


Linezolid resistance in *Enterococcus faecium* and *Enterococcus faecalis* from hospitalized patients in Ireland: high prevalence of the MDR genes *optrA* and *poxtA* in isolates with diverse genetic backgrounds

Sarah A. Egan¹, Anna C. Shore¹, Brian O'Connell^{2,3}, Grainne I. Brennan³ and David C. Coleman^{1*} 

¹Microbiology Research Unit, Division of Oral Biosciences, Dublin Dental University Hospital, University of Dublin, Trinity College, Lincoln Place, Dublin 2, Ireland; ²Department of Clinical Microbiology, School of Medicine, University of Dublin, Trinity College Dublin, St. James's Hospital, Dublin 8, Ireland; ³National MRSA Reference Laboratory, St. James's Hospital, James's Street, Dublin 8, Ireland

*Corresponding author. E-mail: david.coleman@dental.tcd.ie

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Objectives: To investigate the prevalence of the *optrA*, *poxtA* and *cfr* linezolid resistance genes in linezolid-resistant enterococci from Irish hospitals and to characterize associated plasmids.

Methods: One hundred and fifty-four linezolid-resistant isolates recovered in 14 hospitals between June 2016 and August 2019 were screened for resistance genes by PCR. All isolates harbouring resistance genes, and 20 without, underwent Illumina MiSeq WGS. Isolate relatedness was assessed using enterococcal whole-genome MLST. MinION sequencing (Oxford Nanopore) and hybrid assembly were used to resolve genetic environments/plasmids surrounding resistance genes.

Results: *optrA* and/or *poxtA* were identified in 35/154 (22.7%) isolates, the highest prevalence reported to date. Fifteen isolates with diverse STs harboured *optrA* only; one *Enterococcus faecium* isolate harboured *optrA* (chromosome) and *poxtA* (plasmid). Seven *Enterococcus faecalis* and one *E. faecium* harboured *optrA* on a 36 331 bp plasmid with 100% identity to the previously described *optrA*-encoding conjugative plasmid pE349. Variations around *optrA* were also observed, with *optrA* located on plasmids in five isolates and within the chromosome in three isolates. Nine *E. faecium* and 10 *E. faecalis* harboured *poxtA*, flanked by IS1216E, within an identical 4001 bp region on plasmids exhibiting 72.9%–100% sequence coverage to a 21 849 bp conjugative plasmid. *E. faecalis* isolates belonged to ST480, whereas *E. faecium* isolates belonged to diverse STs. Of the remaining 119 linezolid-resistant isolates without linezolid resistance genes, 20 investigated representatives all harboured the G2576T 23S RNA gene mutation associated with linezolid resistance.

Conclusions: This high prevalence of *optrA* and *poxtA* in diverse enterococcal lineages in Irish hospitals indicates significant selective pressure(s) for maintenance.

Introduction

Linezolid is an antibiotic used for infections caused by MDR Gram-positive bacteria, including VRE. Linezolid resistance was first reported in vancomycin-resistant *Enterococcus faecium* (VREfm) in Greece in 2004.^{1,2} Ireland had the highest rate of VREfm bloodstream infections in Europe between 2007 and 2017.³ Although no data are available on linezolid usage in Ireland, an almost 10% increase in the overall use of antimicrobials was noted between 2007 and 2017.⁴ A linezolid usage increase of 40% between 2012 and 2013 was reported in one Irish hospital.⁵ The emergence of

linezolid-resistant enterococci (LRE) during or after linezolid exposure has been described.^{6–10}

Linezolid binds in the V domain of the 23S rRNA component of the 50S ribosomal subunit and inhibits protein synthesis.¹¹ Enterococcal linezolid resistance can be due to G2576T or G2505A mutations in the 23S rRNA binding site or mutations in the genes encoding ribosomal proteins L3 and/or L4.⁷ However, linezolid resistance can develop following acquisition of the resistance genes *optrA*, *poxtA* and variants of the *cfr* gene, which have been described in detail previously.¹² Although the reported number of

E. faecium and *Enterococcus faecalis* isolates harbouring these genes is low, *optrA* has been reported with increased frequency recently. In 2014, it was reported that 3/9 linezolid non-susceptible isolates (MIC ≥ 4 mg/L) were *optrA*-positive *E. faecalis* (two from Ireland).¹³ This increased to 8/17 in 2016.¹⁴ German researchers reported that 6% of 698 LRE recovered between 2007 and 2017 harboured *optrA*.¹⁵

The *poxxA* gene was originally identified in an Italian clinical MRSA in 2018 and subsequently in a porcine *E. faecium*.^{16,17} More recently, *optrA* and *poxxA* were co-located on a conjugative plasmid in a porcine *E. faecalis*.¹⁸ To date, *poxxA* has only been reported in a Greek clinical *E. faecium* in 2018.¹⁹ The *cfr* gene, and its variants *cfr(B)* and *cfr(D)* (GenBank: MG707078.1) have been reported in clinical *E. faecium*,⁵ whereas only *cfr* has been reported in *E. faecalis*.^{5,20,21} The first reported linezolid-resistant VREfm outbreak in Ireland occurred in 2014; it involved 15 patients and was identified as a clonal outbreak using PFGE.²² All isolates harboured the G2576T 23S mutation and were *cfr* negative. Other linezolid resistance genes were not investigated.²² In 2014, the first two *optrA*-positive VREfm were recovered in separate Irish hospitals.¹³ In 2016, the Irish Health Protection Surveillance Centre requested that all LRE identified in Irish hospitals should be sent to the National MRSA Reference Laboratory (NMRSARL) for linezolid resistance gene screening. In 2017, a VREfm clinical isolate harbouring a *cfr*- and *optrA*-encoding plasmid was reported from an Irish hospital.⁵

The purpose of this study was to investigate the molecular mechanisms and spread of linezolid resistance in LRE from Irish hospitals sent to the NMRSARL for linezolid resistance gene screening between June 2016 and August 2019. All isolates harbouring *optrA*, *poxxA* or *cfr* and a selection of isolates without these genes were investigated using WGS.

