Dispersal of linezolid-resistant enterococci carrying poxtA or
483	optrA in retail meat and food-producing animals from Tunisia. J Antimicrob
484	Chemother 74:2865-2869.
485	26. Ruiz-Ripa L, Feßler AT, Hanke D, Sanz S, Olarte C, Eichhorn I, Schwarz S,
486	Torres C. 2019. Detection of <i>poxtA</i> - and <i>optrA</i> -carrying <i>E</i> . faecium isolates
487	in air samples of a Spanish swine farm. J Glob Antimicrob Resist 22:28-31.

488	27. Wang Y, Li X, Fu Y, Chen Y, Wang Y, Ye D, Wang C, Hu X, Zhou L, Du J,
489	Shen J, Xia X. 2020. Association of florfenicol residues with the abundance
490	of oxazolidinone resistance genes in livestock manures. J Hazard Mater
491	399:123059.
492	28. Dias MF, da Rocha Fernandes G, de Paiva MC, de Matos Salim AC, Bueno
493	Santos A, Amaral Nascimento AM. 2020. Exploring the resistome, virulome
494	and microbiome of drinking water in environmental and clinical settings.
495	Water Res 174:115630.
496	29. Torres C, Alonso CA, Ruiz-Ripa L, León-Sampedro R, Del Campo R, Coque
497	TM. 2018. Antimicrobial resistance in Enterococcus spp. of animal origin.
498	Microbiol Spectr 6(4).
499	30. Papagiannitsis CC, Tsilipounidaki K, Malli E, Petinaki E. 2019. Detection
500	in Greece of a clinical Enterococcus faecium isolate carrying the novel
501	oxazolidinone resistance gene poxtA. J Antimicrob Chemother 74:2461-
502	2462.
503	31. Egan SA, Shore AC, O'Connell B, Brennan GI, Coleman DC. 2020.
504	Linezolid resistance in Enterococcus faecium and Enterococcus faecalis
505	from hospitalized patients in Ireland: high prevalence of the MDR genes
506	optrA and poxtA in isolates with diverse genetic backgrounds. J Antimicrob
507	Chemother 75:1704-1711.
508	32. Deshpande LM, Castanheira M, Flamm RK, Mendes RE. 2018. Evolving
509	oxazolidinone resistance mechanisms in a worldwide collection of
510	enterococcal clinical isolates: results from the SENTRY Antimicrobial
511	Surveillance Program. J Antimicrob Chemother 73:2314-2322.
512	33. Elghaieb H, Tedim AP, Abbassi MS, Novais C, Duarte B, Hassen A, Peixe
513	L, Freitas AR. 2020. From farm to fork: identical clones and Tn6674-like

514	elements in linezolid-resistant Enterococcus faecalis from food-producing
515	animals and retail meat. J Antimicrob Chemother 75:30-35.
516	34. He T, Shen Y, Schwarz S, Cai J, Lv Y, Li J, Feßler AT, Zhang R, Wu C,
517	Shen J, Wang Y. 2016. Genetic environment of the transferable
518	oxazolidinone/phenicol resistance gene optrA in Enterococcus faecalis
519	isolates of human and animal origin. J Antimicrob Chemother 71:1466-
520	1473.
521	35. Càmara J, Camoez M, Tubau F, Pujol M, Ayats J, Ardanuy C, Domínguez
522	MÁ. 2019. Detection of the novel optrA gene among linezolid-resistant
523	enterococci in Barcelona, Spain. Microb Drug Resist 25:87-93.
524	36. Zhou W, Gao S, Xu H, Zhang Z, Chen F, Shen H, Zhang C. 2019.
525	Distribution of the optrA gene in Enterococcus isolates at a tertiary care
526	hospital in China. J Glob Antimicrob Resist 17:180-186.
527	37. Freitas AR, Novais C, Ruiz-Garbajosa P, Coque TM, Peixe L. 2009.
528	Dispersion of multidrug-resistant Enterococcus faecium isolates belonging
529	to major clonal complexes in different Portuguese settings. Appl Environ
530	Microbiol 75:4904-4908.
531	38. Di Sante L, Morroni G, Brenciani A, Vignaroli C, Antonelli A, D'Andrea
532	MM, Di Cesare A, Giovanetti E, Varaldo PE, Rossolini GM, Biavasco F.
533	2017. pHT β -promoted mobilization of non-conjugative resistance plasmids
534	from Enterococcus faecium to Enterococcus faecalis. J Antimicrob
535	Chemother 72:2447-2453.
536	39. Morroni G, Brenciani A, Simoni S, Vignaroli C, Mingoia M, Giovanetti E.
537	2017. Commentary: Nationwide Surveillance of Novel Oxazolidinone
538	Resistance Gene optrA in Enterococcus Isolates in China From 2004 to
539	2014. Front Microbiol 8:1631.
	25

