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CORRESPONDENCE

Genomic characterisation of linezolid-resistant *Enterococcus faecalis* from Western Australia 2016–2021

To the Editor,

Linezolid (LNZ) is an oxazolidinone antibiotic and a drug of last resort used to treat infections caused by Gram-positive pathogens that are resistant to multiple classes of antibiotics. In Australia, LNZ was introduced in 2002 and is currently reserved to treat suspected or proven infections caused by methicillin-resistant staphylococci or vancomycin-resistant enterococci. Despite restricted use, LNZ resistance has emerged worldwide, threatening the effective treatment of multidrug-resistant bacterial infections.[1] In Australia, LNZ-resistant enterococci (LRE) are monitored and reported by each state and territory to the National Alert System for Critical Antimicrobial Resistances (CARAlert), as part of the AURA (Antimicrobial Use and Resistance in Australia) surveillance system.

LNZ interferes with bacterial protein synthesis and prevents the formation of the translation initiation complex by binding to the 23S rRNA on the 50S ribosomal subunit. In enterococci, resistance to LNZ is often caused by point mutations in the bacterial 23S rRNA gene; the most common mutation is G2576T. *Enterococcus faecalis* has four copies of the 23S rRNA gene and there is a direct correlation between the number of gene copies carrying the G2576T mutation and the phenotypic level of LNZ resistance.[2] Furthermore, transferable genes encoding LNZ resistance have been identified in enterococci and include *cfr*, *cfr(B)*, *optrA*, and *poxtA*.[3]

Although an increasing number of LNZ-resistant *E. faecalis* (LREfs) has been reported worldwide, little is known about the genetic diversity and resistance mechanism of LREfs in Australia. In this study, we used whole genome sequencing to characterise and determine the mechanism of LNZ resistance in 25 clinical isolates of LREfs isolated in Western Australia (WA) from 2016 to 2021.

Isolates were identified as *E. faecalis* using matrix-assisted laser desorption ionisation (MALDI Biotyper; Bruker Daltonics, Germany). Antimicrobial susceptibility testing was initially performed on the VITEK 2 (bioMérieux, France) automated system using the AST-P612 susceptibility panel. Resistance to LNZ was confirmed by the Etest method (bioMérieux) as per the manufacturer's instructions and results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) breakpoints [susceptible (S) ≤2 mg/L; intermediate (I) 4 mg/L; resistant (R) ≥8 mg/L]. Whole genome sequencing was performed on a NextSeq 500 platform (Illumina, USA) using 150 bp paired-end chemistry. LNZ resistance determinants were identified using the LRE-Finder 1.0 tool[3] which accurately detects the most common mutations associated with LNZ resistance in enterococci. LRE-Finder aligns raw sequence reads (fastq) with k-mer alignment and can detect the number of 23S rRNA gene copies harbouring the relevant mutations. Genomes were aligned and a phylogenetic tree was constructed using the neighbour-joining algorithm. Genetic environments were annotated and visualised using the Geneious Prime software (Biomatters, New Zealand).

Of the 25 isolates, 72% (*n*=18) had a LNZ MIC of 8 mg/L and the remainder had a MIC of 4 mg/L (*n*=3), 16 mg/L (*n*=3) and 64 mg/L (*n*=1). Although 10 unique STs were identified, 48% (*n*=12) of isolates belonged to ST16 (Fig. 1). Most ST16 isolates had a LNZ MIC of 8 mg/L and were phylogenetically related (Fig. 1). Except for LREfs-WA-08, all isolates possessed four wild-type copies of the 23S rRNA gene. LREfs-WA-08, which had the highest LNZ MIC (64 mg/L), harboured the G2576T mutation in three copies of the 23S rRNA gene. Resistance to LNZ in the remaining isolates was due to the presence of the *optrA* gene. The *cfr*, *cfr(B)* and *poxtA* genes were not identified.