Materials and methods

Isolates

Between June 2016 and August 2019, 154 LRE from patients in Irish hospitals were sent to the NMRSARL for linezolid resistance gene PCR screening (Table S1, available as [Supplementary data](#) at JAC Online). Thirty-five of these harboured at least one of the genes *optrA* and *poxxA* and were investigated in detail. Of the remaining 119 isolates without linezolid resistance genes, 20 representatives from a range of isolation dates and hospital locations were also investigated (Table S2). These 55 isolates investigated in detail were recovered in 14 Irish hospitals (H1–H14) (Figure S1). The remaining 99 LRE isolates lacking resistance genes were not investigated further.

Phenotypic and genotypic testing

All isolates were tested for susceptibility to linezolid, vancomycin, chloramphenicol and tetracycline using the VITEK[®]2 system (bioMérieux, France). MICs were interpreted using the EUCAST interpretative criteria.²³ Etests (bioMérieux) were used to assess linezolid MICs between 8 and 256 mg/L. PCRs for enterococcal species and resistance genes (Table S1) were performed using GoTaq DNA polymerase and buffers (Promega Corporation, USA).

Conjugation

Conjugative transfer of plasmids encoding *optrA* and/or *poxxA* harboured by all 35 LRE was undertaken by filter mating using the plasmid-free rifampicin- and fusidic acid-resistant recipient strains *E. faecium* 64/3 and

E. faecalis OG1RF.¹⁵ Putative transconjugants were screened for enterococcal species, *optrA* and *poxxA* by PCR. Transconjugants harbouring *optrA* or *poxxA* underwent WGS and genomes were assembled using SPAdes v3.7.1 and compared with the corresponding recipient strain genomes.

WGS

The 55 LRE and transconjugants underwent WGS using genomic DNA extracted using the *S. aureus* Genotyping Kit 2.0 [Abbott (Alere Technologies), Germany] and the QIAGEN DNeasy Blood and Tissue Kit (QIAGEN, UK). Libraries prepared with the Nextera DNA Flex Library Preparation Kit (Illumina, The Netherlands) underwent paired-end sequencing using the 500-cycle MiSeq Reagent Kit v2 (Illumina). Libraries were scaled to yield $\geq 50\times$ coverage.

For isolates selected for hybrid assembly, DNA was extracted using the GenFind v3 kit (Beckman Coulter, USA). Long-read sequencing was performed in multiplex using MinION sequencing (Oxford Nanopore Technologies, UK), the 1D Genomic DNA sequencing kit (SQK-LSK109) and 1D Native Barcoding Kit (EXP-NBD103). Libraries were sequenced on an Mk1B (MIN101B) MinION platform with a FLO-MIN106D (SpotON R9.4) flow cell and using MinKNOW v1.7.10 (Oxford Nanopore). Basecalls were performed on MinION FAST5 files using Guppy v3.1.5 (Oxford Nanopore) and demultiplexing was performed using qCat v1.0.1 (<https://github.com/nanoporetech/qcat>).

Analysis of WGS data

WGS data were analysed using the enterococcal whole-genome (wg) MLST schemes available in BioNumerics v7.7 (Applied Maths, Belgium). The *E. faecium* scheme consisted of 5489 wgMLST loci [including 1423 core-genome (cg) MLST loci], while the *E. faecalis* scheme consisted of 5285 wgMLST loci.²⁴ Two BioNumerics algorithms were used to generate a consensus wgMLST profile for each isolate, one of which determined locus presence/absence and allelic identity using an assembly-free *k*-mer approach. The other, assembly-based, method used a BLAST approach to detect alleles on contigs assembled using SPAdes v3.7.1, all using default parameters. Minimum-spanning trees (MSTs) were created using BioNumerics based on allelic differences. Illumina WGS data for all isolates were examined for 23S rRNA mutations (G2576T and G2505A) using LRE-Finder (<https://cge.cbs.dtu.dk/services/LRE-finder/>).²⁵

Assembly and analysis of plasmids encoding resistance genes

MinION-generated FASTQ files and MiSeq-generated FASTQ files were used to perform a hybrid assembly using Unicycler.²⁶ The genetic organization of plasmids harbouring *optrA* or *poxxA* was determined following hybrid assembly and annotation using RAST v2.0 (<http://rast.nmpdr.org/>).²⁷ These were used as reference sequences for further analysis. MiSeq reads were mapped against reference plasmid sequences and percentage depth and breadth of coverage was calculated using Burrows–Wheeler aligner, SAMtools and BEDTools coverage.^{28–30} Alignments were viewed for quality using Tablet.³¹

Sequences of plasmids resolved by hybrid assembly (pM16/0594, pM18/0011 and pM17/0314) and DNA regions encoding *optrA* variants have been deposited in GenBank under accession numbers MN831410, MN831411, MN831412, MN831413, MN831414, MN831415, MN831416, MN831417, MN831418 and MN831419.

