A hospital outbreak of linezolid-resistant and vancomycin-resistant ST80 *Enterococcus faecium* harbouring an *optrA*-encoding conjugative plasmid investigated by whole-genome sequencing

S.A. EGAN[a], S. CORCORAN[b], H. McDERMOTT[b], M. FITZPATRICK[c], A. HOYNE[b], O. McCORMACK[b], A. CULLEN[d] G.I. BRENNAN[e], B. O'CONNELL[e,f], D.C. COLEMAN[a*]

[a] *Microbiology Research Unit, Division of Oral Biosciences, Dublin Dental University Hospital, University of Dublin, Trinity College, Lincoln Place, Dublin 2, Ireland*

[b]*Department of Clinical Microbiology, Bon Secours Hospital Dublin, Glasnevin, Dublin 9, Ireland*

[c] *Infection Prevention and Control Department, Bon Secours Hospital Dublin, Glasnevin, Dublin 9, Ireland*

[d]*Pharmacy Department, Bon Secours Hospital Dublin, Glasnevin, Dublin 9, Ireland*

[e] *National MRSA Reference Laboratory, St. James's Hospital, James's Street, Dublin 8, Ireland*

[f] *Department of Clinical Microbiology, School of Medicine, University of Dublin, Trinity College, St. James's Hospital, Dublin 8, Ireland*

Running title: Linezolid-resistant, vancomycin-resistant *E. faecium* outbreak

*Corresponding author. Address: Microbiology Research Unit, Division of Oral Biosciences, Dublin Dental University Hospital, University of Dublin, Trinity College, Lincoln Place, Dublin 2, Ireland. Tel.: +353 1 6127276; fax: + 353 1 6127295.

E-mail address: david.coleman@dental.tcd.ie (D.C. Coleman).

Summary

Background: Linezolid is an antibiotic used to treat infections caused by multi-drug resistant Gram-positive bacteria. Linezolid resistance in enterococci has been reported with increasing frequency, with a recent rise in resistance encoded by *optrA, poxtA* or *cfr*.

Aim: To investigate a hospital outbreak of linezolid- and vancomycin-resistant *Enterococcus faecium* (LVREfm) using whole-genome sequencing (WGS).

Methods: Thirty-nine VREfm from patient screening (19 isolates, 17 patients) and environmental sites (20 isolates) recovered in October 2019 were investigated. Isolates were PCR screened for *optrA, poxtA* and *cfr* and underwent Illumina MiSeq WGS. Isolate relatedness was assessed using *E. faecium* core-genome (cg) MLST. One LVREfm underwent MinION long-read WGS (Oxford Nanopore) and hybrid assembly with MiSeq short-read sequences to resolve an *optrA*-encoding plasmid.

Findings: Twenty isolates (51.3%) were LVREfm and *optrA*-positive, including the LVREfm from the index patient. A closely related cluster of 28 sequence type (ST) 80 isolates was identified by cgMLST, including all 20 LVREfm and eight linezolid-susceptible VREfm, with an average allelic difference of two (range=0-10), indicating an outbreak. Nineteen (95%) LVREfm harboured a 56,684 bp conjugative plasmid (pEfmO_03). The remaining LVREfm exhibited 44.1% sequence coverage to pEfmO_03. The presence of pEfmO_03 in LVREfm and the close relatedness of the outbreak cluster isolates indicated the spread of a single strain. The outbreak was terminated by enhanced IPC and environmental cleaning measures, ceasing ward admissions and ward dedicated staff.

Conclusion: WGS was central in investigating an outbreak of ST80 LVREfm. The rapid implementation of enhanced IPC measures terminated the outbreak.

Keywords: Vancomycin resistant *Enterococcus faecium,* linezolid resistance, conjugative plasmid, *optrA*, nosocomial outbreak

Introduction

Enterococcus faecium is an important nosocomial pathogen causing bacteraemia, abdominal, urinary tract and intravenous catheter-related infections[1]. Acquired resistance to ampicillin, gentamicin (high level) and vancomycin has increased worldwide among hospital-associated *E. faecium*, narrowing treatment options[1]. Ireland had one of the highest rates of vancomycin-resistant *E. faecium* (VREfm) bloodstream infections in Europe between 2006-2018[2]. Furthermore, the population-weighted mean percentage of VREfm across Europe increased from 10.4% in 2014 to 17.3% in 2018[3].

Conventional MLST for *E. faecium* was first described in 2002, consisting of seven housekeeping genes with derived nomenclature managed and assigned via PubMLST.org[4]. Clinical VREfm worldwide assign to sequence types (ST) belonging to the epidemic hospital-adapted lineage clade A1. These strains are generally enriched in mobile genetic elements, putative virulence determinants, and antibiotic resistance determinants[5,6]. Whole-genome sequencing (WGS) studies revealed a polyclonal VREfm population structure with evidence of hospital transmission and inter- and intra-regional spread of VREfm clones[7–9] and no distinct geographical patterns [3]. Enhanced surveillance is required to better understand the epidemiology, clonal diversity and risk factors associated with VREfm[3].

Linezolid is an antibiotic used to treat infections caused by multi-drug resistant Gram-positive bacteria, including VREfm[10]. The emergence of linezolid-resistant enterococci (LRE) during or after linezolid exposure has been well described, with the first description of resistance noted during initial clinical trials[11–15]. Linezolid binds in the V domain of the 23S rRNA component of the 50S ribosomal subunit and inhibits protein synthesis[16]. Enterococcal linezolid resistance results mainly from G2576T or G2505A mutations in the 23S rRNA binding site or mutations in genes encoding ribosomal proteins L3 and/or L4[13]. Linezolid-resistance can also develop following acquisition of the *optrA, poxtA* genes and variants of the *cfr* gene, which are frequently encoded on conjugative plasmids[17].

The *optrA* gene was 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[12,14,15,18–20]. The OptrA protein belongs to the ATP-binding cassette (ABC)-F protein subfamily and mediates resistance to oxazolidinones and phenicols, which share an overlapping binding site at the ribosomal A-site. A recent study indicated the mechanism of *optrA*-mediated antibiotic resistance does not involve active efflux, like other ABC transporters[21]. Current evidence indicates that (ABC)-F proteins like OptrA bind to the ribosome and effect the release of ribosome-targeted antibiotics, thereby rescuing the

translation machinery from antibiotic-mediated inhibition[22]. Although the number of *optrA*-positive enterococci reported to date is low, they have increased recently. In 2014, 3/9 linezolid-resistant isolates (linezolid MIC > 4 mg/L) were *optrA*-positive *E. faecalis* (two from Ireland); this increased to 8/17 in 2016[18,19].

In Ireland in 2014, the first reported linezolid-resistant VREfm (LVREfm) clonal outbreak was reported involving 15 patients and was investigated using PFGE. All isolates harboured the G2576T 23S rRNA mutation and were *cfr*-negative. However, other linezolid resistance genes were not investigated[23]. That same year, two *optrA*-positive VREfm were recovered in separate Irish hospitals[18]. Since centralised screening commenced in 2016, Ireland had the highest reported prevalence of gene-encoded linezolid resistance, with *optrA* and/or *poxtA* identified in 22.7% (35/154) isolates, predominantly encoded on conjugative plasmids in diverse enterococcal lineages[24].

In October 2019, a LVREfm isolate was recovered from a patient in a Dublin hospital. Enhanced patient screening and environmental sampling yielded additional VREfm isolates including LVREfm suggesting an outbreak. In this study we describe the WGS analysis of these LVREfm, identification of an outbreak by a ST80 strain harbouring an *optrA*-encoding conjugative plasmid, and control measures implemented to terminate the outbreak.

Methods

Hospital setting

The outbreak occurred in a level 2, 107-bed hospital in Dublin, Ireland, and was primarily associated with two wards and the X-Ray department. Ward 1 (W1) was a 26-bed unit with single patient en-suite rooms, linked with ward 2 (W2), an oncology day-unit (Figure 1). The hospital specialities include general medical and oncology with a large proportion of patients requiring extensive care.

VREfm surveillance

In October 2019, patient A was admitted to W1 with chronic leg ulcers, cellulitis and an extensive medical history including colon cancer. Patient A had also been admitted the previous month to the high dependency unit and to W1. While an inpatient, patient A visited other departments including X-Ray and required a high level of care. On re-admission 13 days later, patient A was screened and placed on contact isolation precautions due to a history of carriage of multi-drug resistant organisms (MDRO) including VREfm. An LVREfm isolate was recovered from rectal screening on this admission, after which additional emphasis was placed on contact precautions,

hand hygiene and equipment decontamination. Nine days later, patient B (W1) also yielded LVREfm following rectal screening. This prompted the infection prevention and control team (IPCT) to request that all patients on W1 be screened for VREfm carriage, after which three more LVREfm-positive patients were identified (patients C-E, Figure 1, Table I). Additional weekly and discharge screening was introduced. As two LVREfm-positive patients (A & B) were oncology patients, the IPCT introduced screening of all patients attending the oncology out-patient W2, which identified two further LVREfm-positive patients (patients G & H, Figure 1, Table I). Patient G was an in-patient on a different hospital ward (W3) who attended W2 every few weeks from early 2019 until the start of the outbreak. Extended rectal screening across the hospital identified VREfm in a further nine patients (Table I). All VREfm patient screening isolates from the hospital recovered during the suspected outbreak timeline were investigated.

