

Presence of *optrA*-mediated linezolid resistance in multiple lineages and plasmids of *Enterococcus faecalis* revealed by long read sequencing

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Abstract

Transferable linezolid resistance due to *optrA*, *poxA*, *cfr* and *cfr*-like genes is increasingly detected in enterococci associated with animals and humans globally. We aimed to characterize the genetic environment of *optrA* in linezolid-resistant *Enterococcus faecalis* isolates from Scotland. Six linezolid-resistant *E. faecalis* isolated from urogenital samples were confirmed to carry the *optrA* gene by PCR. Short read (Illumina) sequencing showed the isolates were genetically distinct (>13900 core SNPs) and belonged to different MLST sequence types. Plasmid contents were examined using hybrid assembly of short and long read (Oxford Nanopore MinION) sequencing technologies. The *optrA* gene was located on distinct plasmids in each isolate, suggesting that transfer of a single plasmid did not contribute to *optrA* dissemination in this collection. pTM6294-2, BX5936-1 and pWE0438-1 were similar to *optrA*-positive plasmids from China and Japan, while the remaining three plasmids had limited similarity to other published examples. We identified the novel Tn6993 transposon in pWE0254-1 carrying linezolid (*optrA*), macrolide (*ermB*) and spectinomycin [ANT(9)-Ia] resistance genes. *OptrA* amino acid sequences differed by 0–20 residues. We report multiple variants of *optrA* on distinct plasmids in diverse strains of *E. faecalis*. It is important to identify the selection pressures driving the emergence and maintenance of resistance against linezolid to retain the clinical utility of this antibiotic.

INTRODUCTION

Enterococcus faecalis and *Enterococcus faecium* are carried in the intestinal tract and are important opportunistic pathogens in humans [1]. Treatment of enterococcal infections is challenging due to intrinsic or acquired resistance to multiple antimicrobials including aminoglycosides, benzylpenicillin, cephalosporins, fluoroquinolones, macrolides, tetracyclines and trimethoprim. Among the remaining treatment options, clinical *E. faecium* isolates are usually resistant to amoxicillin, and resistance to vancomycin is increasingly common [2]. In contrast, *E. faecalis* typically remains susceptible to amoxicillin and resistance to vancomycin is uncommon. Where vancomycin cannot be used, treatment options against severe enterococcal infections are largely limited to daptomycin, linezolid or combination therapy and are further complicated by issues with efficacy, susceptibility or toxicity [1].

Oxazolidinones such as linezolid block protein synthesis by binding to the 50S ribosomal subunit and inhibit formation of the initiation complex [3]. Linezolid resistance is reported in ≤1% of bloodstream enterococcal isolates in the UK and is an important

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Abbreviations: SNP, single nucleotide polymorphism.

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Repositories: sequencing reads and annotated assemblies for this study are deposited in the European Nucleotide Archive at EMBL-EBI under accession number PRJEB36950 (<https://www.ebi.ac.uk/ena/data/view/PRJEB36950>).

One supplementary table and two supplementary figures are available with the online version of this article.

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antimicrobial for the treatment of multi-drug-resistant Gram-positive infections, including vancomycin-resistant enterococci [4, 5]. The G2576T mutation in the chromosomal 23S rRNA genes can arise *de novo* during extended linezolid therapy [6], although antimicrobial stewardship and infection prevention and control measures appear to be successful in limiting the generation and spread of mutational linezolid resistance in clinical practice [7]. The methyltransferases Cfr, Cfr(B) and Cfr(D), and the ABC-F ribosomal protection proteins OptrA and PoxA also confer resistance to linezolid in enterococci but are carried on mobile genetic elements, which can spread across genetically distinct lineages in the absence of antimicrobial selection [8–14]. Recent international surveillance confirmed that linezolid resistance remains rare, but *optrA* has recently spread to every continent and is the dominant mechanism of linezolid resistance in *E. faecalis* [15]. Surveillance has also detected *optrA* in the UK [16]. Studies into the genetic context of *optrA* have identified the gene on both the chromosome and plasmids, often associated with insertion sequences such as IS1216, a possible vehicle for the rapid spread of *optrA* [17, 18].

We used whole genome sequencing to determine whether Scottish *optrA*-positive *E. faecalis* isolates represent transmission of a single clonal lineage. We hypothesized that spread of *optrA* is driven by a single mobile genetic element, and to investigate this we made hybrid assemblies of short and long read sequencing data to generate complete genomes and to reconstruct the genetic environment of *optrA*.