Results and discussion

Linezolid-resistant isolates

A total of 35/154 (22.7%) LRE (23 *E. faecalis* and 12 *E. faecium*) submitted to the NMRSARL between June 2016 and August 2019

harboured *optrA* (2 *E. faecium* and 13 *E. faecalis*), *poxtA* (9 *E. faecium* and 10 *E. faecalis*) or both *optrA* and *poxtA* (1 *E. faecium*). All 35 isolates were from hospitalized patients in 11 Irish hospitals (H1–H11) (Figure S1) and were phenotypically resistant to linezolid, chloramphenicol and tetracycline (Table S2). This is the highest prevalence of *optrA* and/or *poxtA* in human enterococci reported to date. Six *E. faecium* isolates were vancomycin resistant and harboured *vanA*. The remaining 29 LRE isolates were vancomycin susceptible and lacked *vanA* (Table S2). The majority (19/35) of LRE harboured *poxtA* only; the largest collection of *poxtA*-positive human isolates reported to date. The remaining LRE harboured *optrA* only (14/35), *optrA* and *poxtA* (1/35) or *optrA* and *cfr(D)* (1/35). One VREfm (M19/0595) harboured *poxtA* and the G2576T 23S mutation (4/6 copies mutated) and is the first report of mutational and gene-encoded linezolid resistance in a single enterococcal isolate. The remaining 34 LRE lacked 23S mutations.

All 20/119 LRE investigated that lacked *cfr*, *optrA* and/or *poxtA* (2 *E. faecalis*, 18 *E. faecium*) were phenotypically resistant to linezolid and chloramphenicol (Table S2). All 20 LRE exhibited a varying copy number (1–5) of the G2576T 23S mutation. The majority of the LRE (15/18 *E. faecium*) harboured *vanA* and exhibited vancomycin MICs ≥ 32 mg/L. The remaining five *vanA*-negative isolates (two *E. faecalis* and three *E. faecium*) were vancomycin susceptible (Table S2).

Relatedness of LRE based on WGS

Of the 55 LRE investigated, the 30 *E. faecium* included from 11 hospitals were assigned to 10 STs using traditional MLST, with ST80 predominating (8/30, 26.6%). Seventeen *E. faecium* isolates were differentiated into seven clusters (CI–CVII) using cgMLST (Figure 1a). Clusters CI–CVI contained isolates of the same STs (ST17, ST787, ST789, ST202 and ST203), while cluster CVII consisted of ST203 and ST1588 (a single locus variant of ST203) isolates (Figure 1a). Clusters were differentiated by intracluster and intercluster allelic differences of 0–22 and 38–394, respectively. Isolates exhibiting ≤ 22 allelic differences were deemed closely related, based on previous work.²⁴ Clusters CI and CIII contained isolates from the same hospitals (H2 and H6), all with the G2576T 23S mutation. The remaining five clusters consisted of isolates from two or more hospitals and a mixture of isolates exhibiting linezolid resistance associated with G2576T mutations or a resistance gene (Figure 1a).

Of the 55 LRE investigated, the 25 *E. faecalis* included originated from 10 hospitals and belonged to nine STs using traditional MLST, with ST480 predominating (13/25). Twenty of the *E. faecalis* isolates differentiated into four clusters (CI–CIV) using wgMLST (Figure 1b). The remaining five isolates were distantly related to any other isolate, exhibiting between 339 and 1897 allelic differences. Clusters were defined by the contrasting tight value of intracluster differences (0–43). Each cluster contained isolates from the same STs (ST6, ST21, ST480 and ST768). Only one cluster, CIV, contained two isolates from the same hospital (H2), both with the G2576T 23S mutation. The remaining three clusters contained isolates from 2–8 hospitals. Clusters CII ($n=3$) and CIII ($n=2$) contained only *optrA*-positive isolates, whereas cluster CI ($n=13$) contained only ST480 isolates, 10 and 3 of which harboured *poxtA* or

optrA, respectively. Isolates within cluster CI exhibited an average allelic difference of 29 (range 7–90) (Figure 1b).

Overall the population structure of LRE was polyclonal, but the presence of highly related strains in different hospitals was evident.

Genetic environment surrounding *optrA*

The WGS data of a selection of isolates encoding *optrA* underwent hybrid assembly, namely two *E. faecium* (M16/0594 and M17/0314) and seven *E. faecalis* (M17/0149, M17/0240, M18/0173, M18/0497, M18/0582, M18/0906 and M19/0596). The first plasmid resolved was a 36 331 bp *optrA*-encoding plasmid (pM17/0149) from *E. faecalis* M17/0149, which was the same size and exhibited 100% DNA sequence identity to plasmid pE349, first described from a clinical *E. faecalis* in China and subsequently identified in *E. faecium* and *E. faecalis* from humans and food-producing animals throughout European, American and Asian countries.^{7,9,13,14,32} Of the 16 *optrA*-positive isolates, half (one *E. faecium* and seven *E. faecalis*) harboured plasmids exhibiting 100% sequence coverage to pM17/0149 (pE349-like). Multiple *E. faecalis* STs (ST19, ST21, ST166 and ST768) harboured this pE349-like plasmid, suggesting independent acquisition by different genetic backgrounds (Table S2). For these eight isolates, the genetic environment surrounding *optrA* was designated *optrA_WT* (Figure 2). The remaining eight *optrA*-positive isolates exhibited $<35\%$ sequence identity to pM17/0149 and were selected for WGS hybrid assembly. The genetic environment surrounding *optrA* was examined and *optrA* was identified on plasmids in five isolates (M17/0240, M18/0582, M19/0596, M18/0173 and M17/0314) and within the chromosome in three isolates (M18/0497, M16/0594 and M18/0906) (Figure 2). Three variations surrounding *optrA* were evident on the plasmid-encoded regions: *optrA_I* (M17/0240, M18/0582 and M19/0596); *optrA_II* (M18/0173); and *optrA_III* (M17/0314). In *optrA_I* and *optrA_II*, *fexA* was encoded around 750 bp upstream of *optrA*, similarly to *optrA_WT*, whereas *optrA_III* lacked *fexA*. Variations were distinguished by various flanking IS elements and other genes encompassed between these elements (Figure 2a). Interestingly, isolate M17/0314, harbouring *optrA_III*, also encoded the linezolid resistance gene *cfr(D)* and the macrolide, lincosamide and streptogramin B resistance genes *erm(A)* and *erm(B)*, on a 103 600 bp plasmid (pM17/0314) (Figure S2). In the case of 1/8 *optrA_WT* isolates (M16/0427), 2/3 isolates harbouring *optrA_I* (M17/0240 and M19/0596) and 1 *optrA_III* isolate (M17/0314), *optrA* was successfully conjugated into a recipient strain of the same species (Table S3).