Patient and environmental screening

Extensive environmental screening was undertaken in all inpatient wards and other areas where patients had attended during hospitalisation. Environmental sites were sampled using regular FLOQSwabs® (Copan Diagnostics Inc., California, USA), pre-moistened with sterile water. Individual swab tips were placed into 5 ml of Brain Heart Infusion (BHI) broth (Fannin Ltd., Dublin, Ireland), incubated for 16-18 h at 37^{o}C, after which the cultures were inoculated onto CHROMID® VRE (bioMérieux, France) agar with a 10 μg linezolid disc (Oxoid [Thermo Fischer Scientific], Ireland). Patient rectal screening swabs were also inoculated onto CHROMID® VRE (bioMérieux) agar with a 10 μg linezolid disc (added following identification of index case), and onto CHROMID® CPS® Elite (bioMérieux) agar to ensure bowel flora were present on the swab.

Decontamination and control measures

For the duration of the outbreak W1 was closed to patient admissions and transfers, strict visitor restrictions were implemented, specific staff members were dedicated to W1, cleaning of all equipment in patient areas was increased to twice daily and cleaning of bathroom facilities was increased to four-times daily, using Actichlor Plus (Ecolab Limited, Cheshire, UK) with 1,000 ppm available chlorine. Patient rooms that yielded LVREfm were decontaminated with hydrogen peroxide vapour (HPV) following patient transfer or discharge using a using the Bioquell Rapid Bio-Decontamination Unit (Bioquell Ireland Ltd., Limerick, Ireland).

Phenotypic and genotypic analysis

All isolates were tested for susceptibility to linezolid and vancomycin using the VITEK®2 system (bioMérieux) and results interpreted using the European Committee on Antimicrobial Susceptibility Testing interpretative criteria[25]. All VREfm and LVREfm isolates were referred to the National MRSA Reference Laboratory (NMRSARL), where gradient strips (E-test, bioMérieux) were used to assess linezolid and chloramphenicol MICs. PCRs for identification of enterococcal species and detection of resistance genes (Table SI) were performed using GoTaq DNA polymerase and buffers (Promega Corporation, USA). One additional stored linezolid-susceptible VREfm isolate recovered in October 2018 from patient A, who was deemed the index case in the current outbreak, was also investigated.

Whole-Genome Sequencing

Thirty-nine enterococcal isolates and selected transconjugant derivatives (Table I, Table SII) underwent WGS using genomic DNA extracted with the *S. aureus* Genotyping Kit 2.0 (Abbott [Alere Technologies GmbH], Germany) and the Qiagen DNeasy blood and tissue kit (Qiagen, UK)[24]. 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)[24]. Libraries were scaled to yield ≥50x coverage.

LVREfm isolate O_03 (patient B) was selected for hybrid assembly to determine the genetic organisation of an *optrA*-encoding conjugative plasmid it harboured. For this isolate, DNA was extracted using the Qiagen HMW MagAttract kit (Qiagen). Long-read sequencing was performed using MinION sequencing (Oxford Nanopore Technologies, UK) using the one-dimensional (1D) genomic DNA sequencing kit (SQK-LSK109) and 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 *E. faecium* whole-genome (wg) MLST scheme available in BioNumerics v7.7 (Applied Maths, Belgium), with a filter applied to include only the 1,423 core-genome (cg) MLST loci[26]. Conventional MLST was also applied using Bionumerics to denote STs. Two BioNumerics algorithms were used to generate a consensus cgMLST 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 cgMLST allelic differences. llumina WGS data for all LVREfm were also examined for 23S rRNA mutations (G2576T and G2505A) using LRE-finder (https://cge.cbs.dtu.dk/services/LRE-finder/). All isolates were also compared with an in-house database of 245 VREfm whole-genome sequences from isolates recovered in two other Dublin hospitals between September 2017-October 2019. Data was stored in Ridom SeqSphere+ v6.0.0 and analysed using the *E. faecium* cgMLST scheme[26].

Hybrid assembly of an optrA-encoding plasmid

MinION- and MiSeq-generated FASTQ files were used to perform a hybrid assembly using UniCycler[27]. The genetic organisation of the *optrA*-encoding plasmid pEfmO_03 from LVREfm outbreak isolate O_03 (patient B) was determined and was annotated using RAST v2.0 (http://rast.nmpdr.org/). This was used as a reference sequence for further analysis against which MiSeq reads from other LVREfm were mapped and percentage depth and breadth of coverage calculated using Burrows-wheel aligner, Samtools and BedTools coverage[28–30]. The sequence of pEfmO_03 has been deposited in GenBank, accession number MT261365.

Conjugation

Conjugative transfer of plasmids encoding *optrA* harboured by LVREfm was undertaken by filter mating using the plasmid-free rifampicin- and fusidic acid-resistant recipient strains *E. faecium* 64/3 and *E. faecalis* OG1RF as described previously[31]. Putative transconjugants were screened for enterococcal species and *optrA* by PCR (Table SI). Transconjugants harbouring *optrA* underwent WGS and genomes were assembled using SPAdes v3.7.1 and compared to the corresponding recipient strain genomes.

Results

Patient VREfm

Eight patients were found to be colonised with LVREfm over a 4-week period. The patients were located in or visited hospital wards W1 and W2 (oncology), with one oncology patient located in W3 at the time of screening (Figure 1). A further nine patients yielded VREfm during a period of enhanced screening (Table I). Patient A, from whom the first LVREfm was recovered, had previously been admitted to the hospital high dependency unit the previous month and to W1. The patient was discharged and 13 days later readmitted to W1. Readmission screens yielded

LVREfm. A review of patients who had previously occupied the same bed as patient A revealed no further VREfm. In addition all VREfm isolates recovered over the previous year were reviewed on the VITEK®2 system (bioMérieux) and no linezolid resistance was found. Patient H yielded both a VREfm (O_37a) and a LVREfm (O_37b) from their screening sample (Table I). A review of antimicrobial prescribing for each patient (A-H) involved in the LVREfm outbreak revealed that only patient A had received linezolid.

Environmental screening

Following sampling of 129 environmental sites throughout the hospital, with particular focus on W1, W2 and X-ray, 20 VREfm were recovered, including 14 LVREfm (Figure 1, Table I). In W1, room 12 yielded a LVREfm five days after admission of patient B who also yielded LVREfm. The isolation and drug trolleys, which are moved throughout W1, also yielded LVREfm. The drug trolley in W1 yielded both an VREfm (O_17a) and an LVREfm (O_17b) (Table I). The treatment room, equipment storage room and consumables store room, all of which have a high volume of staff traffic, all yielded LVREfm. The family room on W1 also yielded LVREfm (Figure 1, Table I). In W2, the sluice room, point-of-care-testing (POCT) machine, cleaners store room and the isolation room all yielded LR-VREfm, between 13-20 days after recovery of the first LVREfm from patient A (index case). In X-ray, only room 2 yielded LVREfm (Figure 1, Table I).

Antimicrobial consumption

Analysis of hospital antimicrobial prescribing audit data revealed that linezolid consumption increased from 0.46 defined daily doses per 100 bed days used (DDD/100 BDU) beginning Q4 2016 to 1.14 DDD/100 BDU by Q4 2019 (range; 0.15-1.39 DDD/100 BDU). A steady rise in linezolid consumption was noted from Q2 of 2019 (1.04 DDD/100 BDU) to Q3 of 2019 (1.23 DDD/100 BDU). Consumption of other antimicrobials (vancomycin and daptomycin) also rose in Q3 2019, reflecting increased complexity of patients and increased numbers of patients colonised with MDROs. All prescriptions were deemed appropriate and compliant with the hospital's restricted antimicrobials policy.

WGS of isolates

A total of 37/39 VREfm investigated belonged to ST80; the remaining two isolates were a single-locus variant (SLV) of ST80. Twenty isolates (51.3%) were resistant to linezolid (Table I), all of which harboured *optrA*, but lacked *poxtA*, *cfr* and the 23S rRNA G2576T or G2505A mutations.

