Genomic analysis of 600 vancomycin-resistant Enterococcus faecium reveals a high prevalence of ST80 and spread of similar vanA regions via IS1216E and plasmid transfer in diverse genetic lineages in Ireland

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Background: Vancomycin-resistant Enterococcus faecium (VREfm) cause a wide range of hospital infections. Ireland has had one of the highest invasive VREfm infection rates in Europe over the last decade, yet little is known about Irish VREfm.

Objectives: To investigate the population structure of Irish VREfm, explore diversity by analysing the vanA transposon region and compare Irish, Danish and global isolates.

Methods: E. faecium (n = 648) from five Irish hospitals were investigated, including VREfm [547 rectal screening and 53 bloodstream infection (BSI)] isolates and 48 vancomycin-susceptible (VSEfm) BSI isolates recovered between June 2017 and December 2019. WGS and core-genome MLST (cgMLST) were used to assess population structure. Genetic environments surrounding vanA were resolved by hybrid assembly of short-read (Illumina) and long-read (Oxford Nanopore Technologies) sequences.

Results: All isolates belonged to hospital-adapted clade A1 and the majority (435/648) belonged to MLST ST80. The population structure was highly polyclonal; cgMLST segregated 603/648 isolates into 51 clusters containing mixtures of screening and BSI isolates, isolates from different hospitals, and VREfm and VSEfm. Isolates within clusters were closely related (mean average ≤16 allelic differences). The majority (96.5%) of VREfm harboured highly similar vanA regions located on circular or linear plasmids with multiple IS1216E insertions, variable organization of vanA operon genes and 78.6% harboured a truncated tnpA transposase. Comparison of 648 Irish isolates with 846 global E. faecium from 30 countries using cgMLST revealed little overlap.

Conclusions: Irish VREfm are polyclonal, yet harbour a characteristic plasmid-located vanA region with multiple IS1216E insertions that may facilitate spread.

Introduction

Enterococcus faecium, a resident of the normal human gastrointestinal (GI) flora has emerged as an important nosocomial pathogen responsible for bloodstream-, abdominal-, urinary tract- and IV catheter-related infections.1 Acquired resistance to ampicillin, gentamicin (high level), vancomycin and linezolid has increased worldwide among hospital-associated E. faecium, narrowing treatment options.1–3 Patients colonized in the GI tract with vancomycin-resistant E. faecium (VREfm) act as VREfm reservoirs and disseminators into the hospital environment.9
Vancomycin-resistant *E. faecium* in Irish hospitals

Previous studies identified 2–10 asymptomatic VREfm carriers for each VREfm-infected patient. For over a decade, the Republic of Ireland has reported one of the highest rates of invasive VREfm infections in Europe, ranging between 32.5% and 45.8% (2006–2019).

Molecular typing of *E. faecium* requires specific approaches due to its highly recombinant genome. Typing methods that are effective for discriminating isolates of other nosocomial pathogens (e.g. MRSA) including MLST and PFGE are poorly discriminatory for *E. faecium* compared with WGS approaches, such as core-genome MLST (cgMLST). The dissemination of plasmids encoding vancomycin resistance genes (e.g. vanA) through diverse lineages presents an additional challenge in VREfm surveillance.

Previous *E. faecium* WGS studies revealed a well-defined hospital population, termed clade A1. Clade A1 isolates tend to be enriched with mobile genetic elements (MGEs), ISS and pathogenicity islands. VREfm can arise de novo from vancomycin-susceptible *E. faecium* (VSEfm) following the acquisition of a vanA-encoding MGE. WGS-based VREfm studies revealed a diverse array of genetically distinct clones (poyclonal population) circulating in hospitals, with evidence of both intra- and inter-hospital spread of particular clones, and in some instances the circulation of predominant vanA plasmids. A previous study of VREfm from Irish hospitals provided evidence of a unique vanA transposon with a truncated transposase gene in 21 isolates investigated, distinct from UK isolates.