Three variations of the genetic environment surrounding *optrA* in the chromosome, designated *optrA_IV*, *optrA_V* and *optrA_VI*, were identified in isolates M18/0906, M16/0594 and M18/0497, respectively (Table S2). Both the *optrA_IV* and *optrA_V* variants harboured *optrA*, flanked by *tnpA* and *tnpB* from Tn554 on one end and by ISL3 on the other end. The *optrA_IV* variant encoded *fexA* and *optrA* in the same orientation as *optrA_WT*, but in *optrA_V*, *fexA* was encoded around 2800 bp upstream from *optrA* in the opposite orientation to the arrangement in *optrA_WT* (Figure 2b). In *optrA_VI*, *optrA* was flanked by ISE_{Enfa5} and ISE_{Fa5} and exhibited the same *optrA* and *fexA* arrangement present in *optrA_WT* (Figure 2b). Attempts to transfer *optrA* in the three isolates encoding chromosomal *optrA* by conjugation were unsuccessful.

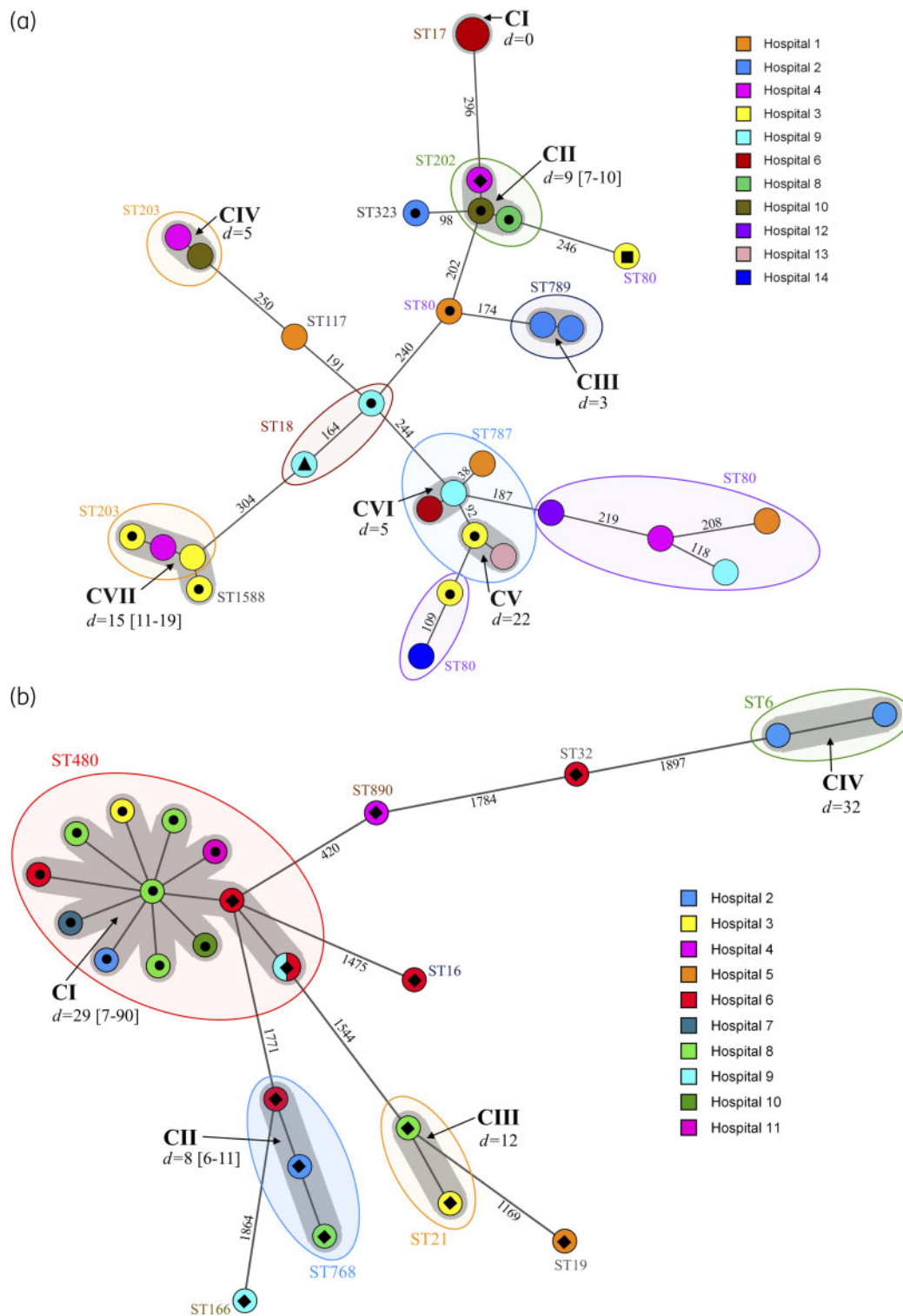


Figure 1. Minimum spanning trees based on (a) cgMLST data from 30 linezolid-resistant clinical *E. faecium* isolates and (b) wgMLST data from 25 linezolid-resistant *E. faecalis* clinical isolates. All isolates were recovered between June 2016 and August 2019 from 14 Irish hospitals, as denoted in the legends. The numbers on the branches represent the number of cgMLST/wgMLST allelic differences. STs are shown in coloured ovals. Grey shadowing around nodes indicates clusters of