METHODS

Bacterial strains

Study isolates were a convenience sample from three regional hospital laboratories during 2014–17; as such they may not reflect the entire Scottish population of *optrA*-positive *E. faecalis*. *E. faecalis* were identified from clinical samples using MALDI-TOF MS or the Vitek-2 GP-ID card (bioMérieux). Initial antimicrobial susceptibility testing was performed with the Vitek-2 AST-607 card; where linezolid resistance was detected the full MIC was determined by agar dilution methodology at the AMRHAI reference laboratory, and susceptibility testing was interpreted with EUCAST breakpoints [19]. Linezolid-resistant isolates were then screened for the genetic determinant of resistance at AMRHAI. Detection of the G2576T mutation (*Escherichia coli* numbering) in the 23S rRNA genes was investigated by PCR-RFLP or by a real-time PCR-based allelic discrimination assay [20, 21]. The *cfr* and *optrA* genes were sought by a multiplex PCR using primers for the detection of *cfr* (*cfr-fw*: 5'-TGAAGTATAAAGCAGGTTGG GAGTCA-3' and *cfr-rev*: 5'-ACCATATAATTGACCACAAGCAGC-3') [22] and *optrA* (*optrA-F*: 5'-GACCGGTGTCCTCTTT-GTCA-3' and *optrA-R*: 5'-TCAATGGAGTTACGATCGCCT-3') (AMRHAI, unpublished).

Access to isolates and clinical data was approved by the NHS Scotland Biorepository Network (Ref. TR000126).

Whole genome sequencing and genomic analysis

Genomic DNA was extracted from pelleted overnight broth cultures using the MasterPure Gram Positive DNA Purification Kit (Cambio), or QiaSymphony DSP DNA Mini Kit (Qiagen). Short read barcoded libraries were prepared using the Nextera XT kit (Illumina) and sequenced with a MiSeq instrument (Illumina) using 250 bp paired-end reads on a 500-cycle v2 kit. Short reads were quality trimmed with Trimmomatic v0.36 and the settings [LEADING:5 TRAILING:5 SLIDINGWINDOW:4:15 MINLEN:100] [23]. Barcoded long read libraries were generated with the 1D Ligation Sequencing Kit (Oxford Nanopore Technologies) and sequenced with an R9.4 flow cell on a MinION sequencer (Oxford Nanopore Technologies). Base-calling and barcode de-multiplexing was performed with Albacore v2.1.3 (Oxford Nanopore Technologies) and the resulting fast5 files were converted to fastq with Poretools v0.6.0 [24], or basecalled and de-multiplexed with Albacore v2.3.3 with direct fastq output. Porechop v0.2.3 (<https://github.com/rrwick/Porechop>) was used to remove chimeric reads and trim adapter sequences. Sequencing reads and annotated assemblies for this study have been deposited in the European Nucleotide Archive at EMBL-EBI under accession number PRJEB36950 (<https://www.ebi.ac.uk/ena/data/view/PRJEB36950>).

Short reads were mapped to the *E. faecalis* reference genome V583 (accession number AE016830) using SMALT v0.7.4 [25]. Mapped assemblies were aligned, and regions annotated as mobile genetic elements in the V583 genome (transposons, integrases, plasmids, phages, insertion sequences, resolvases and recombinases) were removed from the assembly (https://github.com/sanger-pathogens/remove_blocks_from_aln). All sites in the alignment with SNPs were extracted using SNP-sites v2.4.0 [26] and pairwise SNP counts were calculated (https://github.com/simonrharris/pairwise_difference_count).

MLST profiling was performed using SRST2 v0.2.0 [27] and the *E. faecalis* MLST database (<https://pubmlst.org/efaecalis/>) sited at the University of Oxford [28, 29]. Antimicrobial resistance mechanisms were detected using ARIBA v2.12.1 [30] and the ResFinder database v3.0 [31] with the addition of linezolid resistance mutations in the 23S rRNA (G2505A and G2576T based on *E. coli* numbering) and *rplC*, *rplD*, and *rplV* ribosomal protein genes.

Hybrid assembly was performed with Illumina short reads and Nanopore long reads using Unicycler v0.4.7 [32] in standard mode. The resulting assemblies were annotated with Prokka v1.5.1 using a genus-specific RefSeq database [33]. Hybrid assemblies were checked for indel errors using Ideel (<https://github.com/mw55309/ideel>) and UniProtKB TrEMBL database v2019_1. Plasmid comparisons were generated and visualized with EasyFig v2.2.2 [34].