Four different *optrA* allele sequences (1968 bp in length) that differed by a maximum of 6 nucleotides were identified: *optrA*-1 (Accession No. KP399637), *optrA*-5 (Accession No. KT862783), *optrA*-6 (Accession No. KT862784), and *optrA*-7 (Accession No. KT862775). The peptide sequences encoded by *optrA*-1 and *optrA*-5 are identical and differ by two amino acid residues from the peptide sequences encoded by *optrA*-6 and *optrA*-7. The *optrA* gene was chromosomally encoded in 22 isolates and located on a plasmid in the remaining two *optrA*-positive isolates. The chromosome-encoded *optrA* was located on a 12.9 kb transposon, recently characterised as Tn*6674*,[4] which integrated into the *radC* gene (Fig. 2A). In addition to the *tnpA*, *tnpB*, and *tnpC* transposase genes, the *optrA*-carrying transposon also carried the *ant(9)-Ia* (also called *spc*), *erm(A)* and *fexA* resistance genes which confer resistance to spectinomycin, erythromycin and chloramphenicol, respectively. The two isolates harbouring a plasmid-encoded *optrA*, LREfs-WA-01 and LREfs-WA-04, were ST16 and were the only isolates harbouring the *optrA*-7 variant. The genetic environment of the plasmid-encoded *optrA* was identical (100% nucleotide identity) to the genetic environment of *optrA* in p10-2-2 (Accession No. KT862775.1), a plasmid harboured by the ST59 LREfs strain 10-2-2 recovered from a pig in China.[5] The *fexA* and *erm(A)* genes were identified upstream and downstream of the plasmid-encoded *optrA*, respectively (Fig. 2B).

Although there have been reports of enterococcal isolates harbouring two copies of *optrA* (a chromosomal copy and a plasmid-encoded copy),[6] all *optrA*-positive isolates in our collection harboured only one copy of the gene. The *optrA* gene encodes an ATP-binding cassette (ABC)-F protein which binds to the large (50S) ribosomal subunit and protects the ribosome by interfering with the actions of ribosome-targeted antibiotics such as oxazolidinones and phenicols.[7] The *optrA* gene was first described in enterococci isolated in 2009 from humans and food-producing animals in China.[8] The gene was originally identified on a plasmid, pE349 (Accession No. KP399637), harboured by an ST116 LREfs of human origin. The pE349 plasmid contained the same *impB-fexA-optrA* fragment identified in our *optrA*-carrying plasmids, but it did not harbour *erm(A)*.[5] The *optrA* gene has further been detected in various countries in enterococci isolated from food animals, animal

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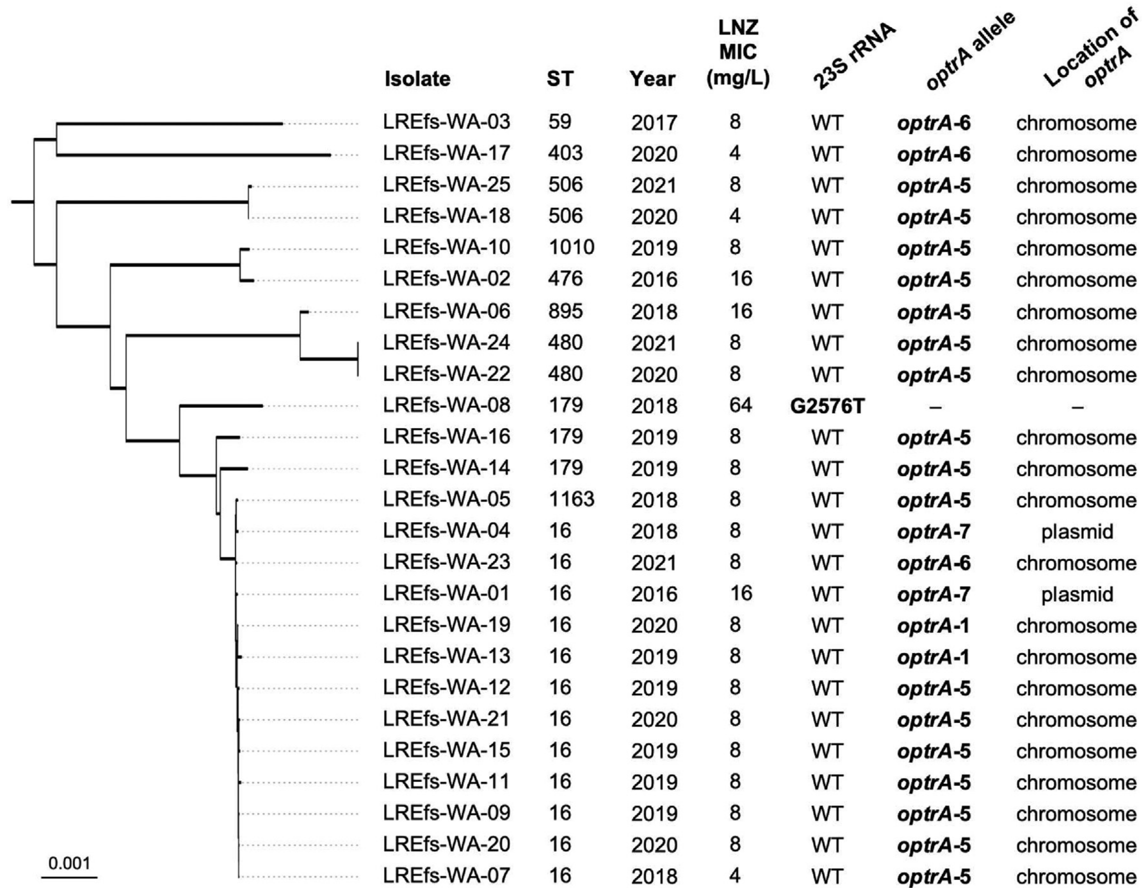