related isolates, which are labelled in bold and denoted CI–VII; ‘ d ’ values indicate average allelic differences and the range in square brackets. Isolate designations are as follows: filled black circle, *poxtA* positive; filled black diamond, *optrA* positive; filled black square, *optrA* positive and *cfr(D)* positive; and filled black triangle, *optrA* positive and *poxtA* positive. Isolates not marked with a symbol were negative for linezolid resistance genes and harboured various copy numbers (1–5) of the G2576T 23S mutation associated with linezolid resistance.

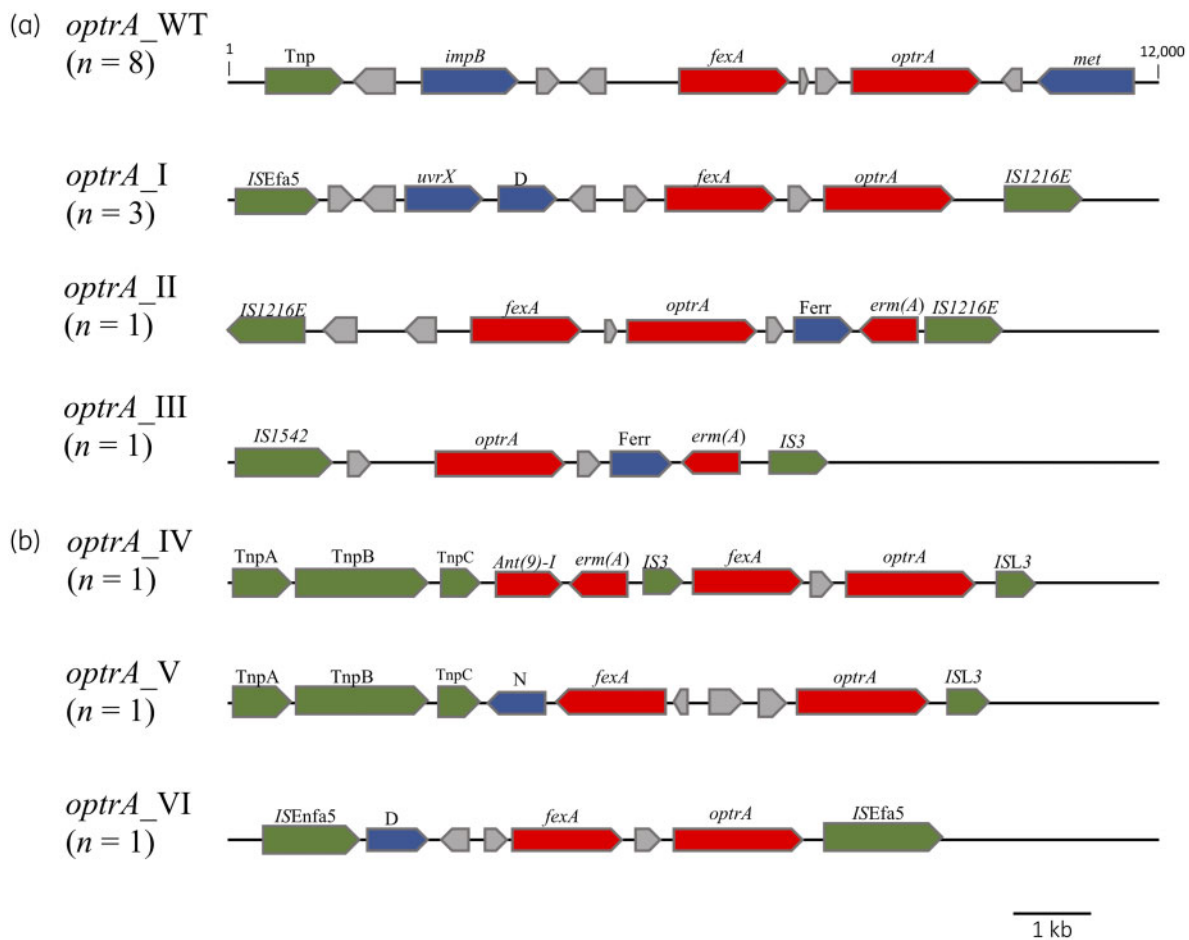


Figure 2. Schematic representation of the *optrA* gene loci in *E. faecalis* and *E. faecium* clinical isolates from Ireland. Different variants of the genetic environment surrounding *optrA* were detected in LRE encoded on plasmids (a) and within the chromosome (b) and were aligned against the prototype *optrA*_WT, first described by Wang et al. in 2015.⁹ Genes of interest and their orientation are shown with arrows and labelled; red indicates antibiotic resistance genes, green indicates ISSs/transposases, blue indicates known proteins and grey indicates hypothetical proteins. Tnp, transposase; met, truncated DNA adenine methylase; *uvrX*, putative UV-damage repair protein; D, DNA-directed RNA polymerase β subunit; Ferr, ferredoxin; N, NAD(P)H oxidoreductase.