RESULTS

Detection of *optrA* in distinct *E. faecalis* strains

There were 14133 isolates of *E. faecalis* during the study period from all sample types: 14 (0.1%) were identified as linezolid-resistant, and eight (57.1%) were confirmed as *optrA*-positive at the AMRHAI reference laboratory. Six *optrA*-positive *E. faecalis* were available for further characterization (Table 1). The earliest isolates in this collection were from the Grampian region in the northeast of Scotland in 2014, 2015 and 2016. Three more isolates were identified in 2017 from the Lothian and Forth Valley regions in east and central Scotland (Table 1), with no clear epidemiological links between the patients. Only one patient had known exposure to linezolid prior to the isolation of an *optrA*-positive *E. faecalis*, two patients were hospitalized at the time of sample collection while the remaining four were from general practice. Samples were collected for symptomatic urinary tract infection or orchitis.

Whole genome sequencing was performed to investigate the genetic relationship between the isolates. *In silico* MLST showed the six isolates belonged to different STs, suggesting they were genetically distinct (Table 1). To further confirm this, we analysed SNPs in the core genomes of the *optrA*-positive isolates and found the isolates differed by a median 18806 SNPs (range 13909–22272). Previous estimates suggest a genetic diversification rate of 2.5–3.4 SNPs/year for *E. faecalis*, highlighting the *optrA*-positive isolates share a very distant common ancestor [35].

optrA is carried on diverse genetic platforms

Hybrid assembly produced complete or near-complete genomes with <3% putative coding sequences shorter than the closest reference match. This indicated the hybrid assembly process removed most indel errors, with 1–5% of coding sequences expected to represent true truncated pseudogenes [36]. The hybrid assemblies contained between one and three plasmids ranging in size from 11 to 80 kb, with *optrA* present on a single complete plasmid in each isolate (pBX5936-1, pBX8117-2, pTM6294-2, pWE0254-1, pWE0438, pWE0851-1; Table S1, available in the online version of this article).

The *optrA*-positive plasmids shared limited sequence similarity to the first described *optrA* plasmid (pE394, accession KP399637), with only the 5–10 kb region surrounding *optrA* and *fexA* (a chloramphenicol/florfenicol exporter) showing >70% nucleotide identity. In all six Scottish *optrA*-positive plasmids, *optrA* and *fexA* were located within 550–750 nt of each other intervened by a single coding sequence (hypothetical function in all but pBX8117-2 which was annotated as a putative NADH reductase). Within the Scottish *optrA*-positive plasmids, pBX5936-1 (69 kb) and pTM6294-2 (53 kb) were most similar, sharing 97% average nucleotide identity over 40 kb of aligned sequence (Fig. 1). pTM6294-2 shared 99.8% identity with a 53 kb *optrA*-positive pheromone responsive plasmid detected in *E. faecalis* from a clinical sample in China (pEF10748), clinical samples in Spain (IsoBar1, IsoBar2 and IsoBar3) and raw dog food in Portugal (pAPT110) [37, 38]. pWE0438 shared 92.3% nucleotide identity over 52 kb with pS7316 from an *E. faecalis* isolated from a hospitalized patient in Japan [39]. In pWE0438, the *optrA* and *fexA* genes were ~3.8 kb upstream of Tn917 carrying *ermB*, and ~1.8 kb downstream of another Tn3-family transposase (Fig. 1). pBX8117-2 carried *optrA* and the novel *cfr(D)* gene (encoding a 23S rRNA methylase that confers phenicol, oxazolidinone, pleuromutilin and streptogramin A resistance) but apart from these genes showed no similarity to another *E. faecium optrA/cfr(D)*-positive plasmid identified in a clinical sample in Ireland (M17-0314) [40]. The other Scottish *optrA*-positive plasmids showed limited similarity to other published examples outside of the *optrA/fexA* region.

IS1216 is often associated with *optrA* and other antimicrobial resistance genes in enterococci. pBX5936-1 and pBX8117-2 had IS1216 flanking the *optrA* and *fexA* region as a putative transposable cassette (Figs 1 and S1). However, IS1216 can mobilize from a single insertion sequence copy [41] and single copies were found close to *optrA* in pTM6294-2 and pWE0851-1 (Figs 1 and S1). BLASTN comparison of pWE0254-1 with the other *optrA*-positive plasmids highlighted a partial IS1216 transposase that was not identified by automated annotation. Immediately upstream of the partial IS1216 was an IS3-family transposase, the insertion of which probably disrupted IS1216. In pWE0254-1 *optrA* and *fexA* were found on a Tn6674-like element carrying macrolide (*ermA*) and spectinomycin (APH(9)-Ia) resistance genes. The element was 98.9% identical to Tn6674 but had a 1.2 kb insertion containing IS3-family transposases (Fig. S1), and was classified as Tn6993 by the Transposon Registry (accession GCA_906464915) [42]. Tn6993 was not inserted into the chromosomal *radC* gene as described for most Tn6674-like elements [43, 44]. A similar element was present in a plasmid from *E. faecalis* in Chinese swine (TBCP-4814-p1, accession MH830363) but this element lacked the *tnpA* gene and the 1.2 kb insertion of Tn6993 (Fig. S1) [45]. pWE0438 had a single copy of IS1216 located ~35 kb from *optrA*, although Tn917 and Tn3-like transposases were detected closer to *optrA* as described above.