540	40. Freitas AR, Tedim AP, Novais C, Lanza VF, Peixe L. 2020. Comparative
541	genomics of global optrA-carrying Enterococcus faecalis uncovers a
542	common chromosomal hotspot for optrA acquisition within a diversity of
543	core and accessory genomes. Microb Genom 6(6).
544	41. Wu Y, Fan R, Wang Y, Lei L, Feßler AT, Wang Z, Wu C, Schwarz S, Wang
545	Y. 2019. Analysis of combined resistance to oxazolidinones and phenicols
546	among bacteria from dogs fed with raw meat/vegetables and the respective
547	food items. Sci Rep 9:15500.
548	42. Clewell DB, Weaver KE, Dunny GM, Coque TM, Francia MV, Hayes F.
549	2014. Extrachromosomal and mobile elements in enterococci: transmission,
550	maintenance, and epidemiology, p 309-134. In Gilmore MS, Clewell DB,
551	Ike Y, Shankar N (ed), Enterococci: from commensals to leading causes of
552	drug resistant infection. Boston, MA, USA: Massachusetts Eye and Ear
553	Infirmary.
554	43. Shang Y, Li D, Shan X, Schwarz S, Zhang SM, Chen YX, Ouyang W, Du
555	XD. 2019. Analysis of two pheromone-responsive conjugative
556	multiresistance plasmids carrying the novel mobile optrA locus
557	from Enterococcus faecalis. Infect Drug Resist 12:2355-2362.
558	44. Huang J, Wang M, Gao Y, Chen L, Wang L. 2019. Emergence of plasmid-
559	mediated oxazolidinone resistance gene poxtA from CC17 Enterococcus
560	faecium of pig origin. J Antimicrob Chemother 74:2524-2530.
561	45. Ripa S, Zampaloni C, Vitali LA, Giovanetti E, Montanari MP, M., Prenna
562	M, Varaldo PE. 2001. SmaI macrorestriction analysis of Italian isolates of
563	erythromycin-resistant Streptococcus pyogenes and correlations with
564	macrolide-resistance phenotypes. Microb Drug Resist 7:65-71.

565	46. Barton BM, Harding GP, Zuccarelli AJ. 1995. A general method for
566	detecting and sizing large plasmids. Anal Biochem 226:235-240.
567	47. Brenciani A, Morroni G, Pollini S, Tiberi E, Mingoia M, Varaldo PE,
568	Rossolini GM, Giovanetti E. 2016. Characterization of novel conjugative
569	multiresistance plasmids carrying cfr from linezolid-resistant
570	Staphylococcus epidermidis clinical isolates from Italy. J Antimicrob
571	Chemother 71:307-313.
572	48. Antonelli A, D'Andrea M, Galano A, Borchi B, Brenciani A, Vaggelli G,
573	Cavallo A, Bartoloni A Giovanetti E, Rossolini GM. 2016. Linezolid-
574	resistant cfr-positive MRSA, Italy. J. Antimicrob. Chemother 71:2349-
575	2351.
576	49. Brenciani A, Morroni G, Vincenzi C, Manso E, Mingoia M, Giovanetti E,
577	Varalado PE. 2016. Detection in Italy of two clinical Enterococcus faecium
578	isolates carrying both the oxazolidinone and phenicol resistance gene optrA
579	and a silent multiresistance gene cfr. J. Antimicrob. Chemother 71: 1118-
580	1119.
581	50. Werner G, Klare I, Witte W. 1997. Arrangement of the vanA gene cluster in
582	enterococci of different ecological origin. FEMS Microbiol Lett 155:55-61.

