Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

# Escalating threat of vanA vancomycin-resistant Enterococcus revealed through integrated statewide genomic surveillance in Australia

**Marwa Talat Alhothali**
The University of Melbourne at the Peter Doherty Institute for Infection & Immunity
**Torsten Seemann**
The University of Melbourne at the Peter Doherty Institute for Infection & Immunity
**Patiyan Andersson**
The University of Melbourne
**Norelle L Sherry**
The University of Melbourne at the Peter Doherty Institute for Infection & Immunity
**Kristy Horan**
The University of Melbourne at the Peter Doherty Institute for Infection & Immunity
**Christopher H Connor**
The University of Melbourne at the Peter Doherty Institute for Infection & Immunity
**Mathilda Wilmot**
The University of Melbourne at the Peter Doherty Institute for Infection & Immunity
**Catherine Glover**
The University of Melbourne at the Peter Doherty Institute for Infection & Immunity
**Donna Cameron**
The University of Melbourne at the Peter Doherty Institute for Infection & Immunity
**Paula Roydhouse**
The University of Melbourne at the Peter Doherty Institute for Infection & Immunity
**Claire L Gorrie**
The University of Melbourne at the Peter Doherty Institute for Infection & Immunity
**Ryan Wick**
The University of Melbourne at the Peter Doherty Institute for Infection & Immunity
**Louise M Judd**
The University of Melbourne
**Benjamin P Howden**
The University of Melbourne at the Peter Doherty Institute for Infection & Immunity
**Courtney R Lane**
c.lane@unimelb.edu.au

The University of Melbourne

**Article**

**Keywords:**

**Posted Date:** November 2nd, 2026

**DOI:** https://doi.org/10.21203/rs.3.rs-11369938/v1

**License:** This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

**Additional Declarations:** No competing interests reported.

# Abstract

*vanA* vancomycin-resistant *Enterococcus faecium* (VREfm) is a major healthcare-associated pathogen. Following notification of *vanA* VRE in Victoria, Australia, statewide genomic surveillance enabled longitudinal characterisation of the VREfm population. We analysed 1618 *vanA* VREfm isolates collected over nearly six years. Overall, 85% of isolates belonged to a genomic cluster, several of which persisted over multiple years and were detected across multiple health networks. ST1424 and ST1421 predominated, while ST817 and ST1478 emerged recently. Genetic resistance determinants associated with last-line antimicrobials were identified in 18.5% of isolates, including expansion of a linezolid-resistant ST817 clone across five health networks. Long-read sequencing identified pELF-like plasmids carrying *vanA*, *optrA* and *cfr(D)* in isolates associated with a multi-hospital linezolid-resistant outbreak, while short-read screening demonstrated increasing prevalence of these elements over time. These findings demonstrate the value of statewide genomic surveillance for understanding pathogen transmission, detecting emerging resistance threats and monitoring the dissemination of clinically important mobile genetic elements.


# Background

Vancomycin-resistant *Enterococcus faecium* (VREfm) is a leading cause of hospital associated infections and is classified by the World Health Organization (WHO) as a high-priority pathogen [1]. Enterococci can acquire resistance to vancomycin through eight *van* operon clusters (*vanA, vanB, vanD, vanE, vanG, vanL, vanM*, and *vanN*). Globally, VREfm have been predominantly mediated by the *vanA* and *vanB* gene clusters[2]. In Australia, while *vanB* remains the dominant genotype in VREfm [3], the prevalence of *vanA* VREfm bacteremia have increased dramatically from 2.6% in 2013 to 26% in 2018[4], indicating its growing clinical and epidemiological importance.

In Victoria, previous genomic studies have provided important insights into the population structure of *vanA* VREfm. A statewide genomic snapshot in 2018, identified ST203, ST80 and ST1421 as the dominant sequence types (STs)[3], while subsequent studies mainly focused on outbreak investigations within individual clinical units[5,6]. Furthermore, a multi-hospital genomics study of several multi-drug-resistant organisms (MDROs) conducted in 2017–2018 revealed that *vanA* VREfm exhibited higher rates of hospital transmission than other target MDROs[7]. These findings directly informed infection control interventions and public health decision making, including changes to screening and cleaning practices, and the declaration of *vanA* VREfm as a notifiable condition by the Victorian Department of Health in 2019[7]. However, longitudinal statewide analyses capturing genomic diversity and epidemiology across multiple health networks remain limited since mandatory notification was instituted.

Despite these efforts, increasing resistance to last-line therapies presents a significant challenge. *VanA* vancomycin-resistant *Enterococcus (*VRE) exhibits high resistance to vancomycin and teicoplanin, limiting treatment options to daptomycin and linezolid[8]. Victorian isolates with reduced susceptibility or

resistance to linezolid or daptomycin are increasingly being reported[9–11]. Genomics studies from Victoria have demonstrated that *vanA* VREfm is strongly associated with daptomycin non-susceptibility through mutations in the *rpoB* gene[9,10], while linezolid resistance arises through diverse genetic mechanisms, including chromosomal mutations and acquired resistance genes such as *optrA*, *cfr* and *poxtA* which are often associated with mobile genetic elements. The emergence of resistance to both of these last line agents within hospital-adapted *Enterococcus faecium* lineages substantially narrows therapeutic options and poses a significant clinical challenge. Therefore, linezolid-resistant VRE is classified as a Critical Antimicrobial Resistance Alert (CARAlert) pathogen in Australia[12], which indicates the importance of early detection and enhanced surveillance of these high-risk lineages.

Whole genome sequencing (WGS) has become an integral component of outbreak investigations, offering high-resolution insights into pathogen genomics and antimicrobial resistance (AMR)[13]. Both global and national studies have highlighted the feasibility of integrating WGS data into routine infection and prevention control practices[7,14,15]. When WGS data is integrated into real-time surveillance programs, it can enable earlier identification of transmission clusters, thus facilitating targeted infection control responses and potentially improving patient safety and reducing overall costs[16].