The remaining 19 VREfm lacked linezolid-resistance genes and were susceptible to linezolid (Table I). Thirty-seven of the VREfm differentiated into four clusters (C1–C4) using cgMLST (Figure 2). The majority of isolates (*N*=28) belonged to C1 and were closely related (average allelic difference of two, range=0-10). C1 consisted of ST80 (*N*=27) isolates, and one isolate deemed a SLV of ST80 and consisted of a mixture of patient (*N*=12) and environmental (*N*=16) isolates. C1 also contained LVREfm isolate O_02, the first *optrA*-positive LVREfm outbreak isolate, recovered from the suspected index case (patient A). A stored VREfm *optrA*-negative isolate (O_01) from patient A recovered a year previously also clustered in C1 (Figure1, Table I). Isolates O_01 and O_02 exhibited only three allelic differences. Two samples (patient H and the W1 drug trolley) each yielded pairs of *optrA*-positive LVREfm and *optrA*-negative VREfm isolates all of which clustered in C1; O_17a (VREfm) and isolate O_17b (LVREfm) from the drug trolley exhibited one allelic difference, whereas isolate O_37a (VREfm) and isolate O_37b (LVREfm) from patient H were indistinguishable. Clusters C2-C4 consisted of *optrA*-negative ST80 VREfm and were deemed unrelated to C1 isolates with intra-cluster allelic differences of 57-388 (Figure 2). A further comparison with a database of sequencing reads from 245 VREfm recovered in two other Irish hospitals revealed that all C1 outbreak isolates grouped in a larger cluster of ST80 isolates. The majority of database isolates (146/245, 59.9%) belonged to ST80, which divided into 11 clusters and 26 singletons, with an inter-cluster allelic differences range of 25-257 (Figure S1). The large cluster, termed ST80 complex type 2933, consisted of the 28 C1 outbreak isolates and seven additional VREfm from another Dublin hospital (Hospital 2) recovered between March-October 2019. This cluster had an average allelic difference of three (range=0-15).

A plasmid encoding optrA

The WGS data of the LVREfm outbreak isolate O_03 (patient B) underwent hybrid assembly and a 56,684 bp plasmid (pEfmO_03) encoding *optrA* and the chloramphenicol resistance gene *fexA* was resolved. The *optrA* gene was flanked by *Tnp*A and *Tnp*B from *Tn*554, and by *IS*Efa15 (Figure S2). A total of 19/20 *optrA*-positive outbreak LR-VREfm harboured plasmids exhibiting ≥99.98% sequence coverage to pEfmO_03. The remaining isolate exhibited 44.1% sequence coverage to pEfmO_03, with 100% coverage across the entire *optrA* encoding region. Plasmid pEfmO_03 was conjugative; transconjugant derivatives of the *E. faecium* 64/3 recipient were obtained with four LVREfm isolates (O_03, O_04, O_13, O_23) and with the *E. faecalis* OG1RF recipient using the LVREfm isolate O_23 as donor (Table SII). All transconjugants harboured

pEfmO_03 and were resistant to linezolid and chloramphenicol (MIC >4 and >32 mg/L, respectively) (Table SII).

Discussion

Linezolid-resistant enterococci harbouring acquired resistance genes have been reported with increasing frequency year on year, since 2014[18,19,24,31,32]. A recent Irish study described the highest prevalence of *optrA* and *poxtA* among LRE reported to date, with *optrA* identified in vancomycin susceptible *E. faecalis* and *E. faecium* isolates with diverse genetic backgrounds. The *poxtA* gene was also identified in nine *E. faecium* isolates, including five LVREfm deemed unrelated by cgMLST, with isolates belonging to several STs (ST80, ST202, ST203 and ST1588)[24]. Previously, *optrA* was reported in four French VREfm recovered between 2013 and 2015, three of which were ST80 and one ST17[32]. The present study represents the first reported hospital outbreak involving *optrA*-positive VREfm, with all isolates belonging to ST80 (or a SLV of ST80) of the hospital-adapted clade A1. All 28 outbreak isolates formed a single cgMLST cluster (C1) and all were highly related (average allelic difference of 2; range = 0-10) (Figure 2). The majority of C1 isolates (20/28) were LVREfm, 19/20 of which harboured an 56,684 bp conjugative plasmid (pEfmO_03) encoding *optrA* and *fexA* (Figure S2). The remaining eight C1 isolates were VREfm and lacked pEfmO_03 but were otherwise indistinguishable or very closely related to the LVREfm.

An allelic difference of ≤20 has been previously proposed as the threshold for determining *E. faecium* isolates as closely related based on cgMLST[26]. Interrogation of a WGS database of 245 VREfm isolates from two other Irish hospitals revealed that the majority of isolates belonged to ST80 (*N*=146), which further divided into 11 clusters, and 16 singletons, with 27 different complex types. All of the isolates in the outbreak cluster (C1) grouped into complex type 2933, along with seven VREfm from another Dublin hospital (Figure S1). These findings demonstrate that VREfm clones can persist over long periods and in different hospital locations, which has been reported previously[8,33]. The average allelic difference between isolates within complex type 2933 was three (range=0-15), showing the closely related nature of the outbreak isolates to isolates from another Dublin hospital. The frequent transfer of patients between hospitals in Ireland (especially in Dublin) could contribute to trafficking of individual strains between hospitals.

The first *optrA*-positive LVREfm outbreak isolate (O_02) recovered from the suspected index patient exhibited only three allelic differences to an *optrA*-negative VREfm (O_01) from the same patient a year earlier, indicating that the index patient harboured the same VREfm strain

for a year. When this strain acquired the *optrA*-encoding plasmid pEfmO_03 was not determined; the patient had no animal/farm exposure and no source of *optrA* was identified in the hospital. The highly related nature of all isolates in cluster C1, together with the finding of an identical *optrA*-encoding conjugative plasmid in all but one LVREfm outbreak isolates indicates the spread of a single strain over the four-week outbreak period. The remaining LVREfm outbreak isolate (O_24) exhibited 44.1% sequence identity to pEfmO_03, with 100% coverage around the entire region surrounding *optrA* and *fexA*, suggesting the loss of some plasmid sequence. The findings of the present study contrast with previous studies of LRE from Irish hospitals, which revealed the presence of the mobile linezolid resistance genes *optrA* and *poxtA* in enterococci with diverse genetic backgrounds[24]. During the present study, two samples (patients H and the drug trolley on W1) yielded isolate pairs, each consisting of an *optrA*-postive LVREfm and *optrA*-negative VREfm isolate. One pair of ST80 isolates (O_37a and O_37b), from patient H, exhibited zero allelic differences and the other pair of ST80 isolates (O_17a and O_17b) exhibited one allelic difference. The *optrA*-positive isolate of each pair harboured pEfmO_03. These findings indicated the loss/gain of the pEfmO_03 plasmid in individual samples.

The suspected index patient, patient A, had previously been treated with linezolid four weeks prior to the recovery of the first LVREfm outbreak isolate from this patient in October 2019. No other patient involved in the outbreak had a history of linezolid treatment. Based on this, the close similarity of all the LVREfm outbreak isolates and the presence of an identical *optrA*-encoding plasmid (pEfmO_03) in 95% (19/20) of LVREfm, strongly suggests that the outbreak was due to the recent transmission of the LVREfm from patient A, either by indirect contact with other patients via the hands of healthcare workers (HCWs) and/or by shedding of the LVREfm into the hospital environment. Interestingly, pEfmO_03 was unique to this outbreak and showed minimal sequence identity (7.8%-18.2%) to the *optrA* genetic environments, both chromosomal and plasmid, described previously in LRE from Ireland[24].

LVREfm environmental isolates in C1 were identified up to 20 days following the initial isolate recovery from patient A, even following enhanced environmental decontamination and increased awareness of the importance of hand hygiene among HCWs. Review of hand hygiene audit records revealed the hospital was compliant with national standards on hand hygiene and achieved >95% compliance. Nonetheless, extensive environmental screening also revealed that sites such as treatment and supply rooms harboured LVREfm. These findings highlight the critical importance of hand hygiene in hospitals and highlight a significant need for ongoing improvements. The appointment of local hand hygiene champions may be beneficial in this regard. The implementation of enhanced IPC measures (improved cleaning of the environment,

the use of HPV decontamination, the scheduling and recording of equipment cleaning, ceasing ward admissions and staff dedicated to W1) was successful in the rapid termination of the outbreak, which was deemed over four weeks after the last LVREfm patient isolate was recovered.

It is likely that linezolid usage was a contributory factor in the emergence of the LVREfm in the outbreak hospital as from Q4 2016, linezolid consumption increased from 0.46 DDD/100 BDU to 1.14 DDD/100 BDU by Q4 2019. This increased linezolid usage reflected increased complexity of patients and colonisation with MDRO's. All prescriptions were deemed appropriate and compliant with restricted antimicrobials policy. This highlights the challenging requirement for more prudent antimicrobial treatment of medically complex patients harbouring MDRO's. Finally, plasmid encoded *optrA* has been reported previously in animal staphylococci [34,35]. It is worrying to consider the possibility that the *optrA*-encoding plasmid identified in the LVREfm isolates in the present study may eventually transfer into staphylococci (e.g. MRSA), or indeed other enterococci, in the hospital environment, further limiting options for treating infections caused by these organisms.

Conclusions

WGS and epidemiological data analysis was central in the rapid identification and characterisation of a clonal ST80 outbreak of LVREfm harbouring a 56,684 bp conjugative plasmid (pEfmO_03) encoding *optrA*. The team approach adopted in the management of this outbreak directed the rapid implementation of enhanced IPC measures including the early detection and aggressive environmental decontamination, which resulted in the timely containment and termination of the outbreak.