The purpose of this study was to investigate the population structure of VREfm from Irish hospitals using WGS, to explore diversity by investigating the vanA region and to identify potential characteristic features associated with Irish VREfm. To place Irish isolates in a global context, the isolates were compared for relatedness with a large collection of international VREfm and VSEfm.

**Materials and methods**

**Isolates**

In total, 648 *E. faecium* from separate patients in five Irish hospitals were investigated. A large acute Dublin hospital (H1) was the main site for the study. H1 isolates were recovered between June 2017 and December 2019 including VREfm from rectal screening (n = 480) and all E. faecium bloodstream infection (BSI) isolates (n = 72). Isolates from four other hospitals included all VREfm screening and *E. faecium* BSI isolates recovered between October 2019 and December 2019; H2 isolates were from Dublin (n = 26), H3 from West (n = 26), H4 from South-East (n = 26) and H5 from South (n = 4) regions of Ireland. In total, 547 VREfm screening, 53 VREfm BSI and 48 VSEfm BSI isolates were investigated (Table S1, available as Supplementary data at JAC Online).

**Species identification and susceptibility testing**

Species identification and vanA/vanB vancomycin resistance gene detection was undertaken by PCR. VANcomycin and teicoplanin susceptibility were determined using the VITEK2 system (bioMérieux, Marcy-l’Étoile, France) using the EUCAST (v11.0) interpretative criteria.

**Conjugation**

Conjugative vanA-encoding plasmid transfer was undertaken by filter mating using the plasmid-free rifampicin- and fusidic acid-resistant recipient *E. faecium* strain 64/3 as described previously. Putative transconjugants were confirmed as *E. faecium* and tested for vanA/vanB by PCR.

**WGS**

All 648 *E. faecium*, the *E. faecium* 64/3 recipient strain and several *E. faecium* 64/3 transconjugants underwent WGS using MiSeq (Illumina, The Netherlands) short-read sequencing as described previously. Briefly, libraries scaled to yield ≥50× coverage were prepared using the Nextera DNA Flex Library Preparation Kit (Illumina) and underwent paired-end sequencing with the 600 cycle MiSeq Reagent Kit v3 (Illumina).

For isolates/transconjugants selected for hybrid assembly, DNA was extracted using the MagAttract HMW kit (QIAGEN, UK) and long-read sequencing performed using the MinION platform (Oxford Nanopore Technologies, UK), using MinKNOW software v1.7.10 (Oxford Nanopore), as described previously.

**Analysis of WGS data**

Quality trimming, de novo assembly using Velvet (v1.1.04) and neighbour-joining phylogenetic tree construction was undertaken in SeqSphere+ (v7.0.4, Ridom GmbH, Germany) using default settings except inclusion of the optimize coverage cut-off parameter with the *E. faecium* cgMLST scheme (1423 loci). Neighbour-joining trees were based on allelic differences using gene-by-gene allele calling against the defined core genes in the SeqSphere+. *E. faecium* cgMLST scheme. Using this approach, alleles are used instead of SNPs or concatenated sequences to mitigate the effects of recombination and to enable a global and public nomenclature (cgMLST.org Nomenclature Server; https://www.cgmlst.org/ncs). Visualization and annotation of trees was undertaken using Interactive Tree Of Life (iTOL) v5. Traditional MLST STs were extracted in silico. Clade A1 isolates were confirmed by comparison with reference clade A1 E. faecium genomes (Aus0004, DO and EB377; GenBank accession numbers: CP003351.1, CP003583.1 and LR135401.1, respectively) by k-mer distance estimation using MASH (v2.2; https://github.com/marbl/Mash).