Understanding the genomic epidemiology of *vanA* VREfm is crucial for identifying closely related isolates, tracking the emergence of antimicrobial resistance and informing effective infection control strategies within and between health care organisations. Here, we present a retrospective genomic analysis of all Victorian *vanA* VREfm isolates processed by the Microbiological Diagnostic Unit-Public Health Laboratory (MDU-PHL) between December 2019 and October 2025. To our knowledge, this represents the first Victorian large-scale, longitudinal genomic surveillance analyses of *vanA* VREfm, leveraging a centralized public health surveillance program that has systematically collected isolates over a nearly six-year period. Using high-resolution genomic analyses, we aim to characterize Victorian *vanA* VREfm isolates, define genomic clusters, critical resistance profiles and define the potential public health value of centralized, statewide prospective genomic surveillance of *vanA* VREfm.

# Methods

## Setting, study design and data collection

Victoria is the second most populous state in Australia, with an estimated resident population of approximately 7 million people in 2025[17]. The MDU-PHL is the primary bacterial reference laboratory for the state of Victoria and a referral centre for CARAlert pathogens[11]. MDU-PHL provides reference microbiology services for notifiable bacterial pathogens and other organisms of public health significance, including antimicrobial resistance surveillance. The laboratory also supports public health microbiology through expertise in pathogen genomics, bioinformatics and the integration of genomic and epidemiological data for outbreak investigation and surveillance[18].

As *vanA* VRE is a notifiable condition in Victoria, laboratories are required to submit identified *vanA* VRE isolates to MDU-PHL, enabling comprehensive statewide surveillance. We included all Victorian *vanA* VRE isolates that have been sequenced at MDU-PHL between December 2019 to October 2025. For patients with multiple screening isolates of the same multilocus sequence type (MLST), only the first isolate was included. Additionally, clinical isolates collected within 14 days of a previous clinical isolate of the same species/MLST from the same patient were excluded.

The reason for sample collection was not provided for many isolates; therefore, it was inferred from specimen type. Specimen types of “rectal swab” or “faeces” were reported as “screening”, and all others were reported as “clinical”. Isolates collected from environmental screening were categorized as “environmental” while isolates without specimen type specified were categorized as “unknown”.

Whole genome sequencing, assembly and quality control

Upon sample receipt at MDU-PHL, a single colony was selected for subculture on horse blood agar and after overnight incubation, 1–2 colonies were selected and placed in lysis buffer for sequencing as previously described[19]. Genomic DNA was extracted from bacterial isolates using the QIAsymphony Virus/Pathogen DSP mini kit (Qiagen) or Chemagic360 virus DNA kit (PerkinElmer). Genomic DNA libraries were then prepared using Nextera XT. Whole genome sequencing was performed on the Illumina Nextseq500 / 550 instrument using 300 cycle kit for 150 bp paired-end reads. Raw reads were trimmed with fastp v0.22.0[20] with the parameters cut_front 3, sliding window size 4, quality 20 and minimum length of 26[21]. Only isolates with an estimated sequencing depth of greater than 40X were retained for further analysis. *De novo* genome assemblies were generated using Shovill v1.4.2[22] with default settings. Assembly summary statistics were generated with SeqKit v2.12.0[23] (seqkit stats) and contigs less than 500bp in size were removed. Assemblies were retained if their total genome size was within the expected range for *Enterococcus faecium* (2.7- 3.3Mbp) and their total contig count did not exceed the 99th percentile (362) of the dataset. Kraken v2.17.1[24] with the GTDB representative genomes database (k2_gtdb_genome_reps_20250609) was used for species identification and potential contamination detection. Species identification was assigned according to the *Enterococcus* species with the highest proportion of kraken2-classified reads, with taxonomic profiles also examined for evidence of mixed-species contamination.

Determination of genomic clusters

Genomic relatedness of isolates was determined with SKA v1.0[25] on trimmed sequencing reads. The *k*mer length was set to 19 and a minor allele frequency filter of 0.01 was applied as described previously[21]. Pairwise single nucleotide polymorphisms (SNPs) distances were generated using SKA distance subcommand, and clusters were defined by hclust function within the stats R package v4.4.1[26] using average-linkage hierarchical clustering, and a cut-off of 15 SNPs, similar to previously applied thresholds[16,27,28].

To determine the relationships between major genomic clusters, pairwise SNP distances between isolates were summarized at the cluster level. For each pair of genomic clusters, the between-cluster distance was calculated as the mean pairwise SNP distance between all isolates within the two clusters. These mean distances were used to construct a cluster-level distance matrix and average-linkage hierarchical clustering was performed with hclust in R. The resulting dendrogram was used for visualization of relative genetic similarity among clusters in Fig. 2.

*In silico* typing and detection of antimicrobial resistance determinants and associated plasmids.

STs were assigned using mlst v2.23.2[29]. AMR genes were identified with abritAMR v1.0.20[30], with the species being set to *Enterococcus faecium*, to enable detection of species-specific AMR point mutations. To estimate the presence and copy number of linezolid resistance associated mutations in the 23S rRNA gene (e.g., G2576T or G2505A), LRE-finder v1.0 [31] was run on the raw sequencing reads. Mutations in the *rpoB* gene resulting in the RpoB substitutions S491F, G482D and H486Y [10], which are associated with daptomycin resistance, were identified by aligning the sequencing reads to the annotated *E. faecium* Aus0085 reference genome (NCBI:CP006620)[32] using Snippy v4.4.5 with default settings[33]. Putative plasmids were predicted from genome assemblies using MOB-suite v3.1.9[34]. Contigs carrying the *vanA* gene were linked with abritAMR results to determine their putative location on chromosome or plasmid.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing (AST) was performed for a subset of isolates using broth microdilution with the Thermo Scientific™ Sensititre™ Gram Positive (GPN3F) MIC Plate, which includes a range of clinically relevant antimicrobials including vancomycin and linezolid. To test for teicoplanin sensitivity, E-test teicoplanin strips (bioMérieux) was used according to the manufacturer's instructions. MIC values were interpreted according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) clinical breakpoints v16.1.[35]