The variation surrounding *optrA* in the investigated LRE, combined with the identification of multiple linezolid resistance genes [*optrA/poxxA* and *optrA/cfr(D)*] in individual isolates, is indicative of an environment with a high selective pressure for linezolid resistance.

Characterization of isolates encoding *poxxA*

The *E. faecium* isolate M16/0594 harboured *optrA*_V in its chromosome and *poxxA*, *tet(M)* and *tet(L)* on a 21 849 bp plasmid (pM16/0594) (Figure 3). Plasmid pM16/0594 was conjugative and transconjugant derivatives were obtained with the *E. faecium* 64/3 recipient. Of the remaining 19 *poxxA*-positive LRE (9 *E. faecium* and 10 *E. faecalis*), 5/19 (all *E. faecium*) exhibited 100% sequence coverage to pM16/0594, while 14/19 (4 *E. faecium* and 10 *E. faecalis*) exhibited between 72.9% and 99.3% coverage. All isolates exhibited 100% sequence coverage to a 4001 bp *poxxA*-encoding region, flanked by two IS1216E elements in parallel orientation; this conserved *poxxA* element has previously been shown to be responsible for horizontal gene transfer of *poxxA*.^{16,33,34} These findings indicate

the spread of *poxxA* in human *E. faecium* and *E. faecalis* in Ireland via this 4001 bp element or by a very similar *poxxA* plasmid. One limitation of this method of read mapping against pM16/0594 is the risk of missing larger plasmids, as 100% coverage will indicate the query sequence is identical to the reference used but cannot indicate if the plasmid is larger. Therefore, isolates with 100% sequence coverage to pM16/0594 have at least a 21 849 bp plasmid region identical to that of pM16/0594, which may be contained within larger plasmids.

The *poxxA*-positive *E. faecalis* isolate M18/0011 exhibiting 73.7% sequence coverage to pM16/0594 was also resolved using hybrid assembly. The plasmid encoding *poxxA* in M18/0011 (pM18/0011) was 3570 bp smaller than pM16/0594 (18 279 bp) and lacked *tet(M)* and *tet(L)*. An identical 4001 bp region encoding *poxxA*, flanked by two IS1216E elements in parallel orientation, was observed in pM18/0011 (Figure S3), albeit in the reverse orientation to the identical region in pM16/0594. Plasmid pM18/0011 was also conjugative and transconjugant derivatives were obtained with the *E. faecalis* OG1RF and *E. faecium* 64/3 recipients (Table S3).