Acknowledgements

We wish to acknowledge the staff of the hospital for their assistance in containing this outbreak and the staff of the National MRSA Reference Laboratory for their support in managing the collection of linezolid-resistant clinical isolates investigated in this study. We wish to acknowledge Dr. Mette Pinholt, Professor Henrik Westh and the bioinformatic staff of the Hvidovre University Hospital, Copenhagen, Denmark, for their assistance and guidance in the bioinformatic analysis of clinical enterococci. We would also like to thank Dr. Jennifer Bender of the Robert Koch Institute, Wernigerode, Germany, for providing us with the recipient strains for filter mating experiments.

Conflict of interest statement

None declared.

Funding source

This work was funded by the Dublin Dental University Hospital Microbiology Research Unit and by Health Research Board grant number ILP-POR-2019-010.

References

[1] Arias CA, Murray BE. The rise of the Enterococcus: beyond vancomycin resistance. Nat Rev Micrbiology 2012;10:266–78. https://doi.org/10.1038/nrmicro2761.

[2] European Centre for Disease Prevention and Control. The European Antimicrobial Resistance Surveillance System EARS-Net Results 2017. https://ecdc.europa.eu/en/antimicrobial-resistance/surveillance-and-disease-data/data-ecdc

[3] European Centre for Disease Prevention and Control. Surveillance of antimicrobial resistance in Europe 2018. https://www.ecdc.europa.eu/en/publications-data/surveillance-antimicrobial-resistance-europe-2018.

[4] Homan WL, Tribe D, Poznanski S, Li M, Hogg G, Spalburg E, et al. Multilocus sequence typing scheme for *Enterococcis faecium*. J Clin Microbiol 2002;40:1963–71. https://doi.org/10.1128/JCM.40.6.1963-1971.

[5] Lebreton F, Schaik W Van, Manson A. Emergence of epidemic multidrug-resistant *Enterococcus faecium* from animal and commensal strains. mBio 2013;4:e00534-13. https://doi.org/10.1128/mBio.00534-13.

[6] Wurster JI, Saavedra JT, Gilmore MS. Impact of antibiotic use on the evolution of *Enterococcus faecium*. J Infect Dis 2016;213:1862–5. https://doi.org/10.1093/infdis/jiv598.

[7] Gorrie C, Higgs C, Carter G, Stinear TP, Howden B. Genomics of vancomycin-resistant *Enterococcus faecium*. Microb Genom 2019;5. https://doi.org/10.1099/mgen.0.000283.

[8] Pinholt M, Gumpert H, Bayliss S, Nielsen JB, Vorobieva V, Pedersen M, et al. Genomic analysis of 495 vancomycin-resistant *Enterococcus faecium* reveals broad dissemination of a vanA plasmid in more than 19 clones from Copenhagen, Denmark. J Antimicrob Chemother 2017;72:40–7. https://doi.org/10.1093/jac/dkw360.

[9] Raven KE, Reuter S, Reynolds R, Brodrick HJ, Russell JE, Török ME, et al. A decade of genomic history for healthcare-associated *Enterococcus faecium* in the United Kingdom and Ireland. Genome Res 2016;26:1388–96. https://doi.org/10.1101/gr.204024.116.

[10] 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;72:354–64. https://doi.org/10.1093/jac/dkw450.

[11] Gonzales RD, Schreckenberger PC, Graham MB, Kelkar S, DenBesten K, Quinn JP. Infections due to vancomycin-resistant *Enterococcus faecium* resistant to linezolid. Lancet 2001;357:1179. https://doi.org/10.1016/S0140-6736(00)04376-2.

[12] Bi R, Qin T, Fan W, Ma P, Gu B. The emerging problem of linezolid-resistant enterococci. J Glob Antimicrob Resist 2018;13:11–9. https://doi.org/10.1016/J.JGAR.2017.10.018.

[13] Bai B, Hu K, Zeng J, Yao W, Li D, Pu Z, 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. https://doi.org/10.1089/mdr.2018.0005.

[14] Cai J, Wang Y, Schwarz S, Lv H, Li Y, Liao K, 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.e1-1095.e4. https://doi.org/10.1016/j.cmi.2015.08.007.

[15] Wang Y, Lv Y, Cai J, Schwarz S, Cui L, Hu Z, 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. https://doi.org/10.1093/jac/dkv116.

[16] Swaney SM, Aoki H, Ganoza MC, Shinabarger DL. The oxazolidinone linezolid inhibits initiation of protein synthesis in bacteria. Antimicrob Agents Chemother 1998;42:3251–5.

[17] Bender JK, Cattoir V, Hegstad K, Sadowy E, Coque TM, Westh H, 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. https://doi.org/10.1016/j.drup.2018.10.002.

[18] Mendes RE, Hogan PA, Jones RN, Sader HS, Flamm RK. 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. https://doi.org/10.1093/jac/dkw052.

[19] Mendes RE, Deshpande L, Streit JM, Sader HS, Castanheira M, Hogan PA, et al. ZAAPS Program 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. https://doi.org/10.1179/1973947812Y.0000000039.

[20] Cavaco LM, Bernal JF, Zankari E, Léon M, Hendriksen RS, Perez-Gutierrez 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. https://doi.org/10.1093/jac/dkw490.

[21] Wang Y, Li X, Wang Y, Schwarz S, Shen J, Xia X. Intracellular accumulation of linezolid and florfenicol in OptrA-producing *Enterococcus faecalis* and *Staphylococcus aureus*. Molecules 2018;23:3195. https://doi.org/10.3390/molecules23123195.

[22] Sharkey LKR, O'Neill AJ. Antibiotic resistance ABC-F proteins: bringing target protection into the limelight. ACS Infect Dis 2018;4:239–46. https://doi.org/10.1021/acsinfecdis.7b00251.

[23] O'Driscoll C, Murphy V, Doyle O, Wrenn C, Flynn A, O'Flaherty N, 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. https://doi.org/10.1016/j.jhin.2015.09.006.

[24] Egan SA, Shore AC, Connell BO, 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 2020:4–11. https://doi.org/10.1093/jac/dkaa075.

[25] European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters Version 9.0. 2019.

[26] de Been M, Pinholt M, Top J, Bletz S, Mellmann A, van Schaik W, et al. Core genome multilocus sequence typing scheme for high-resolution typing of *Enterococcus faecium*. J Clin Microbiol 2015;53:3788–97. https://doi.org/10.1128/JCM.01946-15.

[27] Wick RR, Judd LM, Gorrie CL, Holt KE. Completing bacterial genome assemblies with multiplex MinION sequencing. Microb Genomics 2017:0–6. https://doi.org/10.1099/mgen.0.000132.

[28] Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler Transform. Bioinformatics 2009;25:1754–60.

[29] Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, et al. The Sequence alignment/map (SAM) format and SAMtools. Bioinformatics 2009;25:2078–9.

[30] Quinlan AR, Hall IM. BEDTools: a flexible suite of utilities for comparing genomic features. Bioinformatics 2010;26:841–2. https://doi.org/10.1093/bioinformatics/btq033.

[31] 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;52:819–27.

https://doi.org/10.1016/j.ijantimicag.2018.09.009.

[32] Sassi M, Guérin F, Zouari A, Beyrouthy R, Auzou M, Fines-Guyon M, et al. Emergence of *optrA*-mediated linezolid resistance in enterococci from France, 2006–16. J Antimicrob Chemother 2019;74:1469–1472. https://doi.org/10.1093/jac/dkz097.

[33] Pinholt M, Bayliss SC, Gumpert H, Worning P, Jensen VVS, Pedersen M, et al. WGS of 1058 *Enterococcus faecium* from Copenhagen, Denmark, reveals rapid clonal expansion of vancomycin-resistant clone ST80 combined with widespread dissemination of a *vanA*-containing plasmid and acquisition of a heterogeneous accessory genome. J Antimicrob Chemother 2019;74:1776–85. https://doi.org/10.1093/jac/dkz118.

[34] Fan R, Li D, Wang Y, He T, Feßler AT, Schwarz S, et al. Presence of the *optrA* gene in methicillin-resistant *Staphylococcus sciuri* of porcine origin. Antimicrob Agents Chemother 2016;60:7200–5. https://doi.org/10.1128/AAC.01591-16.

[35] Guo D, Liu Y, han C, Chen Z, Ye X. Phenotypic and molecular characteristics of methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* isolated from pigs: implication for livestock-association markers and vaccine strategies. Infect Drug Resist 2018;11:1299-1307. https://doi.org/10.2147/IDR.S173624

Figure legends

Figure 1. Schematic diagram showing the layout of hospital ward 1, ward 2 and X-ray involved in the *optrA*-positive vancomycin-resistant *Enterococcus faecium* (VREfm) outbreak. The single en-suite rooms in ward 1 are labelled 1-26. Room numbers have been changed to maintain patient anonymity. Other areas of interest are labelled accordingly, or details are provide using the key. Locations where patients (denoted by capital letters A-H) that yielded linezolid-resistant VREfm (LVREfm) isolates were housed are denoted by a filled red circle. Environmental sites that yielded LVREfm environmental isolates are denoted by a filled red square. Patients A, B and F were transferred during course of the outbreak, movements are denoted by corresponding red letter in alternate rooms. Patient G, an oncology in-patient housed on ward 3 (not shown), is shown on ward 2, as this is the likely location for acquisition of LVREfm. Room locations of patients and environmental sites that yielded VREfm are not shown to retain clarity. This information is provided in Table I. Abbreviations: POCT machine, point-of-care-testing machine; Equip. store, equipment storage; consumables, consumable storeroom.