Long-read sequencing, linear plasmids curation, annotation and clustering

Thirteen ST817 isolates carrying the *vanA* gene and representing different combinations of linezolid resistance genetic determinants were selected for long-read sequencing with Oxford Nanopore Technologies (ONT). Isolates were cultured overnight on horse blood agar and DNA was extracted by first lysing bacteria with PowerBead Pro tubes (QIAGEN) in a Precellys followed by spin column DNA purification with Monarch Spin gDNA Extraction Kit (NEB). Ligation libraries were prepared (ONT, SQK-NBD114-96) and sequenced on MinION flow cells (ONT, R10.4.1). Basecalling was performed using Dorado v7.11.2 with the Super-accurate model version dna_r10.4.1_e8.2_400bps_sup@v5.2.0

Reads were filtered with chopper v0.11.0[36] to retain reads with minimum quality score of 10 and minimum length of 1000bp. Filtered reads were assembled with Autocycler v0.6.2[37] using the autocycler_full.sh pipeline. Linear plasmids predicted with Autocycler were visualized and manually

curated with Bandage v0.8.1[38] to resolve divergent paths following the protocol described in Autocycler's documentation[39]. To ensure maximum terminal sequence recovery, genomes were reassembled with Unicycler v0.5.1[40] and the longest resolved linear plasmid was used for further analysis as previously described[41]. All assemblies underwent short-read polishing using Polypolish v0.6.1[42] and pypolca v0.4.0[43]. Quality of assemblies were checked with QUAST v5.3.0[44]. Two isolates were excluded from further analysis because of unresolved assemblies. Complete plasmids were annotated with Bakta v1.12.0[45].

Structural similarity between complete *vanA*-carrying plasmids was assessed using Pling v3.0.2[46] which combines containment distance (shared sequence content) and Double Cut and Join Indel (DCJ-indel) distance (structural evolutionary distance) to infer clusters of related plasmids. Plasmids were grouped into communities according to the default containment distance threshold of 0.5 and a DCJ-indel distance of 4 and a network visualization was generated.

Screening for pELF-like linear plasmids with short reads

To estimate the prevalence of pELF-like plasmids across the dataset, paired-end short-read data were screened using pELF-Finder v0.1.0[47] Python-based software developed in this study to implement and standardize a previously described screening approach[48]. Briefly, pELF-Finder aligns reads to the characteristic pELF1 terminal ends including the hairpin end (position 1 to 799) and the inversion end (position 142792 to 143316) regions, derived from pELF1 (GenBank accession LC495616.1) using Bowtie2 v2.5.5.[49] Then, it uses SAMtools v1.23.1[50] to count the number of reads aligned the two end regions. Isolates with more than 5 reads mapped to both reference hairpin end and inversion end regions were classified as pELF positive.

Data visualization

Figures were generated in R v4.4.1[26] using the following packages: ggplot2 v4.0.1[51], ComplexUpset v1.3.3[52] and patchwork v1.3.2[53]. Plasmid network visualisations were generated with pling and visualized with Cytoscape v3.10.3[54]. Plasmid comparison figure was created with GenoFig v1.1[55].

**Ethics approval**

All data were collected in accordance with the Victorian Public Health and Wellbeing Act 2008. Ethical approval was obtained from the Human Research Ethics Committee, University of Melbourne (Project ID 30320).

# Results

Characterization and temporal distribution of Victorian *vanA* VRE isolates

Between December 2019 and October 2025, a total of 1631 *vanA* VRE isolates were submitted to the MDU-PHL for genomic surveillance. Isolates were received from 39 Victorian health networks, including 32 public health networks and seven private health networks. Participating networks included all major metropolitan tertiary referral centres and multiple regional health services, providing broad statewide coverage.

While most isolates were identified as *E. faecium* (99.2%,1618/1631), a small number belonged to other *Enterococcus* species, including *Enterococcus faecalis* (n = 11), *Enterococcus lactis* (n = 1) and Enterococcus *raffinosus* (n = 1) (Supplementary table S1). Subsequent genomic analysis exclusively used *E. faecium* isolates only.

Monthly non-duplicate *vanA* VREfm isolate counts increased from 18 in Dec 2019 to 56 in September 2025 (Fig. 1, A). As implementation of notification requirement coincided with the onset of the COVID-19 pandemic, when capacity for compliance may have been reduced, MDU-PHL distributed a letter to all Victorian diagnostic laboratories in May 2022 reinforcing isolate submission for genome sequencing. The increase in submissions following that was observed across both screening and clinical isolates, with clinical isolate numbers remaining elevated during the later years of the surveillance period (Fig. 1, B).

Isolates were recovered from a range of specimen types including urine, blood, wounds and faecal samples. Overall, 56.8% (920/1618) of isolates collected were clinically indicated, while 41.2% (667/1618) were obtained through screening. The reason for sample collection varied across health networks, with isolates from Health networks N, T, G and X predominantly collected through screening, whereas isolates from health networks S and L were primarily clinically indicated (Fig. 1, B) (Supplementary Table S2).

Among *E. faecium* isolates, 32 defined sequence types (ST) were identified. The dominant ST was ST1424 (62.4%, n = 1009/1618), followed by ST1421 (11.5%, n = 186/1618), ST1478 (6.2%, n = 100/1618), ST817 (5.2%, n = 84/1618) and ST80 (5.1%, n = 83/1618). Notably, ST817 and other emerging STs including ST1478, were detected more frequently during later years of the surveillance period (Fig. 1, A).

SKA-based genomic clustering of *vanA* VREfm isolates

Using a 15 SNP threshold, we found that 85.7% of isolates clustered with at least one other isolate. In total, 94 genomic clusters were identified, with a median pairwise SNP distance of 8 SNPs between clustered isolates. Clusters 1 and 118 were mostly comprised of isolates from health network N, while cluster 95 was predominantly composed of isolates from health network L. In contrast, larger clusters such as 3, 146, 174, 175 contained isolates originating from multiple different health networks (Fig. 2).