584 Figure legends

586	Figure 1. Maps of two sampling areas located in Ligurian and Adriatic Sea. The
587	yellow pins indicate sites where florfenicol-resistant enterococci were isolated; the
588	white pins indicate sites where no florfenicol-resistant strains were recovered.
589	Geographic coordinates and depth of sampling sites: GEN (44°23'25.26"N/ $\!$
590	8°56'40.56"E – 13,4 m); GES (44°23'22.06"N/ 8°56'44.59"E – 16.1 m); GEF
591	$(44^{\circ}23'21.48"N/8^{\circ}56'39.77"E - 16,2 m); EN (43^{\circ}38'51.06"N/13^{\circ}22'6.66"E - 4 m);$
592	EF (43°38'41.16"N/13°22'22.74"E - 3 m); ES (43°38'37.20"N/13°22'41.46"E - 3.4
593	m); TF (43°36'45.96"N/13°27'12.36"E - 3 m); PN (43°37'21.30"N/13°29'2.10"E -
594	8,8 m); PS (43°37'22.78"N/13°29'26.16"E – 7 m); TN
595	$(43^{\circ}37'17.94"N/13^{\circ}27'26.64"E - 7,6 m); TS (43^{\circ}36'40.02"N/13^{\circ}27'22.08"E - 2,4 m); TS (43^{\circ}36'40.02"N); TS (43^{\circ}3$
596	m).
597	
598	Figure 2. Schematic representation of the optrA-carrying pEfs-EN3 plasmid
598 599	Figure 2. Schematic representation of the <i>optrA</i> -carrying pEfs-EN3 plasmid (16,500 bp) from <i>E. faecalis</i> EN3 (accession no. MT683614).
598 599 600	Figure 2. Schematic representation of the <i>optrA</i>-carrying pEfs-EN3 plasmid(16,500 bp) from <i>E. faecalis</i> EN3 (accession no. MT683614).Arrows indicate the positions and directions of transcription of the different genes.
598 599 600 601	Figure 2. Schematic representation of the <i>optrA</i> -carrying pEfs-EN3 plasmid (16,500 bp) from <i>E. faecalis</i> EN3 (accession no. MT683614). Arrows indicate the positions and directions of transcription of the different genes.
598 599 600 601 602	 Figure 2. Schematic representation of the <i>optrA</i>-carrying pEfs-EN3 plasmid (16,500 bp) from <i>E. faecalis</i> EN3 (accession no. MT683614). Arrows indicate the positions and directions of transcription of the different genes. Figure 3. Schematic representation of the <i>poxtA</i>-carrying pEfm-EF3 plasmid
598 599 600 601 602 603	 Figure 2. Schematic representation of the <i>optrA</i>-carrying pEfs-EN3 plasmid (16,500 bp) from <i>E. faecalis</i> EN3 (accession no. MT683614). Arrows indicate the positions and directions of transcription of the different genes. Figure 3. Schematic representation of the <i>poxtA</i>-carrying pEfm-EF3 plasmid (27,703 bp) from <i>E. faecium</i> EF3 (accession no. MT683615).
598 599 600 601 602 603 604	 Figure 2. Schematic representation of the <i>optrA</i>-carrying pEfs-EN3 plasmid (16,500 bp) from <i>E. faecalis</i> EN3 (accession no. MT683614). Arrows indicate the positions and directions of transcription of the different genes. Figure 3. Schematic representation of the <i>poxtA</i>-carrying pEfm-EF3 plasmid (27,703 bp) from <i>E. faecium</i> EF3 (accession no. MT683615). Arrows indicate the positions and directions of transcription of the different genes.
598 599 600 601 602 603 604 605	 Figure 2. Schematic representation of the <i>optrA</i>-carrying pEfs-EN3 plasmid (16,500 bp) from <i>E. faecalis</i> EN3 (accession no. MT683614). Arrows indicate the positions and directions of transcription of the different genes. Figure 3. Schematic representation of the <i>poxtA</i>-carrying pEfm-EF3 plasmid (27,703 bp) from <i>E. faecium</i> EF3 (accession no. MT683615). Arrows indicate the positions and directions of transcription of the different genes.
598 599 600 601 602 603 604 605	 Figure 2. Schematic representation of the <i>optrA</i>-carrying pEfs-EN3 plasmid (16,500 bp) from <i>E. faecalis</i> EN3 (accession no. MT683614). Arrows indicate the positions and directions of transcription of the different genes. Figure 3. Schematic representation of the <i>poxtA</i>-carrying pEfm-EF3 plasmid (27,703 bp) from <i>E. faecium</i> EF3 (accession no. MT683615). Arrows indicate the positions and directions of transcription of the different genes. Figure 4. Schematic representation of the <i>poxtA</i>-carrying pEh-GE2 plasmid
 598 599 600 601 602 603 604 605 606 607 	 Figure 2. Schematic representation of the <i>optrA</i>-carrying pEfs-EN3 plasmid (16,500 bp) from <i>E. faecalis</i> EN3 (accession no. MT683614). Arrows indicate the positions and directions of transcription of the different genes. Figure 3. Schematic representation of the <i>poxtA</i>-carrying pEfm-EF3 plasmid (27,703 bp) from <i>E. faecium</i> EF3 (accession no. MT683615). Arrows indicate the positions and directions of transcription of the different genes. Figure 4. Schematic representation of the <i>poxtA</i>-carrying pEh-GE2 plasmid (24,793 bp) from <i>E. hirae</i> GE2 (accession no. MT683616).