Figure 2. Minimum spanning tree based on core-genome multilocus sequence typing (cgMLST) data from the 39 ST80 vancomycin-resistant *Enterococcus faecium* (VREfm) isolates recovered from patient rectal screening swabs (19 isolates, 17 patients, denoted by a filled white diamond) and hospital environmental sites (20 isolates) during the hospital outbreak between the 8th of October and the 1st of November 2019. Twenty of the isolates were linezolid-resistant VREfm (LVREfm) and harboured *optrA* as denoted by the colour legend. The first LVREfm outbreak isolate recovered from the suspected index patient in October 2019 is denoted by I. A stored linezolid-susceptible VREfm isolate lacking *optrA* recovered from the same patient a year earlier is denoted by a filled yellow diamond and an I. A green asterisk denotes pairs of isolates; one isolate of each pair was LVREfm (harbouring plasmid pEfmO_03) and the other VREfm (lacking plasmid pEfmO_03). Pairs of isolates included O_17a and O_17b recovered from a drug trolley, also O_37a and O_37b recovered from patient H. The numbers on the branches represent the number of cgMLST allelic differences. Clusters of related isolates are encircled and labelled C1 – C4; *d*= indicates average allelic differences and the range is shown in square brackets.

Table I. Phenotypic and genotypic characteristics of the 38 vancomycin-resistant *Enterococcus faecium* isolates recovered in an outbreak setting in an Irish hospital over four weeks in October 2019, with the addition of one isolate from the index patient from 2018.

***E. faecium* isolate No.**	**Ward/ Room[a]**	**Day since first isolate recovered**	**Source[c]**	**Clinical history**	**LIN MIC mg/L (R> 4 mg/L)[d]**	**VAN MIC mg/L (R> 4 mg/L)[d]**	**CHL MIC mg/L (R> 32 mg/L)[d]**	***optrA***	**ST**	**cgMLST cluster[e]**	**Plasmid sequence similarity (%) to pEfmO_03**
O_01	W1	N/A[b]	Patient A		4.0	≥32	32	-	80	C1	N/A
O_02	W1 9 > 22	0	Patient A	Colon cancer, diabetes, COPD, chronic leg ulcers, multiple MDRO including VRE carriage	8.0	≥32	≥256	+	80	C1	100
O_03	W1 12 > 26	8	Patient B	Metastatic cancer, palliative care	16.0	≥32	≥256	+	80	C1	100
O_04	W1	13	Room 12		16.0	≥32	≥256	+	80	C1	100
O_05	W2	13	Sluice room		16.0	≥32	≥256	+	80	C1	100
O_06	W1	13	Isolation trolleys		16.0	≥32	≥256	+	80	C1	100
O_07	W1 7	13	Patient C	COPD, arthritis, malignancy	8.0	≥32	≥256	+	80	C1	100
O_08	W1	13	Treatment room		16.0	≥32	≥256	+	80	C1	100
O_09	W1 22	14	Patient D	Infected leg ulcers, recurrent UTI's, rheumatoid arthritis	16.0	≥32	≥256	+	80	C1	99.98
O_10	W1	14	Patient		2.0	≥32	16	-	SLV of ST80	N/A	N/A
O_11	W1	14	Patient		2.0	≥32	32	-	80	C4	N/A
O_12	W1	14	Patient		2.0	≥32	32	-	80	C1	N/A
O_13	W1 21	15	Patient E	Metastatic malignancy, palliative care	16.0	≥32	≥256	+	SLV of ST80	C1	100
O_14	W1	16	Equipment store		16.0	≥32	≥256	+	80	C1	99.98
O_15	W1	16	Consumable		8.0	≥32	≥256	+	80	C1	100

			store								
O_16	W1	16	Family room		32.0	≥32	≥256	+	80	C1	100
O_17a	W1	16	Drug trolley		1.0	≥32	16	-	80	C1	N/A
O_17b	W1	16	Drug trolley		16.0	≥32	≥256	+	80	C1	100
O_18	W1	16	Linen room		2.0	≥32	16	-	80	C1	N/A
O_19	W1	16	Night nurse trolley		1.0	≥32	16	-	80	C4	N/A
O_20	W1	16	Cleaners store		2.0	≥32	16	-	80	C1	N/A
O_21	W4	16	Patient		2.0	≥32	32	-	80	C1	N/A
O_22	W2	20	POCT machine		8.0	≥32	≥256	+	80	C1	100
O_23	W2	20	Isolation room		8.0	≥32	≥256	+	80	C1	100
O_24	W1 5 > 26	20	Patient F	Congestive cardiac failure and COPD	16.0	≥32	≥256	+	80	C1	44.1
O_25	W2	20	Cleaner room		8.0	≥32	≥256	+	80	C1	100
O_26	X-ray	20	Room 2		8.0	≥32	≥256	+	80	C1	100
O_27	W2	20	Lobby		2.0	≥32	16	-	80	C4	N/A
O_28	W1	20	Patient		2.0	≥32	64	-	80	C3	N/A
O_29	W1	20	Patient		2.0	≥32	32	-	80	C4	N/A
O_30	W4	20	Patient		1.0	≥32	32	-	80	C1	N/A
O_31	W1	20	New treatment room (room 10)		4.0	≥32	64	-	80	C3	N/A
O_32	W6	20	Bathroom		2.0	≥32	32	-	80	C2	N/A
O_33	X-ray	20	Ultrasound		2.0	≥32	32	-	80	N/A	N/A
O_34	W3	21	Patient		2.0	≥32	32	-	80	C2	N/A
O_35	W5	22	Patient		1.0	≥32	16	-	80	C4	N/A
O_36	W3	23	Patient G	Metastatic malignancies, gastrointestinal upset	8.0	≥32	≥256	+	80	C1	99.98
O_37a	W2	23	Patient H	Breast cancer	2.0	≥32	16	-	80	C1	N/A
O_37b	W2	23	Patient H	Breast cancer	32.0	≥32	≥256	+	80	C1	100

[a] Room numbers have been changed to maintain patient anonymity, x > y indicates room transfers during course of outbreak.
[b] This isolate was recovered from the index case (patient A) one year previous to the outbreak.
[c] All isolates recovered from patients were recovered from rectal swabs. Environmental isolates were recovered from pre-moistened FLOQSwabs® (Copan Diagnostics Inc., California, USA) used to swab area.
[d] Clinical breakpoints (with epidemiological cut-off value used for chloramphenicol), taken from the European Committee on Antimicrobial Susceptibility Testing guidelines[25].
[e] Thirty-seven of the 39 VREfm outbreak isolates were differentiated into four clusters (C1–C4) using cgMLST (Figure 2).
Abbreviations: LIN, Linezolid; VAN, Vancomycin; CHL, Chloramphenicol; W, Ward; N/A, Not applicable; ST, sequence type; MDRO, multiple drug-resistant organisms; COPD, chronic obstructive pulmonary disease.