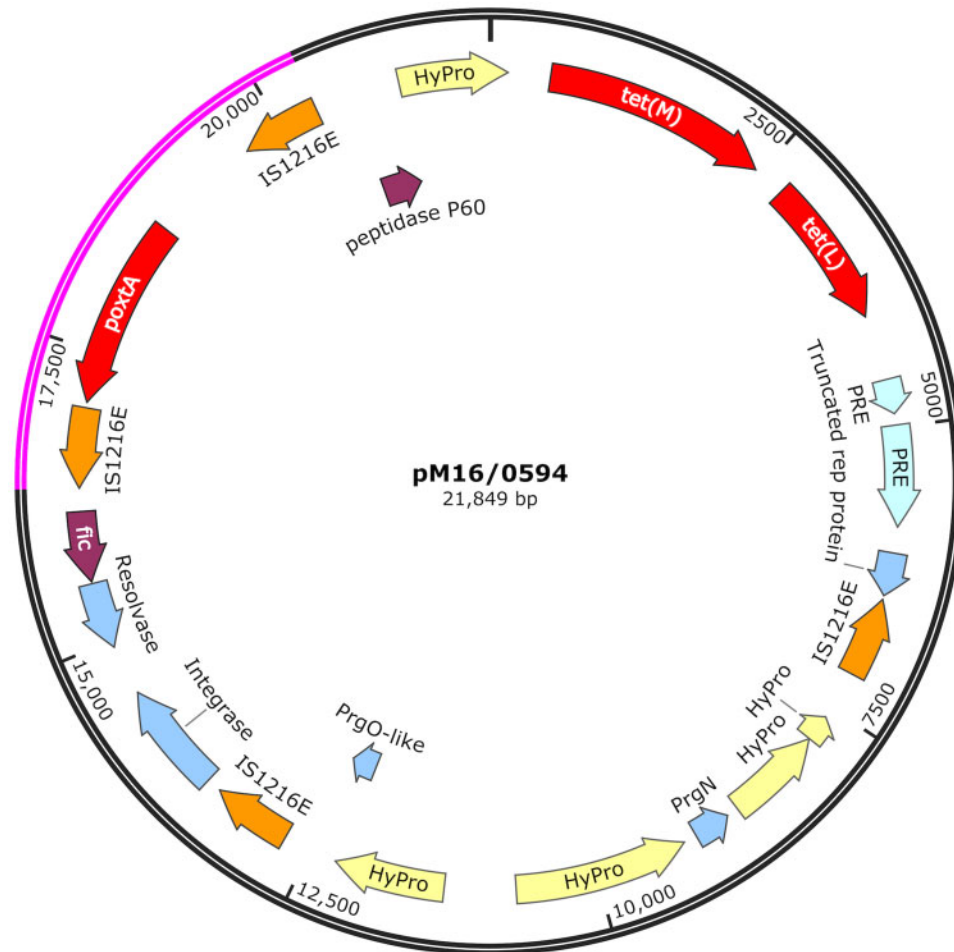


Figure 3. Schematic diagram of the structural organization of plasmid pM16/0594 from *E. faecium* isolate M16/0594, encoding the *poxA* linezolid resistance gene resolved by hybrid assembly of paired-end Illumina MiSeq short reads with Oxford Nanopore Technologies long reads. The 4001 bp highly conserved region around *poxA*, flanked by two *IS1216E* in parallel orientation, is highlighted in pink. Genes of interest and their orientation are represented by arrows as follows: red indicates antibiotic resistance genes, orange indicates ISs, blue indicates rep proteins, purple indicates other known proteins and yellow indicates hypothetical proteins. The plasmid size is labelled (bp). PRE, plasmid recombination enzyme; fic, cell filamentation protein; HyPro, hypothetical protein.

In total, 5/20 *poxA*-positive LRE yielded transconjugant derivatives including *E. faecium* M16/0594 (harbouring pM16/0594) and *E. faecalis* M18/0011 (harbouring pM18/0011), two *E. faecalis* donors (M16/0419, 75.2% like pM16/0594; and M16/0633, 74.5% like pM16/0594) conjugated to *E. faecalis* OG1RF and one *E. faecium* donor (M19/0357, 77.9% like pM16/0594) conjugated to *E. faecium* 64/3 (Table S3). For the remaining 15 *poxA*-positive LRE, conjugation was unsuccessful. The spread of a single clone was indicated in all 10 identified *poxA*-positive *E. faecalis* LRE, as all belonged to ST480 and were closely related, with an average of 22 (range 7–54) wgMLST allelic differences (Figure 1b). The ST480 clone was recovered in seven hospitals (Table S2). ST480 is one of the predominant *optrA*-positive clinical *E. faecalis* clones in France and Germany.^{15,35} In contrast, *poxA* was found to have spread in *E. faecium* via the 4001 bp mobile element or a promiscuous plasmid to multiple STs (ST18, ST80, ST202, ST203, ST323, ST787 and ST1588).

Conclusions

The results of this study revealed the high prevalence and spread of *optrA* and *poxA* among enterococci in Irish hospitals. A major cause for concern is that 26.3% (5/19) of isolates harbouring a *poxA*-encoding plasmid also harboured *vanA*, which poses a significant risk for hospitalized patients, as treatment options for such strains are limited. Linezolid consumption in Irish hospitals is currently not specifically recorded as part of annual national hospital antimicrobial consumption surveillance.⁴ This needs to change and linezolid consumption in hospitals needs to be more rigidly controlled.

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Transparency declarations

None to declare.

Supplementary data

Tables S1 to S3 and Figures S1 to S3 are available as [Supplementary data](#) at JAC Online