Fig. 1 Phylogeny and resistance determinants of linezolid-resistant *E. faecalis* isolated in Western Australia in 2016–2021. The tree was constructed using the neighbour-joining algorithm and 200 bootstrapping values. MIC (minimum inhibitory concentration) values were determined using the E-test. LNZ, linezolid; ST, sequence type; WT, wild type; Year, year of isolation. *optrA*-1 (Accession No. KP399637); *optrA*-5 (Accession No. KT862783); *optrA*-6 (Accession No. KT862784); *optrA*-7 (Accession No. KT862775).

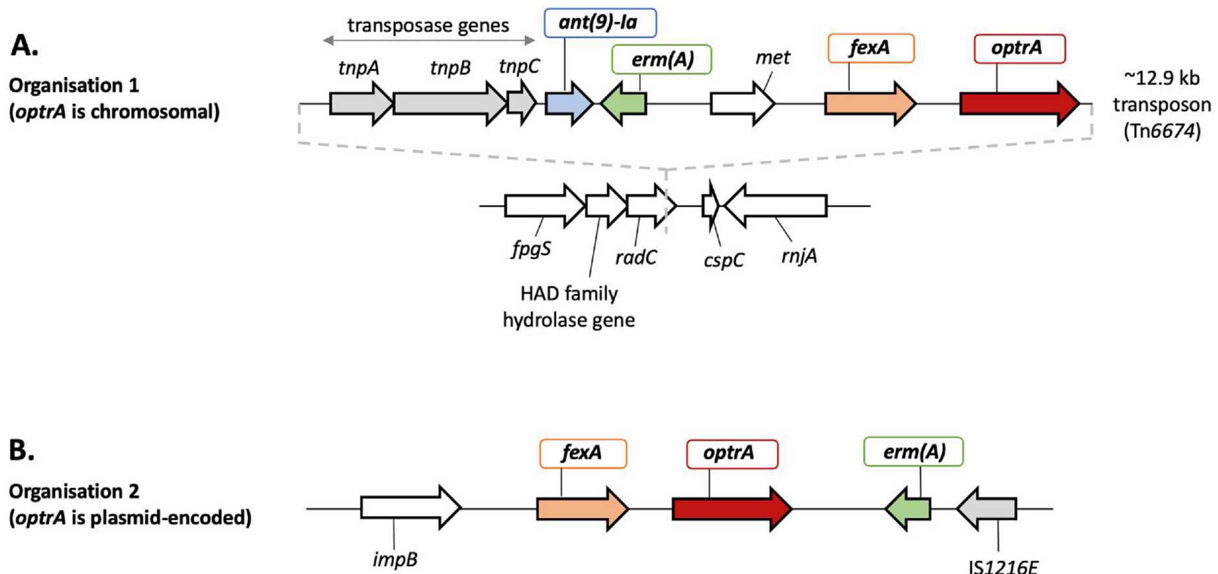

Fig. 2 Schematic representation of the genetic environment of *optrA* in *E. faecalis* investigated in this study. (A) The *optrA* gene is chromosomally encoded and located on a Tn*6674* transposon which also carries transposase genes (*tnpA*, *tnpB*, and *tnpC*), the spectinomycin resistance gene *ant(9)-Ia* (also called *spc*), the macrolide-lincosamide-streptogramin B resistance gene *erm(A)*, and the chloramphenicol-florfenicol resistance gene *fexA*. (B) The *optrA* gene is plasmid-encoded and is flanked by the *fexA* and *erm(A)*. *met* encodes an *S*-adenosylmethionine (SAM)-dependent methyltransferase; *folC* encodes a dihydrofolate synthase; *radC* encodes a DNA repair protein; *cspC* encodes a cold shock protein; *rnjA* encodes ribonuclease J; impB is predicted to encode a plasmid-associated type VI secretion protein.

carcasses, animal food products and wastewater, highlighting the importance of this resistance gene in a One Health context.[9–11]

In our collection, *optrA* was predominantly chromosomal and the gene was carried on the Tn*6674* transposon in conjunction with *fexA*, *erm(A)* and *ant(9)-Ia*. Tn6674 has previously been detected in circular intermediate forms,[4] suggesting chromosomally encoded *optrA* is transposable, which could explain the presence of Tn*6674* in the genetically diverse *E. faecalis* isolates in our study. Tn*6674* was always identified within the *radC* gene which is considered a hotspot for the chromosomal integration of *optrA*-carrying genetic elements. In addition to hospitalised patients, the Tn*6674* transposon has previously been identified in healthy humans and food-producing animals across different continents,[12] highlighting the importance of monitoring this mobile multi-resistance genetic element.