Figure 1

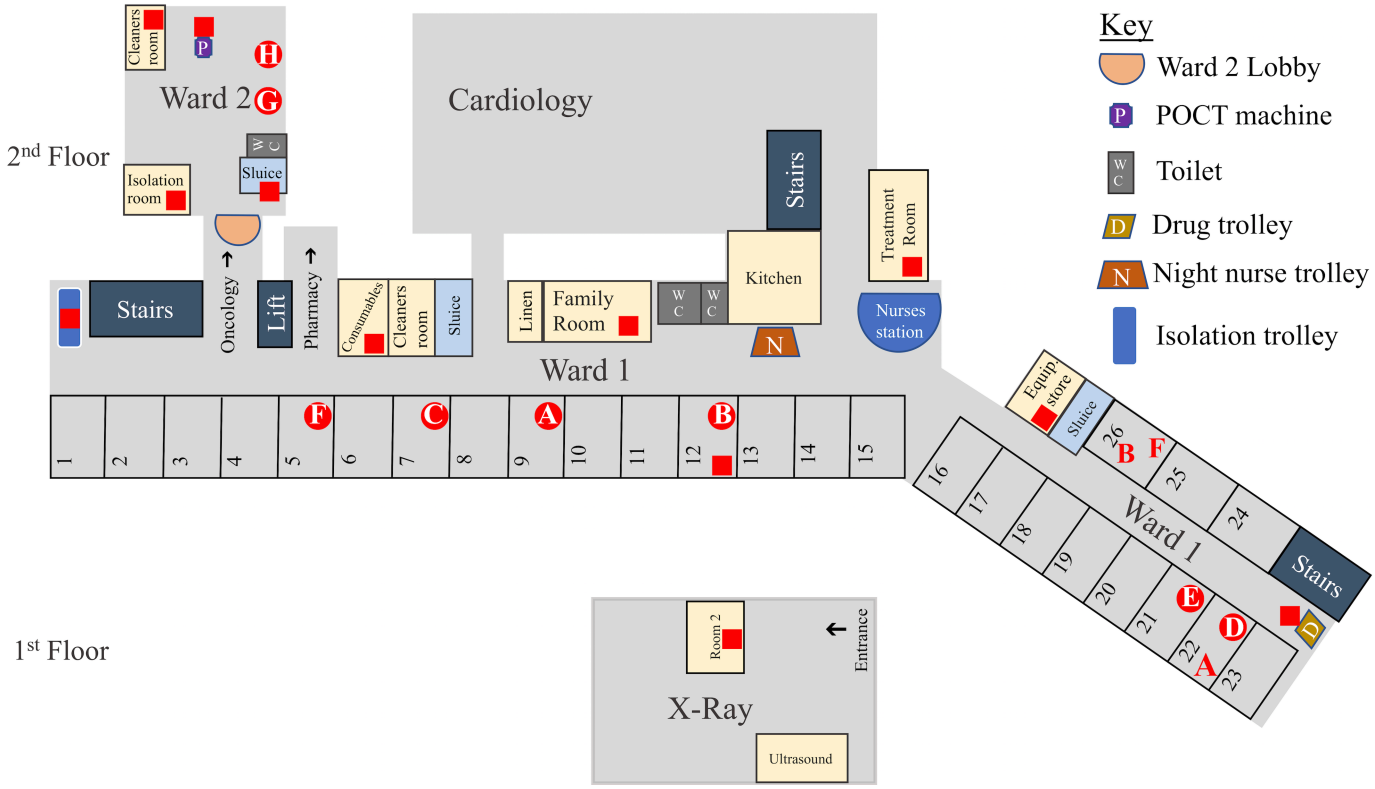
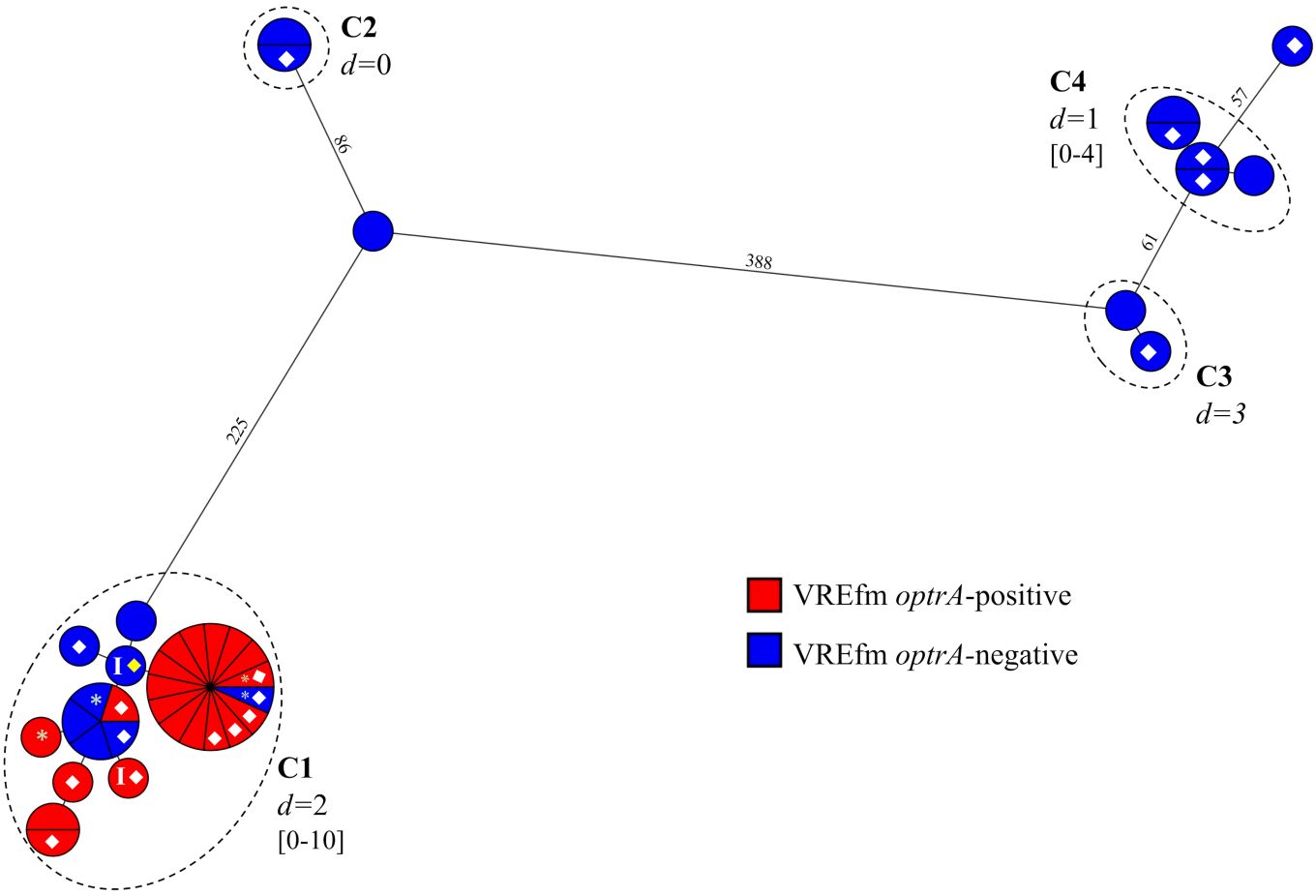

Key
Ward 2 Lobby
POCT machine
Toilet
Drug trolley
Night nurse trolley
Isolation trolley
2nd Floor
Cleaners room
Ward 2
Cardiology
Isolation room
Sluice
Stairs
Oncology
Lift
Pharmacy
Consumables
Cleaners room
Sluice
Linen
Family Room
Kitchen
Stairs
Treatment Room
Nurses station
Ward 1
Equip. store
Sluice
Ward 1
Stairs
1st Floor
Room 2
Entrance
X-Ray
Ultrasound

Figure 2


C2
d=0
98
C4
d=1
[0-4]
57
61
388
C3
d=3
225
C1
d=2
[0-10]
VREfm optrA-positive
VREfm optrA-negative

Supplemental data

Table SI. Phenotypic and genotypic characteristics of the 38 vancomycin-resistant *Enterococcus faecium* isolates recovered in an outbreak setting in an Irish hospital over four weeks in October 2019, with the addition of one isolate from the index patient from 2018.

***E. faecium* isolate No.**	**Ward/ Room[a]**	**Day since first isolate recovered**	**Source[c]**	**Clinical history**	**Lnz MIC mg/L (R≥ 4 mg/L)[d]**	**Vanc MIC mg/L (R≥ 4 mg/L)[d]**	**Chl MIC mg/L (R≥ 8 mg/L)[d]**	***optrA***	**ST**	**cgMLST cluster[e]**	**Plasmid sequence similarity (%) to pEfmO_03**
O_01	W1	N/A[b]	Patient A		4.0	≥32	32	-	80	C1	N/A
O_02	W1 9 > 22	0	Patient A	Colon cancer, diabetes, COPD, chronic leg ulcers, multiple MDRO including VRE carriage	8.0	≥32	≥256	+	80	C1	100
O_03	W1 12 > 26	8	Patient B	Metastatic cancer, palliative care	16.0	≥32	≥256	+	80	C1	100
O_04	W1	13	Room 12		16.0	≥32	≥256	+	80	C1	100
O_05	W2	13	Sluice room		16.0	≥32	≥256	+	80	C1	100
O_06	W1	13	Isolation trolleys		16.0	≥32	≥256	+	80	C1	100
O_07	W1 7	13	Patient C	COPD, arthritis, malignancy	8.0	≥32	16	+	80	C1	100
O_08	W1	13	Treatment room		16.0	≥32	≥256	+	80	C1	100
O_09	W1 22	14	Patient D	Infected leg ulcers, recurrent UTI's, rheumatoid arthritis	16.0	≥32	≥256	+	80	C1	99.98
O_10	W1	14	Patient		2.0	≥32	16	-	SLV of ST80	N/A	N/A
O_11	W1	14	Patient		2.0	≥32	32	-	80	C4	N/A
O_12	W1	14	Patient		2.0	≥32	32	-	80	C1	N/A
O_13	W1 21	15	Patient E	Metastatic malignancy, palliative care	16.0	≥32	≥256	+	SLV of ST80	C1	100
O_14	W1	16	Equipment store		16.0	≥32	≥256	+	80	C1	99.98