To place Irish isolates in a global context, Irish isolates (n = 648) were compared for relatedness with 846 global clade A1 isolates [547 VREfm (304 vanA, 243 vanB) and 299 VSEfm] originating from 30 countries, spanning a 30 year period (1986–2016) from the study of van Hal et al. [Table S2] using cgMLST in SeqSphere+. Irish isolates (n = 640) were also compared with a secondary comparator group consisting of 989 VREfm [862/989 (87%) rectal screening and 27/989 (13%) infection isolates] from patients in four hospitals in Copenhagen, Denmark between 2017 and 2018. The majority (983/989; 99%) were vanA VREfm, while the remaining 6 (1%) were vanB VREfm. Danish VREfm were sequenced as previously described. Read files from both Irish and Danish isolates were trimmed with BBduk (https://sourceforge.net/projects/bbmap/) with the parameters ktrim = r, k = 23, mink = 11, hdist = 1, tbo, qtrim = r and minlength = 30. De novo assembly was performed using SKESA v2.3.1 with default settings except inclusion of the parameter –allow_snps.

**Assembly and analysis of vanA-encoding regions and plasmids**

Long-read MinION and paired-end short-read MiSeq FASTQ files were used for hybrid assemblies using Unicyclic (v0.4.8). The genetic organization of vanA-encoding plasmids from Irish isolates was determined following hybrid assembly and these were used as reference sequences for further analysis. MiSeq reads were mapped against reference plasmid sequences and percentage depth and breadth of coverage calculated using Burrows–Wheeler Aligner, SAMtools and BedTools. Alignment quality was assessed using Tablet. VanA-encoding genomes resolved by hybrid
assembly were annotated using RAST v2.0 (http://rast.nmpdr.org/)\textsuperscript{27} and visualized using SnapGene (GSL Biotech; https://snapgene.com). Irish VREfm and VSEfm sequence reads and sequences resolved by hybrid assembly have been deposited in GenBank under BioProject PRJNA734127. Danish VREfm sequence reads have been deposited in GenBank under BioProjects PRJNA573568, PRJNA688881, PRJNA691722, PRJNA702038 and PRJNA740173.

**Ethics**

Ethical approval for this project was provided by the School of Dental Science & Dublin Dental University Hospital Research Ethics Committee (Reference DSREC2020-01-02).

**Results**

**Genomic epidemiology of Irish hospital *E. faecium***

In total, 648 *E. faecium* isolates (547 screening and 101 BSI) from patients in five Irish hospitals recovered between June 2017 and December 2019 were investigated. The majority (600/648; 92.5\%) were vancomycin resistant, harbouring vanA (vancomycin MIC > 4 mg/L; 547 screening and 53 BSI), while the remaining 48 (7.4\%) (all BSI) were vancomycin susceptible (vancomycin MIC ≤ 4 mg/L). The majority of isolates were from H1 (n = 552), a large Dublin hospital, whereas the remainder were from four other hospitals (H2, H3, H4 and H5; n = 96).

All 648 isolates belonged to clade A1; the majority were identified as ST80 (435/648; 67.1\%) by conventional MLST, with the remaining belonging to ST117 (n = 23), ST203 (n = 61), ST612 (n = 34) and ST787 (n = 28). Four isolates were non-typeable due to a previously described pstS housekeeping gene deletion.\textsuperscript{28} Nine ‘novel’ isolates were submitted to pubMLST.org for ST assignment. Using cgMLST, the 648 isolates segregated into 51 clusters (≤ 24 allelic differences) and 43 singletons, with an inter-cluster allelic difference range of 22–1201. Clusters contained mixtures of screening and BSI isolates, isolates from different hospitals, and VREfm and VSEfm (Figure 1, Table S1).

The two largest clusters consisted of ST80 isolates and were defined by their predominant cgMLST complex types (CTs). Cluster CT1916 [70 VREfm, 1 VSEfm (BSI)] had an allelic difference range of 0–24 (mean 8.8) and contained screening and BSI isolates from four hospitals recovered between December 2017 and November 2019, of which 65/71 were from H1 (Table S1). Two isolates within CT1916 were identified as CT2023 (SJ47) and CT3175 (SJ266). Cluster CT2933 (59 VREfm and 5 BSI VSEfm) had an average allelic difference range of 0–22 (mean 5.5) and contained screening and BSI isolates recovered from four hospitals between May 2019 and November 2019. Two isolates (SJ55, SJ64) within CT2933 were not assigned a CT.