optrA sequences vary between isolates

Comparison of the *optrA* sequence from each isolate to the first identified *optrA* from pE394 revealed different variants at the nucleotide and amino acid levels: WE0254 and TM6294 had one synonymous nucleotide substitution, BX5936 had a single non-synonymous nucleotide substitution, WE0851 had two non-synonymous nucleotide substitutions, WE0348 had three non-synonymous and one synonymous substitution, and BX8117 had 20 non-synonymous and a further 17 synonymous substitutions (Table S1, Fig. S2). The degree of sequence variation between the six FexA proteins was less than that seen in Optra. Comparison

Table 1. Details of the *optrA*-positive *E. faecalis* characterized in this study

Isolate	Year	Region	Clinical sample	Patient source	MLST	Acquired linezolid resistance genes			Mutations in 23S rRNA		Mutations in ribosomal proteins*			MIC (mg l ⁻¹)			
						<i>cftr</i>	<i>cftr</i> (B)	<i>cftr</i> (D)	<i>optrA</i>	<i>poxtA</i>	G2505A	G2576T	L3		L4	L22	CHL
WE0851	2014	Grampian	Urine	GP	480	-	-	-	-	-	-	-	T150A	F101L	-	≥64	8
WE0254	2015	Grampian	Urine	GP	19	-	-	-	-	-	-	-	T150A	F101L	-	≥64	8
WE0438	2016	Grampian	Urine	Hospital	330	-	-	-	-	-	-	-	T150A	F101L	-	≥64	8
TM6294	2017	Forth Valley	Urine	Hospital	585	-	-	-	-	-	-	-	T150A	F101L	-	≥64	8
BX5936	2017	Lothian	Semen	GP	894	-	-	-	-	-	-	-	T150A	F101L	-	≥64	8
BX8117	2017	Lothian	Urine	GP	16	-	-	+	-	-	-	-	T150A	F101L	-	≥64	8

GP, general practice.

*The mutations identified here have never been detected in the absence of other resistance mechanisms in linezolid-resistant isolates, and have been detected in linezolid-susceptible isolates. Their role in linezolid resistance is unclear [54].