Cluster persistence varied over time. Isolates belonging to cluster 3 (ST1424) were continuously detected throughout the surveillance period whereas clusters 161 (ST817), 171 (ST1424), 172 (ST1478),

and 175 (ST1424) emerged more recently from mid-2023 onwards. Environmental samples collected from Health network N were clustered with patient isolates in cluster 1 (ST1424), 34 (ST2681) and 57 (ST1424) while the ones collected from Health Network U were in cluster 161 (ST817) (Fig. 2).

Prevalence of AMR genetic determinants and mobile genetic elements (MGEs)

Phenotypic susceptibility data was available for a subset of the VREfm isolates, including all isolates that reported to be linezolid resistant by the referring laboratory and a convenience sample of additional isolates. Phenotypically tested isolates were temporally distributed over the study period (Supplementary Fig. 1). Among the 93 isolates tested for vancomycin susceptibility, all but one isolate were resistant, with MIC values ranging from > 4 mg/L to > 128 mg/L. All 10 isolates tested for teicoplanin susceptibility were resistant, with MIC values between 12 mg/L and > 256 mg/L (Supplementary Table S3).

Overall, 18.5% (n = 300/1618) of *E. faecium* isolates harboured at least one genetic determinant associated with reduced susceptibility to daptomycin and/or linezolid. Linezolid resistance-associated genetic determinants were identified in 7.2% (117/1618) of all VREfm isolates. Among the 92 isolates with phenotypic testing of linezolid susceptibility, 46 (50%) were resistant (MICs > = 8 mg/L) (Supplementary Table S3) and all resistant isolates carried at least one linezolid resistance associated genetic determinant. However, 6 of the 46 phenotypically susceptible isolates also carried a linezolid resistance associated genetic determinant (Fig. 3).

The most frequently identified resistance determinant was the daptomycin associated RpoB S491F substitution, detected in 10.8% (n = 175/1618) of VREfm isolates. The most common resistance profile involved the linezolid-associated genetic determinants G2576T 23S rRNA mutation, *optrA and cfr(D)* genes together with the daptomycin-resistance associated RpoB S491F substitution (Fig. 3). These isolates were predominantly found among the ST817 cluster 161.

All isolates were predicted to carry one or more plasmids by MOB-suite analysis. Contigs carrying the *vanA* gene identified from abritAMR output were located on predicted putative plasmids in 97.3% (1575/1618) of *vanA* VREfm isolates.

Genomic and phenotypic characterization of a linezolid-resistant ST817 *vanA* VREfm cluster 161

In January 2024, MDU-PHL detected an increase in the number of ST817 *optrA*-carrying *vanA* VREfm isolates from a single health network U (Fig. 2). Following detection of this resistant phenotype, classified as a CARAlert pathogen, MDU-PHL notified the affected hospital and the Victorian Department of Health to initiate further investigation. Based on epidemiological investigations by the affected health network, a linezolid-resistant *vanA* VREfm outbreak was subsequently declared.

The increase in linezolid-resistant ST817 isolates prompted further genomic and phenotypic investigation. A total of 57 ST817 isolates were found to be carrying the *optrA* gene and/or *cfr(D)* or G2576T 23S rRNA mutation, and 36 of which underwent linezolid susceptibility testing, and all were

resistant. Notably, all those isolates also co-carried the daptomycin resistance associated RpoB S491F substitution (Fig. 3). As a part of the outbreak investigation, three environmental samples were collected from the rim of toilet (n = 2) and a bedside table (n = 1) of a single room at the affected hospital and were submitted for sequencing. Our genomic analysis confirmed that environmental isolates clustered with the outbreak-associated patient isolates and those isolates were a part of a broader genomic cluster designated as cluster 161 in this study. This cluster subsequently expanded beyond the index health network, with related isolates detected across five additional health networks by May 2024 and continued to be detected until May 2025 (Fig. 2, inset plot).

Co-localization of linezolid resistance genes and *vanA* gene cluster on pELF-like linear plasmids

To investigate the genomic context of the *vanA* gene operon and linezolid resistance genes *optrA* and *cfr(D)*, 11 of the ST817 VREfm isolates representing different resistance profiles, genomic clusters, and singleton isolates were selected for long-read sequencing (Supplementary Table S4). These included isolates carrying linezolid resistance determinants as well as isolates carrying the *vanA* gene only. In all 11 isolates, the *vanA* gene cluster was plasmid-associated and located on either circular or linear plasmids. Based on structural similarity analysis using pling, the *vanA*-carrying plasmids formed two major communities with a containment distance between 0.00 and 0.1 and a high structural similarity with DCJ-indel between 0 and 4. The three circular *vanA* plasmids were clustered within community 1 (Fig. 4, A), while 7 of the 9 linear *vanA* plasmids formed a distinct community 2 (Fig. 4, B); the remaining two linear plasmids were singletons.

Among *vanA* plasmids from isolates belonging to cluster 161, the linezolid resistance genes *cfr(D)* and/or *optrA* were co-located with the *vanA* gene on linear plasmids ranging from 111,005 bp to 145,515bp in size. Blast analysis demonstrated that these linear plasmids were highly similar to previously described pELF-like linear plasmids reported from Canada (accession CP197454.1) (Fig. 4, C), India (accession CP072588.1), and Italy (accession PP566944.1), sharing 84% query coverage and 99.9% nucleotide identity. One of the singleton linear plasmids, from isolate DMG2600921, was more similar to plasmids from India (CP059807.1 and CP059785.1). This isolate carried only the *vanA* and *cfr(D)* gene and lacked the *optrA* gene. In isolate DMG2600920, the *vanA* gene was located on a circular plasmid (28,489 bp) while the *cfr(D)* was located on the linear plasmid (95029 bp). This linear plasmid shared 84% query coverage with ~ 99.8% similarity with pELF-plasmids from the USA (CP086645.1 and CP115972.1).