The majority of ST80 isolates (412/435; 94.7\%) divided into 27 clusters of ≥ 2 isolates by cgMLST (Figure S1), but clusters differed widely, with an inter-cluster allelic difference range of 26–268. Within clusters, close relatedness between screening and BSI isolates, isolates from different hospitals and between VREfm and VSEfm was evident; for example, ST80 CT1598 [intra-cluster allelic difference range 0–16 (mean 5.2)] contained 54 isolates from 14 H1 wards and three H3 isolates. CT1598 also contained one VSEfm (BSI_SJ72) with zero allelic differences to VREfm within the cluster (Figure S1).

Irish, global and Danish *E. faecium* comparison

In order to place Irish isolates in a global context, 648 Irish isolates were compared with 846 global *E. faecium* from 30 countries, which revealed very little overlap between the two groups of isolates (Figure 2). None of the Irish isolates clustered with any of the 101 isolates investigated from the UK, the closest geographic neighbour of Ireland. A small number of Irish isolates (10 in total) were identified in four clusters of related isolates consisting of both Irish and global isolates (Figure 2). Cluster 1 consisted of two isolates separated by 20 allelic differences including one Irish ST18 CT3387 VREfm and one ST18 CT173 VREfm (vanA) from Slovenia. Cluster 2 consisted of three isolates including two Irish ST262 CT3166 VREfm and one German ST262 CT1016 VREfm (vanA), which was separated by 20 allelic differences from the nearest Irish isolate. Cluster 3 consisted of 19 isolates including one Irish ST1478 CT24 VREfm and 18 global isolates from five countries with a maximum allelic difference between the isolates of 30. The global isolates in cluster 3 included four Norwegian ST117 CT24 VSEfm, one German ST117 CT24 VREfm (vanA), one German ST117 CT1020 VREfm (vanA), one German ST1486 CT178 VREfm (vanB), five Spanish ST117 CT24 isolates (two VREfm and three VSEfm), two Danish ST117 CT24 VREfm (both vanA), one ST1201 CT24 Danish VREfm (vanA), one Belgian ST117 CT24 VREfm (vanB) and two Dutch ST117 CT24 VREfm (both vanB). The closest global isolate to the Irish isolate in cluster 3 was a Norwegian isolate, which differed by seven allelic differences. Cluster 4 consisted of 22 ST203 CT20 isolates including 6 Irish isolates (5 VREfm and 1 VSEfm) and 16 global isolates from six countries. The global isolates in cluster 4 included one Norwegian VSEfm, two German VREfm (both vanA), one Danish VREfm (vanA), one Belgian VREfm (vanA), three Dutch VREfm (all vanA) and eight Australian VREfm (all vanA). The closest global isolate to an Irish isolate in cluster 4 was the Belgian isolate, which differed by four allelic differences.

*E. faecium* (n = 640) were also compared with 989 Danish VREfm recovered from four hospitals during 2017–18. Denmark was chosen as a direct comparator due to its increase in VREfm prevalence, caused by the introduction of an ST80 clone.\textsuperscript{8} The minimum spanning tree of Danish VREfm revealed 30 clusters and 37 singletons. The largest clusters were ST1421, ST203, ST80 and ST117 containing 420, 294, 107 and 56 isolates, respectively. The comparison of Irish and Danish isolates revealed 64 clusters and 69 singletons. Clusters were predominantly country-specific, with only three containing isolates from both countries (Figure S2).