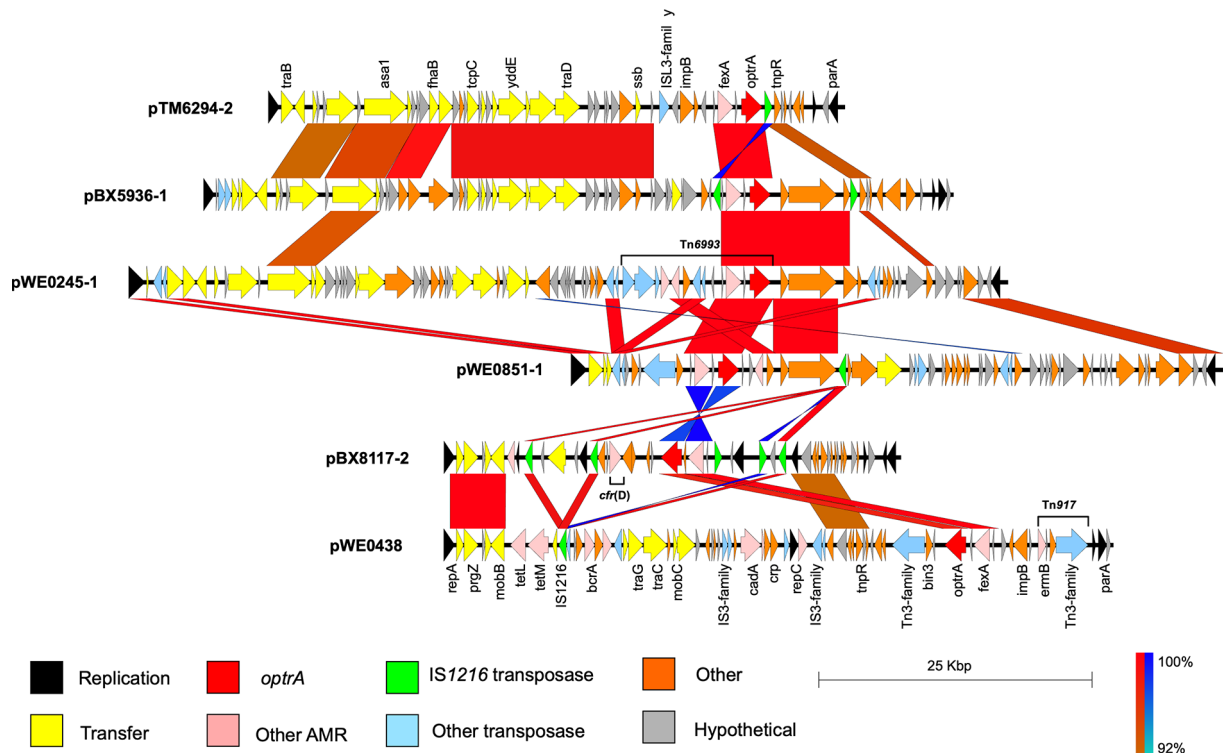


Fig. 1. Alignment of full *oprA*-positive plasmid sequences. While some sequence similarity is seen between pTM6294-2 and pBX5936-1, in general identity is low between the *oprA*-positive plasmids, indicating *oprA* has mobilized to multiple plasmid backbones. Arrows indicate coding sequences, coloured blocks between each sequence indicate regions with BLASTN sequence identity $\geq 90\%$ and length >680 bp. Blue identity blocks indicate inverted sequence.

with the first reported FexA sequence (AJ549214) showed four common non-synonymous variants in all strains (amino acid changes A34S, L39S, I131V and V305I), with all but BX8117 having an additional D50A variant.

DISCUSSION

This study found *oprA* present in diverse genetic lineages of *E. faecalis* and carried on largely unrelated plasmids in six isolates from Scotland. pTM6294-2, pBX5936-1 and pWE0438 shared homology with plasmids identified in China or Japan, highlighting the wide dispersal of *oprA*. However, the other Scottish plasmids had limited similarity to other published examples, suggesting a diverse reservoir of *oprA*-carrying genetic elements. We identified *oprA* often carried with a number of other resistance genes, including in a novel multiresistance transposon Tn6993 in pWE0254-1, and the recently described *cfr(D)* in pBX8117-2. Despite differences in *oprA* sequences and carriage of other linezolid determinants such as *cfr(D)*, all isolates showed low-level linezolid resistance of 8 mg l^{-1} (Table 1).

Freitas *et al.* [44] recently analysed all publicly available *oprA*-positive genome sequences and categorized the genetic environment of *oprA*. Group 1 includes Tn6674-like platforms, of which WE0254 is a representative (Fig. S1). However, in the original scheme all Group 1 elements were integrated into the chromosome, while in WE0254 the *oprA* element Tn6993 is inserted into a plasmid. Group 2 includes *oprA-fexA-impB* platforms, represented in the Scottish isolates by TM6294 and WE0438 (Fig. S1). Group 3 includes platforms containing the *araC* regulatory element and is not represented in the Scottish *oprA*-positive isolates characterized here. The three remaining Scottish isolates could not be grouped based on the Freitas scheme, highlighting the need for further studies and public access to complete genome sequences to determine the true diversity of *oprA*-positive platforms.

Many studies of *oprA* to date are from China and tend to show a higher prevalence of *oprA* in isolates from animals rather than humans [11, 46, 47]. Additionally, florfenicol use in agriculture is linked to *oprA* detection in farm animals [48, 49]. However, increasing reports describe rapid increases in *oprA* detection from human samples in many countries [15, 50, 51]. *oprA*-positive isolates are often resistant to multiple antibiotic classes used in animal and human health, allowing significant opportunity for co-selection of *oprA*-positive strains both in animal and in human settings. More recently, *oprA* has been identified in clinical vancomycin-resistant *E. faecium* isolates, with very limited treatment options [50, 52, 53].

Our study is limited in scale as we only include isolates from three regional clinical laboratories, and therefore larger studies are required to infer national patterns. However, our finding that *optrA* is present as different gene variants, carried on different mobile genetic elements, in unrelated strains of *E. faecalis* suggest a diverse *optrA* reservoir that is only partly investigated in this study.