O_15	W1	16	Consumable store		8.0	≥32	32	+	80	C1	100
O_16	W1	16	Family room		32.0	≥32	≥256	+	80	C1	100
O_17a	W1	16	Drug trolley		1.0	≥32	16	-	80	C1	N/A
O_17b	W1	16	Drug trolley		16.0	≥32	32	+	80	C1	100
O_18	W1	16	Linen room		2.0	≥32	16	-	80	C1	N/A
O_19	W1	16	Night nurse trolley		1.0	≥32	16	-	80	C4	N/A
O_20	W1	16	Cleaners store		2.0	≥32	16	-	80	C1	N/A
O_21	W4	16	Patient		2.0	≥32	32	-	80	C1	N/A
O_22	W2	20	POCT machine		8.0	≥32	≥256	+	80	C1	100
O_23	W2	20	Isolation room		8.0	≥32	≥256	+	80	C1	100
O_24	W1 5 > 26	20	Patient F	Congestive cardiac failure and COPD	16.0	≥32	≥256	+	80	C1	44.1
O_25	W2	20	Cleaner room		8.0	≥32	≥256	+	80	C1	100
O_26	X-ray	20	Room 2		8.0	≥32	≥256	+	80	C1	100
O_27	W2	20	Lobby		2.0	≥32	16	-	80	C4	N/A
O_28	W1	20	Patient		2.0	≥32	64	-	80	C3	N/A
O_29	W1	20	Patient		2.0	≥32	32	-	80	C4	N/A
O_30	W4	20	Patient		1.0	≥32	32	-	80	C1	N/A
O_31	W1	20	New treatment room (room 10)		4.0	≥32	64	-	80	C3	N/A
O_32	W6	20	Bathroom		2.0	≥32	32	-	80	C2	N/A
O_33	X-ray	20	Ultrasound		2.0	≥32	32	-	80	N/A	N/A
O_34	W3	21	Patient		2.0	≥32	32	-	80	C2	N/A
O_35	W5	22	Patient		1.0	≥32	16	-	80	C4	N/A
O_36	W3	23	Patient G	Metastatic malignancies, gastrointestinal upset	8.0	≥32	≥256	+	80	C1	99.98
O_37a	W2	23	Patient H	Breast cancer	2.0	≥32	16	-	80	C1	N/A
O_37b	W2	23	Patient H	Breast cancer	32.0	≥32	≥256	+	80	C1	100

[a] Room numbers have been changed to maintain patient anonymity, x > y indicates room transfers during course of outbreak.
[b] This isolate was recovered from the index case (patient A) one year previous to the outbreak.
[c] All isolates recovered from patients were recovered from rectal swabs. Environmental isolates were recovered from pre-moistened FLOQSwabs® (Copan Diagnostics Inc., California, USA) used to swab area.
[d] Clinical breakpoints taken from the European Committee on Antimicrobial Susceptibility Testing guidelines[1].
[e] Thirty-seven of the 39 VREfm outbreak isolates were differentiated into four clusters (C1–C4) using cgMLST (Figure 1).
Abbreviations: Lnz, Linezolid; Vanc, Vancomycin; Chl, Chloramphenicol; W, Ward; N/A, Not applicable; ST, sequence type; MDRO, multiple drug-resistant organisms; COPD, chronic obstructive pulmonary disease.

Table SII. Primers used in the present study

Primer purpose	Gene amplified	Primer pair	Nucleotide Sequence (5'-3')	Product size (bp)	Nucleotide coordinates	PCR conditions	Reference
Multiplex PCR to confirm enterococcal species and *van* gene type	$ddl_{E.\ faecium}$	Efm-1	TAGAGACATTGAATATGCC	529	210949-210967[b]	94°C for 2 min. 30 cycles of 94°C for 1 min, 54°C for 1 min, 72°C for 1 min. Final elongation of 10 min at 72°C	Dutka-Malen et al., 1995 [2]
		Efm-2	ACCTAACATCGTGTAAGCT[a]		211460-211478[b]		
	$ddl_{E.\ faecalis}$	Efs-1	ATCAAGTACAGTTAGTCT	941	802443-802460[c]		
		Efs-2	ACGATTCAAAGCTAACTG		803366-803383[c]		
	vanA	*VanA*-1	GGGAAAACGACAATTGC	732	10540-10556[d]		
		VanA-2	GTACAATGCGGCCGTTA		9825-9841[d]		
	vanB	*VanB*-1	ATGGGAAGCCGATAGTC	635	2213806-2213822[b] 2213188-2213204[b]		
		VanB-2	GATTTCGTTCCTCGACC				
Multiplex PCR for detection of linezolid-resistance genes following filter mating	*optrA*	*optrA*-F	GAAGAAGGAACTGGTGAAAGTGAG	1103	217-240[e]	94°C for 2 min. 30 cycles of 94°C for 1 min, 61°C for 1 min, 72°C for 1 min. Final elongation 10 min at 72°C	In-house primers (This study)
		optrA-R	GTGTCATTTAGCTCAGGGTATTCG		1296-1319[e]		
	poxtA	*poxtA*-F	TATTGTCGGCGTGAACGGAG	1355	90-109[f]		
		poxtA-R	TCTGCGTTTCTGGGTCAAGG		1425-1444[f]		
Multiplex PCR	*cfr*	*cfr*-F	TGCTACAGGCGACATTGGAT	137	357-376[g]	95°C for 2 min.	NMRSARL

Primer purpose	Gene amplified	Primer pair	Nucleotide Sequence (5'-3')	Product size (bp)	Nucleotide coordinates	PCR conditions	Reference
used in NMRSARL to screen linezolid-resistant enterococci		*cfr*-R	GACGGTTGGCTAGAGCTTCA		474-493[g]	25 cycles of 95°C for 15 s, 53°C for 15 s, 68°C for 90 s. Final elongation of 5 min at 68°C	primers
	optrA	*optrA*-F	ACCGGTGTCCTCTTTGTCAG	369	1374-1393[e]		
		optrA-R	TCAATGGAGTTACGATCGCCTT		1721-1742[e]		
	poxtA	*poxtA*-F	TCAGAGCCGTACTGAGCAAC	167	1274-1293[f]		
		poxtA-R	CGTTTCTGGGTCAAGGTGGT		1421-1440[f]		

[a] Primer designed in-house, due to error in original manuscript which was followed by publication of a correction.
[b] Nucleotide coordinates based on *E. faecium* Aus0004, GenBank accession number CP003351.
[c] Nucleotide coordinates based on *E. faecalis* V583, GenBank accession number NC_004668.
[d] Nucleotide coordinates based on *E. faecium* V24, GenBank accession number KX574671.
[e] Nucleotide coordinates based on *optrA* gene, GenBank accession number KY579372.
[f] Nucleotide coordinates based on *poxtA* gene, GenBank accession number MF095097.
[g] Nucleotide coordinates based on *cfr* gene, GenBank accession number NC_023913.1.
Abbreviation: NMRSARL, National MRSA Reference Laboratory.

Table SIII. MIC profiles of transconjugant derivatives of *E. faecium* 64/3 and *E. faecalis* OG1RF

Strain/isolate/transconjugant	Gene conjugated	Linezolid MIC (mg/L) (R ≥ 4 mg/L)[a]	Vancomycin MIC (mg/L) (R ≥ 4 mg/L)[a]	Chloramphenicol MIC (mg/L) (R ≥ 8 mg/L)[a]
Donor: VREfm isolate O_03	N/A	16.0	≥32	≥256
Recipient: *E. faecium* 64/3	N/A	4	≤1	8
Transconjugant: O_03:*Efm* 64/3 TC2	*optrA*	32	≤1	≥256
Donor: VREfm isolate O_04	N/A	16.0	≥32	
Recipient: *E. faecium* 64/3	N/A	4	≤1	8
Transconjugant: O_04:*Efm* 64/3 TC2	*optrA*	16	≤1	≥256
Donor: VREfm isolate O_13	N/A	16.0	≥32	
Recipient: *E. faecium* 64/3	N/A	4	≤1	8
Transconjugant: O_13:*Efm* 64/3 TC1	*optrA*	16	≤1	≥256
Donor: VREfm isolate O_23	N/A	8.0	≥32	
Recipient: *E. faecium* 64/3	N/A	4	≤1	8
Transconjugant: O_23:*Efm* 64/3 TC2	*optrA*	32	1	≥256
Donor: **VREfm isolate O_23**	N/A	8.0	≥32	≥256
Recipient: *E. faecalis* OG1RF	N/A	≤2	2	≤4
Transconjugant: O_23:*Efs* OG1RF TC2	*optrA*	48	4	≥256

[a] Clinical breakpoints taken from the European Committee on Antimicrobial Susceptibility Testing guidelines[1].
Abbreviations: MIC, minimum inhibitory concentration; R, resistant; N/A, not applicable.

References

[1] European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters Version 10.0. 2020. http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_10.0_Breakpoint_Tables.pdf

[2] Dutka-Malen S, Evers S, Courvalin P. Detection of glycopeptide resistance genotypes and identification to the species level of clinically relevant enterococci by PCR. J Clin Microbiol 1995;33:24–7.