In conclusion, LNZ resistance in our collection was predominantly mediated by the presence of *optrA*. The *optrA* gene clusters harboured other resistance genes such as *fexA* and *erm(A)*, and in the case of chromosomally encoded *optrA*, also harboured *ant(9)-Ia* (or *spc*). However, the isolate with the highest LNZ MIC was *optrA*-negative and resistance was due to the G2576T mutation in three of its four 23S rRNA gene copies. Although *optrA* can be detected using *de novo* draft assemblies, mutations in 23S must be determined by analysing the raw sequence reads using a tool such as LRE-Finder because consensus sequences produced by assemblers ignore less abundant mutations. Based on our findings, similar genomic characterisation studies on LREfs in other parts of Australia should be conducted. Furthermore, as LNZ resistance has been associated with food-producing animals in other countries, a One Health surveillance approach is warranted.

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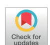
Macrolide resistant *Mycoplasma pneumoniae* in Auckland, New Zealand



To the Editor,

Mycoplasma pneumoniae is an important cause of community acquired pneumonia and other respiratory infections, particularly in school-aged children and young adults.[1] *Mycoplasma pneumoniae* respiratory disease typically has a mild presentation, although severe and fatal cases also occur.[1] It is a fastidious and slow growing organism, so the primary method for diagnosing acute infection is through nucleic acid amplification tests on respiratory specimens.[1] Macrolide antimicrobials are typically the treatment of choice, having the greatest *in vitro* potency and the ability to be safely used in young children.[1,2] However, treatment with macrolides has been complicated by the emergence of resistance, which occurs in association with mutations in the peptidyltransferase loop of Mycoplasma 23S rRNA, primarily at positions 2063 and 2064.[1,2] Worldwide, the prevalence of macrolide resistance displays significant geographic variation, with rates up to 90% in Asia, typically less than 10% in Europe, 7.5% in the United States (with substantial regional differences), while a study in South Australia detected no resistance.[1–4] Current data suggest macrolide resistance is associated with a prolonged duration of fever and hospitalisation in children, although an increase in severe complications or mortality has not been described.[1,5,6] In New Zealand, macrolides are frequently used in the empiric antimicrobial treatment of community acquired pneumonia and for the specific treatment of *M. pneumoniae*. However, there has been an absence of local data on macrolide susceptibility. To help guide therapy and provide a baseline resistance estimate, this study determined the prevalence of macrolide resistance associated mutations in *M. pneumoniae* in the Auckland region of New Zealand.