**Structural characterization of the vanA region in Irish VREfm**

The extrachromosomal vanA-harbouring regions of four VREfm, SJ10 (ST789, CT1601), SJ11 (non-typeable by conventional MLST, CT24), BSI_SJ40 (ST80, CT1598) and RC_19_023 (ST80, CT1916) were resolved using hybrid assembly. The vanA regions varied in size (SJ10vanA, 11,022 bp; SJ11vanA, 13,252 bp; BSI_SJ40vanA, 13,269 bp; RC_19_023vanA, 12,883 bp) and differed from the Tn1546 vanA operon prototype from VREfm BM4147 (GenBank access number: M97297). All four vanA regions harboured large deletions in the tnpA transposase gene, mainly in the 3′ end, the extent of which varied (Figures 3 and 4). Other differences included
Figure 1. Neighbour-joining tree (NJT) based on cgMLST of 648 clade A1 E. faecium isolates, recovered between June 2017 and December 2019 from five Irish hospitals. The 648 isolates divided into 51 clusters and 43 singletons, with an inter-cluster allelic difference range of 22–1201. Clusters are shaded in grey and predominant CTs labelled accordingly. Isolates within clusters were closely related; the allelic differences between isolates in the three largest clusters were as follows: CT1916, range 0–24, mean 8.8 (71 isolates); CT1598, range 0–16, mean 5.2 (55 isolates); CT2933, range 0–22, mean 5.5 (64 isolates clustering, two not assigned a CT). Scale bar represents the phylogenetic distance between isolates based on cgMLST. Metadata are represented encircling the NJT as denoted by the colour legends, data represented are (a) STs, (b) vancomycin phenotype, (c) source of isolate, and (d) origin hospital location of isolates.*Indicates isolates SJ47 (CT2023) and SJ266 (CT3175) within ST80 CT1916 cluster.
multiple IS1216E insertions, different orientations of the vanA operon genes and in the case of SJ11 vanA, BSI_SJ40 and RC_19_023 vanA, a cadmium efflux accessory protein gene insertion (Figure 3). RC_19_23 vanA lacked vanY but remained phenotypically resistant to vancomycin and teicoplanin. Both SJ11 and BSI_SJ40 exhibited 100% sequence identity to SJ10 vanA. The vanA region was compared across all VREfm investigated and 96.6% (580/600) of isolates harboured a highly similar vanA region, as demonstrated by >90% sequence identity, indicating enrichment of IS1216E around vanA in Irish VREfm (Figure 3).

Terminal 5’ and 3’ 1000 bp sequences from the TN1546 prototype tnpA transposase gene (2967 bp) were used as references to compare the tnpA region across all VREfm. The majority (578/600; 96.3%) harbour a tnpA region with >99% sequence identity to the 5’ tnpA reference. In contrast, the majority (472/600; 78.6%) lacked the 3’ tnpA region with <40% sequence identity to the 3’ tnpA reference. These findings demonstrated the majority of VREfm harboured a tnpA gene with large 3’-end deletions.

**Plasmids encoding vanA**

Attempts to close plasmids harbouring SJ10vanA, SJ11vanA, BSI_SJ40vanA and RC_19_023vanA by hybrid assembly were unsuccessful but in each case yielded vanA on a single large contig.
Figure 3. Schematic diagram showing the structural organization of vanA transposon regions closed by hybrid assembly from VREfm isolates from Irish hospitals. (a) SJ10vanA ST789, CT1601 (11,022 bp)
(b) SJ11vanA Non-typeable, CT24 (13,252 bp)
(c) BSI_SJ40vanA ST80, CT1598 (13,269 bp)
(d) pSJ82vanA ST203, CT20 (10,823 bp)
(e) pSJ245vanA ST117, CT2929 (14,168 bp)
(f) RC-19-039vanA ST17, CT2934 (13,601 bp)
(g) RC-19-023vanA ST80, CT1916 (12,883 bp)
(h) Tn1546 vanA prototype (10,851 bp)