609	Fable 1. Linezolid	resistance genes,	antimicrobial	susceptibility profiles,	, typing data and g	genes location.
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Strain	Species	Sampling	Sample	Oxazolidinone			MIC (mg/L)						Typing		S1-PFGE and	
		site		resistance genes											hybridization	
				optrA	cfr	poxtA	FFC ^a	CHL	LZD	TZD	ТЕ	VAN	SmaI-	MLST	optrA	poxtA
													PFGE			
EN3	E. faecalis	EN	sediment	+	-	-	128	128	4	4	128	1	-	ST585	20 ^b ,140	-
EF3	E. faecium	EF	sediment	-	-	+	64	16	8	2	128	1	С	ST1710	-	30, c^{c}
ES2	E. faecium	ES	sediment	-	-	+	32	16	8	2	128	1	C ₁	ST1710	-	15, 30, c
TF3	E. faecium	TF	sediment	-	-	+	64	32	2	2	>128	0.5	В	ST1711	-	30, 50, 80, c
GE5	E. hirae	GEN	sediment	-	-	+	64	64	4	2	128	0.5	A ₁	-	-	25, 100
GE2	E. hirae	GES	zooplankton	-	-	+	64	64	8	3	128	0.5	А	-	-	25, 100

611

612 ^{*a*}FFC, florfenicol; CHL, chloramphenicol; LZD, linezolid; TDZ, tedizolid; TE, tetracycline; VAN, vancomycin; ^{*b*}Estimated plasmid size (in kb)

613 ^cc, chromosome. MIC resistance breakpoints (EUCAST or CLSI): FFC, not applicable; CHL, $R \ge 32 \text{ mg/L}$; LZD, R > 4 mg/L; TDZ, $S \le 0.5 \text{ mg/L}$

614 (only for *E. faecalis*); TE, $R \ge 16 \text{ mg/L}$; VAN, R > 4 mg/L.

615 Table 2. Florfenicol and linezolid MICs, resistance genotypes and genes location for relevant
 616 transconjugants.

10	Donor				Recipient	Transfer frequency	Transconjugant			
010										
619		MIC (mg/L)		LZD resistance			MIC (mg/L)		LZD resistance	S1-PFGE and
620		FFC ^a	LZD	genotype			FFC	LZD	genotype	hybridization
621	E. faecalis EN3	128	4	optrA	E. faecium $64/3^b$	ND ^c	-	-	-	-
622	E. faecium EF3	64	8	poxtA	E. faecium 64/3	5 x 10 ⁻⁵	64	4	poxtA	30^d , c ^e
623	E. faecium ES2	32	8	poxtA	E. faecium 64/3	6.5 x 10 ⁻¹	64	4	poxtA	30, c
624	E. faecium TF3	64	2	poxtA	E. faecium 64/3	1.1 x 10 ⁻¹	32	2	poxtA	30, c
625	E. hirae GE5	64	4	poxtA	E. faecium 64/3	7.5 x 10 ⁻⁵	64	4	poxtA	25, c
626	E. hirae GE2	64	8	poxtA	<i>E. faecium</i> 64/3	3 x 10 ⁻⁶	64	4	poxtA	25, c
627										

^{*a*}FFC, florfenicol; LZD, linezolid. ^{*b*}The MICs of FFC and LZD for *E. faecium* 64/3 were 4 mg/L and 1 mg/L, respectively. ^{*c*}ND, not detectable; ^{*d*}Estimated plasmid size (in kb); ^{*e*}c, chromosome

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