Prevalence of pELF-like plasmids among Victorian *vanA* VREfm isolates

We screened all available short-read sequencing data from Victorian *vanA* VREfm isolates collected between 2020 and 2025 to identify putative pELF-like plasmids. The overall prevalence was 11% (178/1618) among VREfm isolates. The proportion of isolates carrying a putative pELF-like plasmid increased from 3.3% in 2020 to 18.4% in 2024, before declining to 10.4% in 2025 (Supplementary Fig. 2). However, the 2025 estimate may underestimate the true annual prevalence, as the dataset only included isolates collected up to October 2025. Putative pELF-like plasmids were identified predominantly among

isolates carrying *optrA* and/or *cfr(D)* or *cfr* linezolid resistance genes. Among the 124 pELF-positive isolates harbouring at least one daptomycin or linezolid resistance genetic determinant, 94 (75.8%) carried one or more linezolid resistance gene. However, pELF-like plasmids were also detected in 30 of 124 (24.2%) pELF-positive isolates lacking known linezolid resistance genes (Fig. 3). Notably, pELF-positive isolates were distributed across multiple genomic clusters and were not restricted to the outbreak-associated ST817 cluster 161.

# Discussion

Almost six years of statewide genomic surveillance revealed a substantial and sustained increase in *vanA* VREfm across Victoria, accompanied by the expansion of multiple genomic clusters and the emergence of resistance to last-line antimicrobials. Although improved ascertainment following mandatory notification and reinforcement of isolate submission requirements likely contributed to this increase, the continued rise in clinical isolate numbers together with the persistence and expansion of multiple genomic clusters suggest ongoing transmission also played an important role. COVID-19 pandemic-related disruptions to antimicrobial stewardship and infection prevention and control programs may have facilitated the acceleration of AMR, potentially further explaining the rise in isolate counts from mid-2022[56].

The proportion of isolates derived from clinical specimens versus screening varied substantially between health networks, reflecting differences in local sampling and surveillance practices. For example, isolates collected from health networks S and L were mostly clinically indicated. This reliance on clinical samples alone may lead to overlooking important reservoirs of VRE, thereby reducing the effectiveness of infection control measures[27]. In contrast, incorporating rectal screening isolates into genomic surveillance framework has been shown to improve detection of VRE transmission and better inform targeted infection prevention interventions[27,57]. Therefore, standardisation and optimisation of VRE screening practices across health networks, while recognising resource constraints of individual health care facilities, may improve the interpretability and impact of genomic surveillance data.

In this study, most *vanA* VREfm isolates belonged to the *pstS-null* sequence types ST1424 and ST1421 consistent with the expansion of these *vanA*-associated STs in Australia since 2016[58]. Notably, the largest genomic cluster identified in this study (cluster 175) emerged in mid-2023 and was predominantly composed of ST1424 isolates. This cluster may represent an expansion of a descendent sublineage arising from an existing ST1424 population already circulating within Victoria. In contrast, ST1478 and ST817 represent more recently emerging sequence types within the Victorian VREfm population. The emergence of ST1478, which is also a *pstS*-null, mirrors observations from Canada where this ST has also increased in prevalence[59,60]. In parallel, ST817 emerged during recent years of the surveillance and was associated with a multi-healthcare-network linezolid-resistant outbreak. Collectively, this highlights the evolving population structure of the Victorian VREfm and demonstrates the capacity of new lineages to establish and expand within healthcare networks.

MLST is commonly used to describe population structure,[61] however, frequent recombination events affecting the MLST loci, and the high genome plasticity of *E. faecium*, limits the sensitivity and accuracy of this method [62]. Core genome MLST (cgMLST) provides greater discriminatory power and can be used to group genetically related isolates, but may not always provide sufficient resolution to robustly identify putative transmission[63]. Higher resolution analysis is commonly performed using pairwise SNP distances derived from reference-based core genome alignments[63]. However, reference-based SNP distances can be influenced by reference selection and the genetic diversity of the dataset[64]. To overcome these limitations, we applied a reference-free split kmer-based approach (SKA) to estimate pairwise SNP distances between isolates. SKA is rapid, computationally efficient, and easy to implement, making it well suited for routine genomic surveillance[21].

We observed that 85% of *vanA* VRE isolates belonged to genomic clusters. Several clusters persisted for multiple years and were detected across multiple health networks, suggesting sustained circulation of successful VREfm lineages within the healthcare system. This persistence may be facilitated by prolonged asymptomatic gastrointestinal carriage and the ability of VRE to survive in healthcare environments[65,66], while inter-institution patient movement has been identified as a key driver of multi-institution dissemination of VRE[67]. Detecting these patterns of persistence and dissemination, therefore requires coordination of genomic surveillance at the State level.

The predominance of the RpoB S491F substitution in our collection mirrors recent global observations identifying it as the most common daptomycin resistance-associated RpoB substitution across diverse VREfm sequence types and geographic locations[10]. More broadly, the detection of resistance-associated mutations across multiple genomic clusters along with the substantially higher proportion of isolates with linezolid resistance-associated genetic determinants (7.2%) compared with previous Victorian estimates (0.26%)[10], suggests an increasing accumulation of resistance to last-line antimicrobials within the Victorian VREfm population. This trend is particularly concerning given the already limited therapeutic options for VRE.

The most common resistance combination, comprising the *optrA* and *cfr(D)* genes, the G2576T 23S rRNA mutation and the RpoB S491F substitution, was predominantly associated with a single cluster (cluster 161). This high-risk clone exhibited sustained clonal expansion over a nearly 2-year period and was detected across five health networks. At the health network where this cluster was initially identified, environmental isolates were found to be closely related to patient isolates indicating a role of hospital environment in VRE spread, consistent with observations from local and international VRE transmission studies in hospital settings[68–70]. Enhanced environmental cleaning including bleach-based and hydrogen peroxide vapour-based decontamination has been associated with reduced VRE acquisition and resolving VRE outbreaks[71,72]. These findings reinforce the importance of environmental cleaning as a part a multifaceted strategy for controlling VRE transmission in healthcare settings.