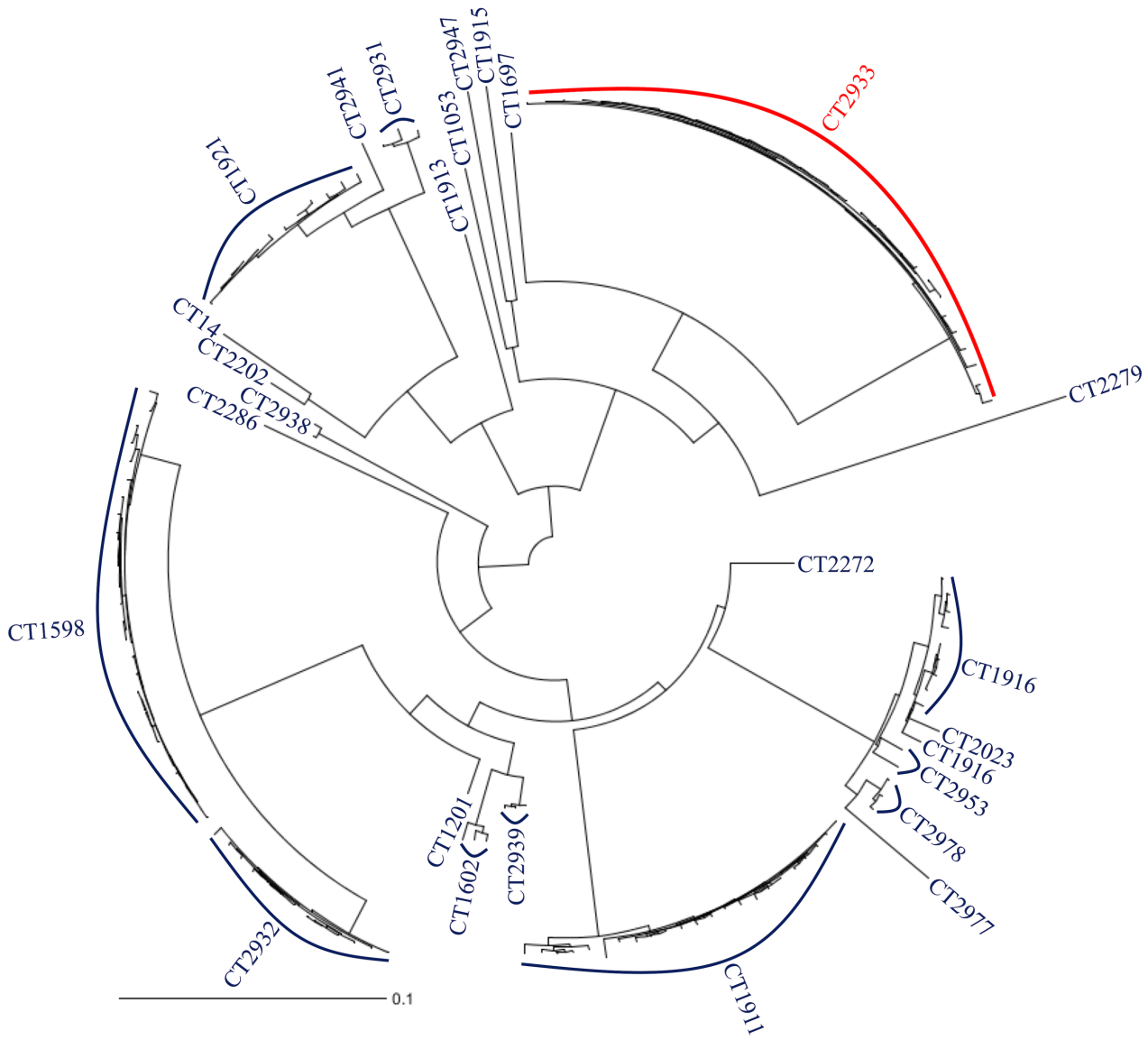


Figure S1. Neighbour-joining tree based on cgMLST of 174 ST80 vancomycin-resistant *Enterococcus faecium* (VREfm) isolates, including the 28 outbreak cluster C1 isolates from the present study and 146 isolates from two other Irish Hospitals, recovered between September 2017-October 2019. The 174 isolates divided divided into 11 clusters and 26 singletons, with an inter-cluster allelic differences range of 25-257. All of the isolates in the outbreak cluster (C1) from the present study grouped into complex type 2933 along with five linezolid susceptible VREfm from another Dublin hospital. Complex type 2933 is highlighted in red. Isolates within complex type 2933 had an average allelic difference of three (range=0-15) All other complex types are highlighted in blue. Scale bar represents the phylogenetic distance between isolates based on cgMLST.

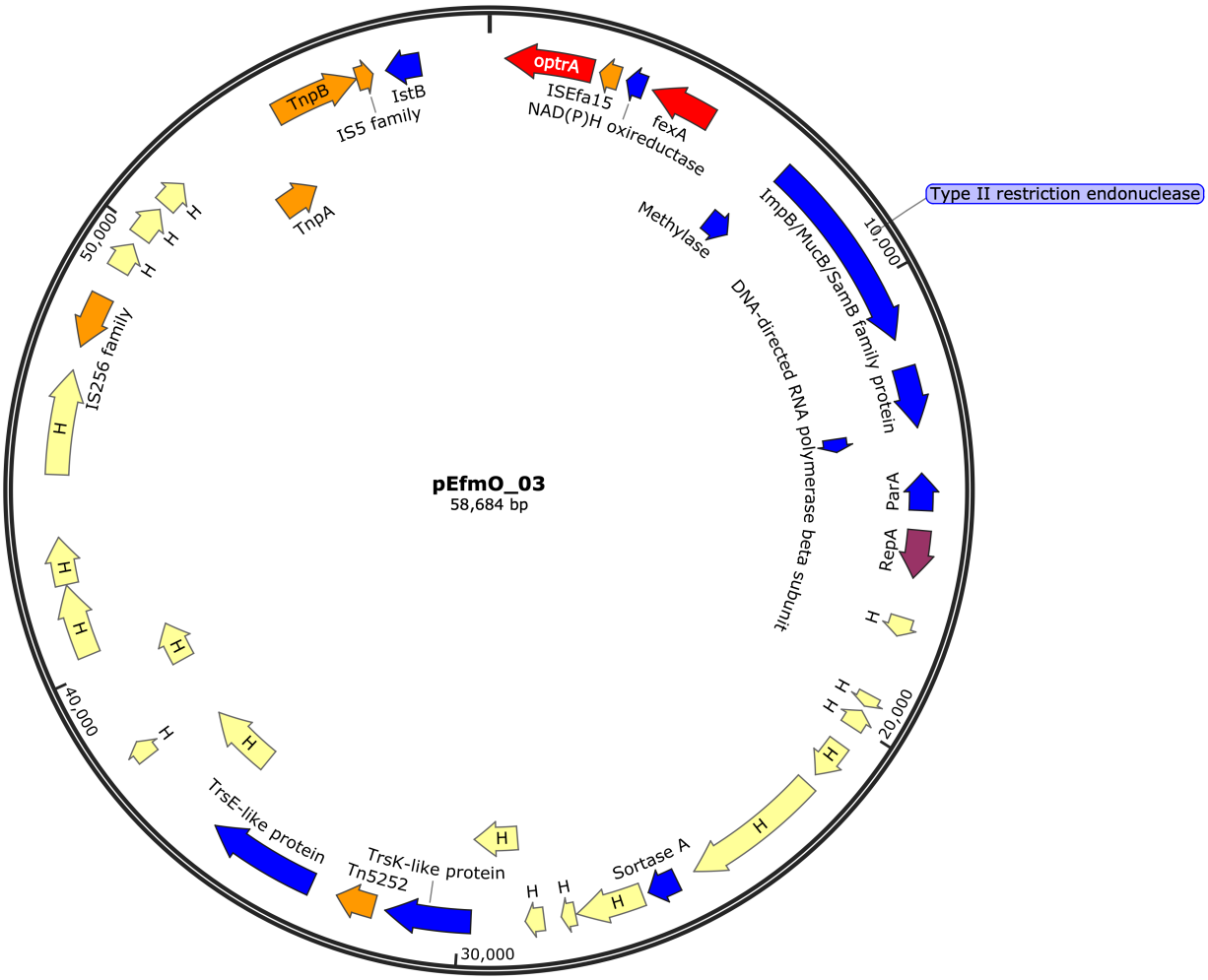


Figure S2. Schematic diagram of the structural organisation of plasmid pEfmO_03 from vancomycin-resistant *E. faecium* isolate O_03 encoding the *optrA* linezolid resistance gene resolved by hybrid assembly of paired-end Illumina MiSeq short reads with Oxford Nanopore Technologies long reads. Genes of interest and their orientation are represented by arrows as follows: red indicates antibiotic resistance genes, orange indicates insertion sequences/transposases, blue indicates known proteins and yellow indicates hypothetical proteins. The plasmid size is labelled indicating number of base pairs (bp). Abbreviations: H; hypothetical protein.