As well as *optrA*, the *cfr* and *poxtA* genes are emerging transferable linezolid resistance mechanisms. Further studies from a One Health perspective are warranted to understand the selection pressures driving transferable linezolid resistance, and the transmission dynamics of these strains to avoid further spread of oxazolidinone resistance within *E. faecalis* and other Gram-positive bacteria.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

Ethical statement

Access to isolates and clinical data was approved by the NHS Scotland Biorepository Network (Ref TR000126).

References

- García-Solache M, Rice LB. The Enterococcus: a model of adaptability to its environment. *Clin Microbiol Rev* 2019;32:e00058-18.
- ECDC. Surveillance of antimicrobial resistance in Europe – Annual report of the European Antimicrobial Resistance Surveillance Network (EARS-Net) 2017; 2019. <http://www.ecdc.europa.eu/sites/portal/files/documents/EARS-Net-report-2017-update-jan-2019.pdf>
- Zahedi Bialvaei A, Rahbar M, Yousefi M, Asgharzadeh M, Samadi Kafil H. Linezolid: a promising option in the treatment of Gram-positives. *J Antimicrob Chemother* 2017;72:354–364.
- Public Health England. English surveillance programme for antimicrobial utilisation and resistance (ESPAUR) Report 2018-2019. London, UK: PHE; 2019. <https://www.gov.uk/government/publications/english-surveillance-programme-antimicrobial-utilisation-and-resistance-espaur-report>
- Health Protection Scotland. Scottish One Health Antimicrobial Use and Resistance in 2018 Annual Report. Glasgow, UK: HPS; 2019. <https://www.hps.scot.nhs.uk/web-resources-container/scottish-one-health-antimicrobial-use-and-antimicrobial-resistance-in-2018>
- Mendes RE, Deshpande LM, Jones RN. Linezolid update: stable in vitro activity following more than a decade of clinical use and summary of associated resistance mechanisms. *Drug Resist Updat* 2014;17:1–12.
- Abbo L, Shukla BS, Giles A, Aragon L, Jimenez A, et al. Linezolid- and Vancomycin-resistant *Enterococcus faecium* in solid organ transplant recipients: infection control and antimicrobial stewardship using whole genome sequencing. *Clin Infect Dis* 2019;69:259–265.
- Antonelli A, D'Andrea MM, Brenciani A, Galeotti CL, Morroni G, et al. Characterization of *poxtA*, a novel phenicol-oxazolidinone-tetracycline resistance gene from an MRSA of clinical origin. *J Antimicrob Chemother* 2018;73:1763–1769.
- Deshpande LM, Ashcraft DS, Kahn HP, Pankey G, Jones RN, et al. Detection of a New *cfr*-like gene, *cfr(B)*, in *Enterococcus faecium* isolates recovered from human specimens in the United States as Part of the SENTRY Antimicrobial Surveillance Program. *Antimicrob Agents Chemother* 2015;59:6256–6261.
- Diaz L, Kiratisin P, Mendes RE, Panesso D, Singh KV, et al. Transferable plasmid-mediated resistance to linezolid due to *cfr* in a human clinical isolate of *Enterococcus faecalis*. *Antimicrob Agents Chemother* 2012;56:3917–3922.
- Wang Y, Lv Y, Cai J, Schwarz S, Cui L, 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;70:2182–2190.
- Pang S, Boan P, Lee T, Gangatharan S, Tan SJ, et al. Linezolid-resistant ST872 *Enterococcus faecium* harbouring *optrA* and *cfr* (D) oxazolidinone resistance genes. *Int J Antimicrob Agents* 2020;55:105831.
- Long KS, Poehlsgaard J, Kehrenberg C, Schwarz S, Vester B. The Cfr rRNA methyltransferase confers resistance to Phenicol, Lincosamides, Oxazolidinones, Pleuromutilins, and Streptogramin A antibiotics. *Antimicrob Agents Chemother* 2006;50:2500–2505.
- Guerin F, Sassi M, Dejoies L, Zouari A, Schutz S, et al. Molecular and functional analysis of the novel *cfr*(D) linezolid resistance gene identified in *Enterococcus faecium*. *J Antimicrob Chemother* 2020;75:1699–1703.
- Deshpande LM, Castanheira M, Flamm RK, Mendes RE. Evolving oxazolidinone resistance mechanisms in a worldwide collection of enterococcal clinical isolates: results from the SENTRY Antimicrobial Surveillance Program. *J Antimicrob Chemother* 2018;73:2314–2322.
- Health Protection Scotland. Oxazolidinone-resistance due to *optrA* in *Enterococcus faecalis*. *HPS Wkly Rep* 2016;50:230–231.
- Cai J, Wang Y, Schwarz S, Zhang G, Chen S, et al. High detection rate of the oxazolidinone resistance gene *optrA* in *Enterococcus faecalis* isolated from a Chinese anorectal surgery ward. *Int J Antimicrob Agents* 2016;48:757–759.
- He T, Shen Y, Schwarz S, Cai J, Lv Y, et al. Genetic environment of the transferable oxazolidinone/phenicol resistance gene *optrA* in *Enterococcus faecalis* isolates of human and animal origin. *J Antimicrob Chemother* 2016;71:1466–1473.
- EUCAST. Breakpoint tables for interpretation of MICs and zone diameters. Version 8.0. Version 2018.
- Woodford N, Tysall L, Auckland C, Stockdale MW, Lawson AJ, et al. Detection of oxazolidinone-resistant *Enterococcus faecalis* and *Enterococcus faecium* strains by real-time PCR and PCR-restriction fragment length polymorphism analysis. *J Clin Microbiol* 2002;40:4298–4300.
- Werner G, Strommenger B, Klare I, Witte W. Molecular detection of linezolid resistance in *Enterococcus faecium* and *Enterococcus faecalis* by use of 5' nuclease real-time PCR compared to a modified classical approach. *J Clin Microbiol* 2004;42:5327–5331.