Both the *vanA* and *optrA* genes are frequently plasmid-borne in enterococcal species[11,73–75]. Consistent with this, the *vanA* gene was predicted to be located on a putative plasmid in 97.3% of VREfm isolates. Long-read sequencing of a small subset of ST817 isolates confirmed that the *vanA* gene was carried on either a small circular plasmid or a linear one. We identified pELF-like plasmids co-carrying the *vanA*, *cfr(D)* and *oprtA* genes in isolates associated with a multi-hospital linezolid-resistant *vanA* VREfm outbreak. Since the first report of pELF-plasmids in Japan in 2019[74], similar plasmids have been reported in the USA[76], India[77], Italy[78] and Canada[48]. The pELF-like plasmids identified in this study shared > 99% nucleotide identity with a linear plasmid identified in a Canadian ST817 isolate collected prior to the initial detection of the Victorian outbreak-related isolates and showed high similarity (> 99%) with other pELF-like plasmids reported internationally, highlighting the potential role of linear plasmids in disseminating linezolid resistance genes globally.

Putative pELF-like plasmids were identified in 11% of Victorian *vanA* VREfm isolates based on short-read screening, with prevalence increasing from 3.3% in 2020 to 18.4% in 2024. Although this prevalence is lower than that reported in Canada which reached 72% in 2024, the upward trend of these elements suggests that they are becoming more established within the local Victorian VREfm population. Their detection across multiple genomic clusters, together with evidence that pELF-like plasmids exhibit elevated horizontal self-transmissibility, have a limited fitness cost,[79] and confer a nutrient utilisation advantage to the host strain[80], raises concern about their ongoing spread. The high similarity of the Victorian pELF-like plasmids to those reported globally further supports the role of these elements in the dissemination of clinically important AMR genes. These findings, therefore, are relevant beyond the Victorian setting, highlighting the importance of monitoring of pELF-like plasmids as a part of the ongoing surveillance programs both locally and globally. To facilitate standardized surveillance of these emerging mobile genetic elements, we developed pELF-Finder, an open-source tool that implements the short-read screening approach used in this study, enabling rapid and reproducible detection of pELF-like plasmids from Illumina sequencing data and enabling their surveillance across different settings and jurisdictions.

In the current *vanA* VRE genomic surveillance framework, genomic data routinely communicated to the Victorian Department of Health are limited to basic descriptors such as sequence types, with detailed cluster analyses performed only upon request. For example, when the expansion of a high-risk resistance phenotype is suspected, as observed for the linezolid-non-susceptible cluster described in this study. From a public health perspective, our findings inform ongoing efforts to integrate statewide genomic surveillance into routine infection prevention and control practice for *vanA* VRE. Given the centralized nature of this surveillance program, systematic and prospective reporting of genomic clusters could facilitate earlier detection of multi-institutional *vanA* VRE spread and enable timely and coordinated responses across affected health networks, informing the prioritization of infection prevention and control efforts in settings experiencing ongoing transmission.

There is an increasing interest and need to transition *vanA* VRE genomic surveillance toward routine and prospective reporting. Australia already has an established national infrastructure for genomic data sharing through AusTrakka[81], which provides a scalable platform for communicating genomic cluster information to public health authorities and healthcare institutions in real time. It has been successfully used during the COVID-19 pandemic to identify inter-jurisdictional transmission and consequently informing critical public health decisions such as border closures.[82]

Similar genomics-informed statewide surveillance approaches have also been successfully applied to other high-priority healthcare-associated pathogens, including carbapenemase-producing organisms (CPOs) and *Candida auris (C. auris)*. For example, the centralised and prospective genomic and epidemiological surveillance program for CPOs in Victoria has been effectively implemented to identify transmission networks, support outbreak investigations and inform infection prevention and control interventions[83]. In parallel, national initiatives have also been established to support coordinated genomic surveillance of emerging pathogens such as *C. auris*[84]. These experiences demonstrate the feasibility of incorporating genomic data into public health surveillance frameworks.

Detailed epidemiological investigations of CPOs or *C. auris* are currently undertaken for every individual case. This “search and contain” approach is resource intensive but feasible because these pathogens remain low prevalence and are often associated with serious clinical and public health consequences[83]. On the other hand, the higher incidence of *vanA* VRE in Victoria makes case-level investigation impractical and unsustainable. However, linkage to administrative datasets, such as the Victorian Admitted Episodes dataset (VAED)[85] may enable the identification of time and location of likely transmission events, allowing initiation of targeted epidemiological investigations and infection control measures. Feasibility of linkage to VAED data has previously been demonstrated for CPO surveillance in Victoria where it improved efficiency of epidemiological data collection (Epidemiologist Mathilda Wilmot, personal communication, July 2026).

Leveraging existing surveillance infrastructure such as that developed through AusTrakka, while developing VRE-specific frameworks for genomic data reporting and infection control, may support earlier hospital level investigations to identify transmission clusters and high-risk settings. In this context, the findings of this study provide an evidence base to inform the development of the Victorian *vanA* VRE guidelines, including standardization of screening practices and interpretation of genomic data. Standardised guidance across hospitals may improve the quality of surveillance, enhance comparability of genomic findings and strengthen institutional infection prevention and control responses.

This study has some limitations. First, for isolates where the reason for sample collection was missing, it was inferred from the specimen source, which may have resulted in misclassification between clinical and screening samples. Second, phenotypic antimicrobial susceptibility data were available for only a subset of isolates, limiting direct correlation between genotypic and phenotypic resistance profiles and the representativeness of the phenotypic data. Third, detailed epidemiological metadata, including

patient location at time of sample collection, bed movements, inter-facility transfer information and travel history were not available, precluding patient-to-patient transmission analysis or detailed investigation of outbreaks. Finally, the use of short read sequencing data for most isolates restricted our ability to resolve the genetic context of resistance genetic determinants and to assess the role of mobile genetic elements in the dissemination of *vanA* VRE across the statewide dataset. Long-read sequencing was performed only for a small subset of outbreak-associated ST817 isolates, which is not representative of the broader Victorian VREfm population.