*Figure 3.* Schematic diagram showing the structural organization of vanA transposon regions closed by hybrid assembly from VREfm isolates from Irish hospitals. (a) SJ10 (SJ10vanA, 11,022 bp); (b) SJ11 (SJ11vanA, 13,252 bp); (c) BSI SJ40 (BSI SJ40vanA, 13,269 bp); (d) SJ82 (10,823 bp vanA region from plasmid pSJ82vanA); (e) SJ245 (14,168 bp vanA region from plasmid pSJ245vanA); (f) RC_19_039 (13,601 bp vanA region from plasmid pRC_19_039); (g) RC_19_023 (RC_19_023vanA, 12,883 bp); and (h) the structural organization of the prototype vanA transposon Tn1546 from VREfm BM6147 (GenBank accession number: M97297). VREfm isolates were recovered from H1 (a–e), H3 (f) and H4 (g). Genes and their orientation are denoted by directional arrows and labelled with corresponding gene names. A reference size scale bar is shown at the bottom of the diagram. The extent of the transposase tnpA gene sequences deleted (Δ) in the various assemblies is shown in Figure 4.
that failed to circularize [89 310 bp (SJ10), 116 749 bp (SJ11), 131 341 bp (BSI_SJ40) and 109 124 bp (RC_19_023)]. These contigs are likely to be plasmids that exhibit linear topology as reported recently.30–32 Using the SJ10 \textit{vanA} contig as a reference plasmid-like sequence, it was clear this element was highly similar throughout all VREfm, with 95.8% (575/600) exhibiting >85% sequence identity. The SJ10\textit{vanA} plasmid-like contig exhibited 99.9% and 99.8% sequence similarity to the \textit{vanA} contigs from SJ11 and BSI_SJ40, respectively. Plasmids encoding \textit{vanA} were successfully transferred from SJ11 (116 749 bp plasmid) and BSI_SJ40 (131 341 bp plasmid) to recipient strain \textit{E. faecium} 63/4 by conjugation, but not from SJ10 or RC_19_023 \textit{vanA} (see below).

Three \textit{vanA}-encoding plasmids were successfully resolved by hybrid assembly from VREfm isolates from Irish hospitals. The arrows indicate the direction of transcription. Deletions are shown in red, bp substitutions are indicated in yellow and bp insertions are indicated in green. The extent of sequences deleted (Δ) is shown beneath each \textit{tnpA} gene in bp. (a) WT \textit{tnpA} (transposase) gene (2967 bp) from the prototype \textit{vanA} transposon \textit{Tn}1546;29 (b) Δ\textit{tnpA} gene from isolate SJ10 (SJ10\textit{vanA}, 1221 bp); (c) Δ\textit{tnpA} gene from isolate SJ11 (SJ11\textit{vanA}, 1680 bp); (d) Δ\textit{tnpA} gene from isolate BSI_SJ40 (BSI_SJ40\textit{vanA}, 2925 bp); (e) Δ\textit{tnpA} gene from plasmid pSJ245\textit{vanA} in isolate SJ245 (pSJ245\textit{vanA}, 2160 bp); (f) Δ\textit{tnpA} gene from isolate RC_19_039 (RC_19_039\textit{vanA}, 1680 bp); and (g) Δ\textit{tnpA} gene from isolate RC_19_023 (RC_19_023\textit{vanA}, 1680 bp). VREfm isolates were recovered from H1 (b–e), H3 (f) and H4 (g). A reference size scale bar is shown at the bottom of the diagram.
(Figures S3–S5). Plasmid pSJ82vanA lacked a tnpA transposase gene, whereas plasmids pSJ245vanA and pRC_19_039vanA each harboured a truncated tnpA (Figures 3 and 4).

Plasmid pSJ82vanA was identified in five screening VREfm (SJ3, SJ12, SJ17, SJ77 and SJ82), all with 100% sequence coverage identity to pSJ82vanA. All five were indistinguishable by cgMLST (ST203, CT20) and were recovered from five H1 wards over 14 months (Table S1), indicating inter-ward spread and persistence over time. The vanA region of pSJ245vanA exhibited 95.5% similarity to the SJ10vanA reference, but there was much rearrangement in the vanA region in SJ245, including the insertion of ISefaS (Figure 3). Plasmid pSJ245vanA was identified in five ST117 isolates (SJ220, SJ273, SJ274, JH085 and SJ24) with >99% sequence identity, four of which were recovered within 7 days in two H1 wards and one 4 months later on another ward (Table S1). All five were indistinguishable by cgMLST and formed a phylogenetic cluster (ST117, CT2929; Figure 1). These findings indicate the spread of a single clone in H1 and highlight the usefulness of pairing cgMLST and plasmid analysis. Plasmid pRC_19_039vanA was identified in five ST17 CT2934 isolates (JH010, JH022, BSI_SJ49, RC_19_033 and RC_19_039) by >99% sequence identity. Three originated from H1 with evidence of persistence on Ward 6 from September 2018 to October 2019; the remaining two isolates were from H3 (Table S1).