22. Kehrenberg C, Schwarz S. Distribution of florfenicol resistance genes *fxaA* and *cfr* among chloramphenicol-resistant *Staphylococcus* isolates. *Antimicrob Agents Chemother* 2006;50:1156–1163.
23. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 2014;30:2114–2120.
24. Loman NJ, Quinlan AR. Poretools: a toolkit for analyzing nanopore sequence data. *Bioinformatics* 2014;30:3399–3401.
25. Ponstingl H, Ning Z. SMALT. Wellcome Trust Sanger Institute; 2014. <http://www.sanger.ac.uk/science/tools/smalt-0> [accessed 21 June 2017].
26. Page AJ, Taylor B, Delaney AJ, Soares J, Seemann T, et al. *SNP-sites*: rapid efficient extraction of SNPs from multi-FASTA alignments. *Microb Genom* 2016;2:e000056.
27. Inouye M, Dashnow H, Raven L-A, Schultz MB, Pope BJ, et al. SRST2: rapid genomic surveillance for public health and hospital microbiology labs. *Genome Med* 2014;6:1–16.
28. Jolley KA, Maiden MCJ. BIGSdb: scalable analysis of bacterial genome variation at the population level. *BMC Bioinformatics* 2010;11:595.
29. Ruiz-Garbajosa P, Bonten MJM, Robinson DA, Top J, Nallapareddy SR, et al. Multilocus sequence typing scheme for *Enterococcus faecalis* reveals hospital-adapted genetic complexes in a background of high rates of recombination. *J Clin Microbiol* 2006;44:2220–2228.
30. Hunt M, Mather AE, Sánchez-Busó L, Page AJ, Parkhill J, et al. ARIBA: rapid antimicrobial resistance genotyping directly from sequencing reads. *Microb Genom* 2017;3:e000131.
31. Zankari E, Hasman H, Kaas RS, Seyfarth AM, Agersø Y, et al. Genotyping using whole-genome sequencing is a realistic alternative to surveillance based on phenotypic antimicrobial susceptibility testing. *J Antimicrob Chemother* 2013;68:771–777.
32. Wick RR, Judd LM, Gorrie CL, Holt KE. Unicycler: Resolving bacterial genome assemblies from short and long sequencing reads. *PLoS Comput Biol* 2017;13:e1005595.
33. Pruitt KD, Tatusova T, Brown GR, Maglott DR. NCBI reference sequences (RefSeq): current status, new features and genome annotation policy. *Nucleic Acids Res* 2012;40:D130–5.
34. Sullivan MJ, Petty NK, Beatson SA. Easyfig: a genome comparison visualizer. *Bioinformatics* 2011;27:1009–1010.
35. Raven KE, Reuter S, Gouliouris T, Reynolds R, Russell JE, et al. Genome-based characterization of hospital-adapted *Enterococcus faecalis* lineages. *Nat Microbiol* 2016;1:15033.
36. Goodhead I, Darby AC. Taking the pseudo out of pseudogenes. *Curr Opin Microbiol* 2015;23:102–109.
37. Zou J, Tang Z, Yan J, Liu H, Chen Y, et al. (n.d.) Dissemination of linezolid resistance through sex pheromone plasmid transfer in *Enterococcus faecalis*. *Front Microbiol*;11.
38. Freitas AR, Finisterra L, Tedim AP, Duarte B, Novais C, et al. Linezolid- and multidrug-resistant enterococci in raw commercial dog food. Multidrug-Resistant Enterococci in Raw Commercial Dog Food, Europe, 2019–2020. *Emerg Infect Dis* 2021;27:2221–2224.
39. Iimura M, Hayashi W, Arai E, Natori T, Horiuchi K, et al. Identification of a multiresistant mosaic plasmid carrying a new segment of IS1216E-flanked *optrA* with integrated Tn551-ermB element in linezolid-resistant *Enterococcus faecalis* human isolate. *J Glob Antimicrob Resist* 2020;22:697–699.