In summary, this study illustrates how genomic data generated through centralized surveillance can be leveraged to better understand the epidemiology of *vanA* VRE at the state level. Although retrospective, the identification of persistent multi-institution clusters and emergence of resistance to last-line agents on plasmids, highlights opportunities where timely and prospective reporting of genomic information could support early investigation and coordinated responses across health networks.

## Declarations

# Competing interests

The authors declare no competing interests.

# Author Contribution

MTA, TS, PA, BPH and CRL conceptualised and designed project. PS and LJ performed laboratory testing and sequencing, supervised by NS. MTA and TS performed and reviewed all bioinformatic analysis. CLG, RW, CHC and KH advised on genomic analysis and reviewed long read sequencing results. MTA and CRL performed and reviewed all epidemiological analysis. MW, CG, NS and DC provided epidemiological data and advised on outbreak investigation and public health implementation. MTA performed all data management, visualisation and drafted initial manuscript. CRL, BPH, TS and PA reviewed and revised manuscript. All authors reviewed the final manuscript. Courtney R Lane and Benjamin P Howden contributed equally to this work.

# Acknowledgement

The authors would like to thank the Victorian diagnostic microbiology laboratories for sending isolates as part of the Victorian state surveillance program. We would also like to thank the laboratory staff at MDU-PHL for receiving, culturing and sequencing these isolates analysed in this study. MDU-PHL is funded by the Department of Health, Victoria. BPH and NLS received Investigator Grants (GNT1196103 to BPH, GNT2033803 to NLS) from the National Health and Medical Research Council, Australia. MTA is a recipient of an educational scholarship from the Saudi Public Health Authority.

# Data Availability

All short and long raw sequencing reads analysed in this study have been submitted to NCBI with the associated accession numbers listed in supplementary table S5.

# References


1. Markwart, R., Willrich, N., Haller, S., Noll, I., Koppe, U., Werner, G., Eckmanns, T., & Reuss, A. (2019). The rise in vancomycin-resistant Enterococcus faecium in Germany: Data from the German Antimicrobial Resistance Surveillance (ARS). *Antimicrobial Resistance & Infection Control*, *8*(1), 147. https://doi.org/10.1186/s13756-019-0594-3

2. Mirzaie, S., Faghiri, I., Askari Badouei, M., & Madani, S. A. (2023). Molecular detection and occurrence of vancomycin resistance genes (van A, B, C1, C2/C3) among Enterococcus species isolated from farm ostriches. *Veterinary Medicine and Science*, *9*(1), 226–233. https://doi.org/10.1002/vms3.1010

3. Lee, R. S., Gonçalves da Silva, A., Baines, S. L., Strachan, J., Ballard, S., Carter, G. P., Kwong, J. C., Schultz, M. B., Bulach, D. M., Seemann, T., Stinear, T. P., & Howden, B. P. (2018). The changing landscape of vancomycin-resistant Enterococcus faecium in Australia: A population-level genomic study. *Journal of Antimicrobial Chemotherapy*, *73*(12), 3268–3278. https://doi.org/10.1093/jac/dky331

4. *AGAR Surveillance Outcome Programs 2022 Report | Australian Commission on Safety and Quality in Health Care*. (n.d.). Retrieved January 23, 2024, from https://www.safetyandquality.gov.au/publications-and-resources/resource-library/agar-surveillance-outcome-programs-2022-report-0

5. Douglas, A. P., Marshall, C., Baines, S. L., Ritchie, D., Szer, J., Madigan, V., Chan, H. T., Ballard, S. A., Howden, B. P., Buising, K., & Slavin, M. A. (2019). Utilizing genomic analyses to investigate the first outbreak of vanA vancomycin-resistant Enterococcus in Australia with emergence of daptomycin non-susceptibility. *Journal of Medical Microbiology*, *68*(3), 303–308. https://doi.org/10.1099/jmm.0.000916

6. Hughes, A., Ballard, S., Sullivan, S., & Marshall, C. (2019). An outbreak of vanA vancomycin-resistant Enterococcus faecium in a hospital with endemic vanB VRE. *Infection, Disease & Health*, *24*(2), 82–91. https://doi.org/10.1016/j.idh.2018.12.002

7. Sherry, N. L., Gorrie, C. L., Kwong, J. C., Higgs, C., Stuart, R. L., Marshall, C., Ballard, S. A., Sait, M., Korman, T. M., Slavin, M. A., Lee, R. S., Graham, M., Leroi, M., Worth, L. J., Chan, H. T., Seemann, T., Grayson, M. L., Howden, B. P., & Controlling Superbugs Study Group. (2022). Multi-site implementation of whole genome sequencing for hospital infection control: A prospective genomic epidemiological analysis. *The Lancet Regional Health. Western Pacific*, *23*, 100446. https://doi.org/10.1016/j.lanwpc.2022.100446

8. Iqbal, F., Alocious, A., Joy, S. C., Stanly, E. A. R., Rajesh, V., Unnikrishnan, M. K., Steinke, D., & Chandra, P. (2024). Vancomycin-resistant enterococci: A rising challenge to global health. *Clinical Epidemiology and Global Health*, *28*, 101663. https://doi.org/10.1016/j.cegh.2024.101663

9. Li, L., Higgs, C., Turner, A. M., Nong, Y., Gorrie, C. L., Sherry, N. L., Dyet, K. H., Seemann, T., Williamson, D. A., Stinear, T. P., Howden, B. P., & Carter, G. P. (2021). Daptomycin Resistance Occurs Predominantly in vanA-Type Vancomycin-Resistant Enterococcus faecium in Australasia and Is Associated With Heterogeneous and Novel Mutations. *Frontiers in Microbiology*, *12*. https://doi.org/10.3389/fmicb.2021.749935