To investigate whether the large transferable vanA-encoding contigs identified in isolates SJ11 and BSI_SJ40 by hybrid assembly were linear plasmids, the sequences of the contigs were compared with the corresponding sequences of linear plasmids pELF1 (143 316 bp), pELF2 (108 102 bp) and pELF_USZ (101 837 bp) recently described in VREfm isolates from Japan, 3,10 Switzerland, respectively. 11 Comparing the vanA contigs from SJ11 (116 749 bp) and BSI_SJ40 (131 341 bp) revealed a clear homology (Figure S6). Each plasmid and contig harboured genes encoding putative replication initiation proteins and proteins with a putative role in DNA partitioning and transfer. These findings strongly indicated that the vanA-encoding contigs identified in isolates SJ11 and BSI_SJ40 were linear plasmids, which were named pELF_SJ11 and pELF_BSI_SJ40, respectively (Figure S6). Details of pELF_SJ11 and pELF_BSI_SJ40 and putative genes encoding plasmid recombination and conjugation are provided in Tables S3 and S4. An alignment of pELF_SJ11 and pELF_BSI_SJ40 with the vanA-encoding contig from SJ10 is shown in Figure S7.

Transmission of vanA via IS1216E translocation and plasmid conjugation

Conjugation of vanA to the E. faecium 64/3 recipient was successful for four VREfm to the E. faecium 64/3 screens isolates investigated (SJ11 (non-typeable, CT2), SJ82 (ST203, CT20), SJ245 (ST117, CT2929) and SJ267 (ST18, CT1898)) and the BSI isolate BSI_SJ40 (ST80, CT1598). Transconjugants were phenotypically resistant to vancomycin and teicoplanin and showed the identical sequence coverage to the SJ10vanA reference as their corresponding donor isolate (81.2%–100%) (Table S5). The donor isolates SJ11, SJ267 and BSI_SJ40 and their corresponding E. faecium 64/3 transconjugant derivatives showed near identical coverage to the SJ10vanA plasmid-like contig (Table S5). Linear plasmids pELF_SJ11 and pELF_BSI_SJ40, identified in donor isolates SJ11 and BSI_SJ40, respectively, were identified in their corresponding E. faecium 64/3 transconjugant derivatives and exhibited 99.9% and 100% coverage to the respective parental linear plasmids. Plasmid pSJ245 in isolate SJ245 was also shown to be conjugal, with the corresponding E. faecium 64/3 transconjugant derivatives SJ245:Efm 64/3 TC1 and SJ245:Efm 64/3 TC2 exhibiting 100% sequence coverage to pSJ245. Both transconjugants showed 95.5% sequence coverage to the reference SJ10vanA, identical to the coverage in the donor isolate SJ245 (Table S5).

The transconjugant SJ82:Efm 64/3 TC1 underwent hybrid assembly and the vanA plasmid identified (pSJ82vanA TC) was much larger (175 921 bp) than plasmid pSJ82vanA (48 934 bp) in donor isolate SJ82 (Figure S5). Examination of the hybrid assembly of SJ28 revealed another large plasmid, termed pSJ82_B (232 026 bp), in addition to pSJ82vanA. Analysis of pSJ82_TC revealed that it was composed of the vanA operon from pSJ82vanA flanked on either side by IS1216E (9826 bp) inserted within a section of pSJ82_B (166 095 bp) (Figures 3d and 5).

