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P2331 Genomic investigation of linezolid-resistant *Enterococcus faecalis* carrying the *optrA* gene

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Background: Linezolid is reserved for treating severe, antibiotic-resistant, Gram-positive bacterial infections in humans. Linezolid resistance is caused by mutations in 23S rRNA or acquisition of *cfr, optrA,* or *poxtA* genes. Although enterococcal linezolid resistance remains rare (generally <1%), the plasmid-mediated *optrA* gene has been detected in many countries. We performed whole genome sequencing of linezolid resistant *Enterococcus faecalis* isolates to determine if the *optrA* gene was present in a single genetic clone and whether it was carried by transmissible genetic elements.

Materials/methods: Isolates were identified as linezolid- and chloramphenicol-resistant in routine diagnostic laboratories, and confirmed to carry the *optrA* gene by Public Health England AMRHAI Reference Unit. Illumina MiSeq short reads were mapped to a reference strain with SMALT and a core genome phylogeny made. Hybrid assemblies of short reads and Oxford Nanopore MinION long reads were generated with Unicycler and annotated with Prokka.

Results: The *optrA* gene was found in six linezolid-resistant *E. faecalis* isolated between 2014 and 2017. All isolates were from genitourinary samples and were susceptible to amoxicillin and vancomycin. No direct epidemiological links were identified between patients, only one had recent linezolid exposure. Core genome phylogeny confirmed the isolates were genetically unrelated, belonging to distinct MLST profiles. The *optrA* gene was found on a plasmid in each isolate, and these plasmids had limited sequence similarity. It was notable that the *optrA* gene was always co-located with the *fexA* phenicol resistance gene and flanked by two copies of IS*1216*E. Four *optrA* amino acid sequence variants were detected, differing from each other at 1-3 amino acids. One of these variants had not been described previously.

Conclusions: This is the first genomic investigation of *optrA*-mediated oxazolidinone resistance in *E. faecalis* using hybrid assembly of short and long sequencing reads. Our sequencing approach allowed the assembly of complete bacterial genomes and investigation of plasmids carrying *optrA*. We report multiple *optrA* variants in diverse *E. faecalis* strain and plasmid backgrounds, suggesting multiple introductions into the population and/or ongoing transfer of the gene. The reservoir and mechanism of *optrA* selection in humans is unclear and requires investigation to avoid widespread linezolid resistance.