40. Egan SA, Shore AC, O'Connell B, Brennan GI, Coleman DC. 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. *J Antimicrob Chemother* 2020;75:1704–1711.
41. Partridge SR, Kwong SM, Firth N, Jensen SO. Mobile genetic elements associated with antimicrobial resistance. *Clin Microbiol Rev* 2018;31:e00088–17.
42. Tansirichaiya S, Rahman MA, Roberts AP. The transposon registry. *Mob DNA* 2019;10:40.
43. Li D, Li X-Y, Schwarz S, Yang M, Zhang S-M, et al. Tn 6674, a novel enterococcal *optrA*-carrying multiresistance transposon of the TN 554 family. *Antimicrob Agents Chemother* 2019;AAC.00809-19, aac;AAC.00809-19v1.
44. Freitas AR, Tedim AP, Novais C, Lanza VF, Peixe L. Comparative genomics of global *optrA*-carrying *Enterococcus faecalis* uncovers a common chromosomal hotspot for *optrA* acquisition within a diversity of core and accessory genomes. *Microb Genom* 2020;6.
45. Chen L, Han D, Tang Z, Hao J, Xiong W, et al. Co-existence of the oxazolidinone resistance genes *cfr* and *optrA* on two transferable multi-resistance plasmids in one *Enterococcus faecalis* isolate from swine. *Int J Antimicrob Agents* 2020;56:105993.
46. Shang Y, Li D, Hao W, Schwarz S, Shan X, et al. A prophage and two ICESa2603-family integrative and conjugative elements (ICEs) carrying *optrA* in *Streptococcus suis*. *J Antimicrob Chemother* 2019;74:2876–2879.
47. Hao W, Shan X, Li D, Schwarz S, Zhang S-M, et al. Analysis of a *poxtA*- and *optrA*-co-carrying conjugative multiresistance plasmid from *Enterococcus faecalis*. *J Antimicrob Chemother* 2019;74:1771–1775.
48. Munk P, Knudsen BE, Lukjancenko O, Duarte ASR, et al. Abundance and diversity of the faecal resistome in slaughter pigs and broilers in nine European countries. *Nat Microbiol* 2018;3:898–908.
49. Zhao Q, Wang Y, Wang S, Wang Z, Du X, et al. Prevalence and abundance of florfenicol and linezolid resistance genes in soils adjacent to swine feedlots. *Sci Rep* 2016;6:1–7.
50. Sassi M, Guérin F, Zouari A, Beyrouthy R, Auzou M, et al. Emergence of *optrA*-mediated linezolid resistance in enterococci from France, 2006–16. *J Antimicrob Chemother* 2019;74:1469–1472.
51. Bender JK, Fleige C, Lange D, Klare I, Werner G. Rapid emergence of highly variable and transferable oxazolidinone and phenicol resistance gene *optrA* in German *Enterococcus* spp. clinical isolates. *Int J Antimicrob Agents* 2018;52:819–827.
52. Egan SA, Corcoran S, McDermott H, Fitzpatrick M, Hoyne A, et al. Hospital outbreak of linezolid-resistant and vancomycin-resistant ST80 *Enterococcus faecium* harbouring an *optrA*-encoding conjugative plasmid investigated by whole-genome sequencing. *J Hosp Infect* 2020;105:726–735.
53. Lazaris A, Coleman DC, Kearns AM, Pichon B, Kinnevey PM, 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;72:3252–3257.
54. Cui L, Wang Y, Lv Y, Wang S, Song Y, et al. Nationwide surveillance of novel oxazolidinone resistance gene *optrA* in *Enterococcus* isolates in China from 2004 to 2014. *Antimicrob Agents Chemother* 2016;60:7490–7493.