References

- Zahedi Bialvaei A, Rahbar M, Yousefi M et al. Linezolid: a promising option in the treatment of Gram-positives. *J Antimicrob Chemother* 2017; **72**: 354–64.
- Bersos Z, Maniati M, Kontos F et al. First report of a linezolid-resistant vancomycin-resistant *Enterococcus faecium* strain in Greece. *J Antimicrob Chemother* 2004; **53**: 685–6.
- ECDC. The European Antimicrobial Resistance Surveillance System EARS-Net Results. 2017. <https://ecdc.europa.eu/en/antimicrobial-resistance/surveillance-and-disease-data/data-ecdc>.
- HSE/HPSC. Hospital Antimicrobial Consumption Surveillance. 2018. http://www.hpsc.ie/a-z/microbiologyantimicrobialresistance/europeansurveillanceofantimicrobialconsumptionesac/PublicMicroB/SACHC/SACHC_Current.pdf.
- Lazaris A, Coleman DC, Kearns AM et al. Novel multiresistance *cfr* plasmids in linezolid-resistant methicillin-resistant *Staphylococcus epidermidis* and vancomycin-resistant *Enterococcus faecium* (VRE) from a hospital outbreak: co-location of *cfr* and *optrA* in VRE. *J Antimicrob Chemother* 2017; **72**: 3252–7.
- Gonzales RD, Schreckenberger PC, Graham MB et al. Infections due to vancomycin-resistant *Enterococcus faecium* resistant to linezolid. *Lancet* 2001; **357**: 1179.
- Bi R, Qin T, Fan W et al. The emerging problem of linezolid-resistant enterococci. *J Glob Antimicrob Resist* 2018; **13**: 11–9.
- Bai B, Hu K, Zeng J et al. Linezolid consumption facilitates the development of linezolid resistance in *Enterococcus faecalis* in a tertiary-care hospital: a 5-year surveillance study. *Microb Drug Resist* 2019; **25**: 791–8.
- Wang Y, Lv Y, Cai J et al. A novel gene, *optrA*, that confers transferable resistance to oxazolidinones and phenicols and its presence in *Enterococcus faecalis* and *Enterococcus faecium* of human and animal origin. *J Antimicrob Chemother* 2015; **70**: 2182–90.
- Cai J, Wang Y, Schwarz S et al. Enterococcal isolates carrying the novel oxazolidinone resistance gene *optrA* from hospitals in Zhejiang, Guangdong, and Henan, China, 2010–2014. *Clin Microbiol Infect* 2015; **21**: 1095.e1–4.
- Swaney SM, Aoki H, Ganoza MC et al. The oxazolidinone linezolid inhibits initiation of protein synthesis in bacteria. *Antimicrob Agents Chemother* 1998; **42**: 3251–5.
- Bender JK, Cattoir V, Hegstad K et al. Update on prevalence and mechanisms of resistance to linezolid, tigecycline and daptomycin in enterococci in Europe: towards a common nomenclature. *Drug Resist Updat* 2018; **40**: 25–39.
- Mendes RE, Hogan PA, Jones RN et al. Surveillance for linezolid resistance via the Zyvox Annual Appraisal of Potency and Spectrum (ZAAPS) programme (2014): evolving resistance mechanisms with stable susceptibility rates. *J Antimicrob Chemother* 2016; **71**: 1860–5.
- Mendes RE, Deshpande L, Streit JM et al. ZAAPS programme results for 2016: an activity and spectrum analysis of linezolid using clinical isolates from medical centres in 42 countries. *J Antimicrob Chemother* 2018; **24**: 328–37.
- Bender JK, Fleige C, Lange D et al. Rapid emergence of highly variable and transferable oxazolidinone and phenicol resistance gene *optrA* in German *Enterococcus* spp. clinical isolates. *Int J Antimicrob Agents* 2018; **52**: 819–27.
- Antonelli A, D'Andrea MM, Brenciani A et al. Characterization of *poxxA*, a novel phenicol–oxazolidinone–tetracycline resistance gene from an MRSA of clinical origin. *J Antimicrob Chemother* 2018; **73**: 1763–9.
- Brenciani A, Fioriti S, Morroni G et al. Detection in Italy of a porcine *Enterococcus faecium* isolate carrying the novel phenicol-oxazolidinone-tetracycline resistance gene *poxxA*. *J Antimicrob Chemother* 2019; **74**: 817–8.
- Hao W, Shan X, Li D et al. Analysis of a *poxxA*- and *optrA*-co-carrying conjugative multiresistance plasmid from *Enterococcus faecalis*. *J Antimicrob Chemother* 2019; **74**: 1771–5.
- Papagiannitsis CC, Tsilipounidaki K, Malli E et al. Detection in Greece of a clinical *Enterococcus faecium* isolate carrying the novel oxazolidinone resistance gene *poxxA*. *J Antimicrob Chemother* 2019; **74**: 2461–2.
- Diaz L, Kiratisin P, Mendes RE et al. Transferable plasmid-mediated resistance to linezolid due to *cfr* in a human clinical isolate of *Enterococcus faecalis*. *Antimicrob Agents Chemother* 2012; **56**: 3917–22.
- Bender JK, Fleige C, Klare I et al. Detection of a *cfr(B)* variant in German *Enterococcus faecium* clinical isolates and the impact on linezolid resistance in *Enterococcus* spp. *PLoS One* 2016; **11**: e0167042.
- O'Driscoll C, Murphy V, Doyle O et al. First outbreak of linezolid-resistant vancomycin-resistant *Enterococcus faecium* in an Irish hospital, February to September 2014. *J Hosp Infect* 2015; **91**: 367–70.
- EUCAST. Breakpoint Tables for Interpretation of MICs and Zone Diameters, Version 9.0. 2019. <http://www.eucast.org>.
- de Been M, Pinholt M, Top J et al. Core genome multilocus sequence typing scheme for high-resolution typing of *Enterococcus faecium*. *J Clin Microbiol* 2015; **53**: 3788–97.
- Hasman H, Clausen P, Kaya H et al. LRE-Finder, a Web tool for detection of the 23S rRNA mutations and the *optrA*, *cfr*, *cfr(B)* and *poxxA* genes encoding linezolid resistance in enterococci from whole-genome sequences. *J Antimicrob Chemother* 2019; **74**: 1473–6.
- Wick RR, Judd LM, Gorrie CL et al. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. *PLoS Comput Biol* 2017; **13**: 1–22.
- Overbeek R, Olson R, Pusch GD et al. The SEED and the Rapid Annotation of microbial genomes using Subsystems Technology (RAST). *Nucleic Acids Res* 2014; **42**: D206–14.
- Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler Transform. *Bioinformatics* 2009; **25**: 1754–60.
- Li H, Handsaker B, Wysoker A et al. The Sequence Alignment/Map (SAM) format and SAMtools. *Bioinformatics* 2009; **25**: 2078–9.
- Quinlan AR, Hall IM. BEDTools: a flexible suite of utilities for comparing genomic features. *Bioinformatics* 2010; **26**: 841–2.

- 31** Milne I, Stephen G, Bayer M *et al.* Using Tablet for visual exploration of second-generation sequencing data. *Brief Bioinform* 2013; **14**: 193–202.
- 32** Cavaco LM, Bernal JF, Zankari E *et al.* Detection of linezolid resistance due to the *optrA* gene in *Enterococcus faecalis* from poultry meat from the American continent (Colombia). *J Antimicrob Chemother* 2016; **72**: 678–83.
- 33** Lei C-W, Kang Z-Z, Wu S-K *et al.* Detection of the phenicol-oxazolidinone-tetracycline resistance gene *poxxA* in *Enterococcus faecium* and *Enterococcus faecalis* of food-producing animal origin in China. *J Antimicrob Chemother* 2019; **74**: 2459–61.
- 34** Huang J, Wang M, Gao Y *et al.* Emergence of plasmid-mediated oxazolidinone resistance gene *poxxA* from CC17 *Enterococcus faecium* of pig origin. *J Antimicrob Chemother* 2019; **74**: 2524–30.
- 35** Sassi M, Guérin F, Zouari A *et al.* Emergence of *optrA*-mediated linezolid resistance in enterococci from France, 2006–16. *J Antimicrob Chemother* 2019; **74**: 1469–72.