Mobilization of the vanA region from pSJ82vanA into pSJ82_B was evident by the identical sequence coverage of the parent and the transconjugant to the SJ10vanA reference, with both having 81.2% sequence coverage (Table S4). The pSJ82vanA vanA region likely moved via IS1216E-mediated translocation to pSJ82_B and there was loss of 65 931 bp of pSJ82_B, giving rise to the hybrid plasmid pSJ82_TC (Figure 5).

Discussion

Ireland has consistently reported one of the highest invasive VREfm infection rates in Europe over the last decade. 7 This study used WGS analysis to determine that the population structure of E. faecium from Irish hospitals consisted of diverse clonal lineages, correlating with previous WGS-based European studies of clade A1 E. faecium, 6,7,9,33 with ST80 accounting for the majority of isolates (61.7%). The predominance of ST80 in hospital H1 (66.5%) was reflected in the four other hospitals (H2, H3, H4 and HS) (75%). Isolates belonging to the same ST determined by conventional MLST were distantly related by cgMLST (Figure 1).

A threshold of ≤20 cgMLST allelic differences has been previously proposed to describe E. faecium isolates as indistinguishable or highly related. 5,6 Clusters of related isolates could be defined by the CT to which the majority of isolates in that cluster belonged. The majority of VREfm harboured identical or highly related vanA regions, with 96.6% exhibiting >90% sequence identity to the SJ10vanA reference (Table S1, Figure 3). This finding of identical/ closely related vanA regions in genetically highly related and unrelated isolates provides a challenge for surveillance. Along with expansion of existing VREfm clones in the hospital environment, the transfer of a vanA MGE could give rise to new VREfm clones from VSEfm. The circulation of a common vanA element among diverse VREfm lineages was also observed previously in Denmark, where 81% of isolates investigated harbour an identical vanA plasmid. 8 In comparison with this Danish vanA plasmid (pV24-5), the Irish isolates had a median sequence coverage of 69.3% (range 10.8%–91.8%), indicating this plasmid has not spread widely among Irish VREfm.

Another notable feature of Irish VREfm was the persistence of clones in hospitals over extended periods, e.g. the ST80 CT1598 cluster contained highly related isolates recovered from 14 wards in H1 and three isolates from H3, recovered over 25 months.

Vancomycin-resistant E. faecium in Irish hospitals
Figure 5. Schematic diagrams of the structural organization of (a) plasmids pSJ82_B and pSJ82vanA from VREfm donor strain SJ82, and (b) the vancomycin resistance plasmid pSJ82_TC from the transconjugant SJ82:Efm 64/3 TC1. Diagrams are partially annotated for clarity. Genes of interest and their orientation are represented by arrows: red indicates antibiotic resistance genes, orange indicates ISs/transposases and blue indicates genes encoding known proteins. The regions from plasmids resolved by hybrid assembly of paired-end Illumina MiSeq short reads with Oxford Nanopore Technologies long reads present in the donor isolate SJ82 (i.e. pSJ82_B and pSJ82vanA) that are present in the hybrid transconjugant plasmid pSJ82_TC are marked as follows: pSJ82_B region highlighted in blue and pSJ82vanA region highlighted in red. Plasmid regions in pSJ82vanA and pSJ82_B shaded in black were not present in pSJ82_TC.
Conclusions

Irish VREfm are diverse yet harbour a characteristic vanA region with a truncated transposase and multiple insertions of IS1216E that may facilitate spread. The vanA region was identified on several closed conjugal plasmids and on conjugal plasmids with linear topology. A comparison of Irish, global and Danish VREfm/VSEfm showed little overlap indicating that local evolution is important in the epidemiology of VREfm. Current Irish guidelines only recommend VREfm screening of patients upon hospital admission to high-risk areas. Implementing universal VRE admission screening would very likely help to mitigate the spread.

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Supplementary data

None of the authors have any conflicts of interest to declare.

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