10. Turner, A. M., Li, L., Monk, I. R., Lee, J. Y. H., Ingle, D. J., Portelli, S., Sherry, N. L., Isles, N., Seemann, T., Sharkey, L. K., Walsh, C. J., Reid, G. E., Nie, S., Eijkelkamp, B. A., Holmes, N. E., Collis, B., Vogrin, S., Hiergeist, A., Weber, D., … Carter, G. P. (2024). Rifaximin prophylaxis causes resistance to the last-resort antibiotic daptomycin. *Nature*, *635*(8040), 969–977. https://doi.org/10.1038/s41586-024-08095-4

11. Beh, J. Q., Daniel, D. S., Judd, L. M., Wick, R. R., Kelley, P., Cronin, K. M., Sherry, N. L., Howden, B. P., Connor, C. H., & Webb, J. R. (2025). Genomics to understand the global landscape of linezolid resistance in Enterococcus faecium and Enterococcus faecalis. *Microbial Genomics*, *11*(6), 001432. https://doi.org/10.1099/mgen.0.001432

12. *National Alert System for Critical Antimicrobial Resistances (CARAlert) | Australian Commission on Safety and Quality in Health Care*. (n.d.). Retrieved January 4, 2026, from https://www.safetyandquality.gov.au/our-work/antimicrobial-resistance/antimicrobial-use-and-resistance-australia-aura/hospital-and-community-antimicrobial-resistance/national-alert-system-critical-antimicrobial-resistances-caralert

13. Tiwari, S., Dhakal, T., Kim, B.-J., Jang, G. S., & Oh, Y. (2025). Genomics in Epidemiology and Disease Surveillance: An Exploratory Analysis. *Life*, *15*(12), 1848. https://doi.org/10.3390/life15121848

14. *Clinical Implementation of Routine Whole-genome Sequencing for Hospital Infection Control of Multi-drug Resistant Pathogens | Clinical Infectious Diseases | Oxford Academic*. (n.d.). Retrieved January 5, 2026, from https://academic.oup.com/cid/article/76/3/e1277/6691363#395219355

15. Rath, A., Kieninger, B., Mirzaliyeva, N., Schmid, S., Mester, P., & Schneider-Brachert, W. (2024). The genome-oriented surveillance of vancomycin-resistant enterococci shows a clear misclassification of nosocomial transmission events. *Clinical Microbiology and Infection*, *30*(8), 1086–1088. https://doi.org/10.1016/j.cmi.2024.04.010

16. Sundermann, A. J., Kumar, P., Griffith, M. P., Waggle, K. D., Rangachar Srinivasa, V., Raabe, N., Mills, E. G., Coyle, H., Ereifej, D., Creager, H. M., Ayres, A., Van Tyne, D., Pless, L. L., Snyder, G. M., Roberts, M., & Harrison, L. H. (2025). Real-Time Genomic Surveillance for Enhanced Healthcare Outbreak Detection and Control: Clinical and Economic Impact. *Clinical Infectious Diseases*, ciaf216. https://doi.org/10.1093/cid/ciaf216

17. *National, state and territory population, December 2025 | Australian Bureau of Statistics*. (2026, June 18). https://www.abs.gov.au/statistics/people/population/national-state-and-territory-population/latest-release

18. Ratwatte, R. (2025, June 30). *About Us*. School of Biomedical Sciences. https://biomedicalsciences.unimelb.edu.au/departments/microbiology-Immunology/research/services/microbiological-diagnostic-unit-public-health-laboratory/about-us

19. Sherry, N. L., Lee, R. S., Gorrie, C. L., Kwong, J. C., Stuart, R. L., Korman, T. M., Marshall, C., Higgs, C., Chan, H. T., Graham, M., Johnson, P. D. R., Leroi, M. J., Reed, C., Richards, M. J., Slavin, M. A., Worth, L. J., Howden, B. P., & Grayson, M. L. (2021). Pilot study of a combined genomic and epidemiologic surveillance program for hospital-acquired multidrug-resistant pathogens across multiple hospital networks in Australia. *Infection Control & Hospital Epidemiology*, *42*(5), 573–581. https://doi.org/10.1017/ice.2020.1253

20. Chen, S., Zhou, Y., Chen, Y., & Gu, J. (2018). fastp: An ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics*, *34*(17), i884–i890. https://doi.org/10.1093/bioinformatics/bty560

21. Connor, C. H., Higgs, C. K., Horan, K., Kwong, J. C., Grayson, M. L., Howden, B. P., Seemann, T., Gorrie, C. L., & Sherry, N. L. (2025). Rapid, reference-free identification of bacterial pathogen transmission using optimized split k-mer analysis. *Microbial Genomics*, *11*(3), 001347. https://doi.org/10.1099/mgen.0.001347

22. Seemann, T. (2017). *Shovill* [Perl; Github]. https://github.com/tseemann/shovill

23. Shen, W., Sipos, B., & Zhao, L. (2024). SeqKit2: A Swiss army knife for sequence and alignment processing. *iMeta*, *3*(3), e191. https://doi.org/10.1002/imt2.191

24. Wood, D. E., Lu, J., & Langmead, B. (2019). Improved metagenomic analysis with Kraken 2. *Genome Biology*, *20*(1), 257. https://doi.org/10.1186/s13059-019-1891-0

25. Harris, S. R. (2018). *SKA: Split Kmer Analysis Toolkit for Bacterial Genomic Epidemiology* (p. 453142). bioRxiv. https://doi.org/10.1101/453142

26. R Core Team. (2026). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing. https://www.R-project.org/

27. Sundermann, A. J., Rangachar Srinivasa, V., Mills, E. G., Griffith, M. P., Evans, E., Chen, J., Waggle, K. D., Snyder, G. M., Pless, L. L., Harrison, L. H., & Van Tyne, D. (2025). Genomic sequencing surveillance of patients colonized with vancomycin-resistant enterococci improves detection of healthcare-associated transmission. *BMC Global and Public Health*, *3*(1), 69. https://doi.org/10.1186/s44263-025-00186-2

28. Schrader, S. M., Baker, M. A., Rhee, C., Klompas, M., Taffner, S., Pearson, Z., Worley, J., Bry, L., Kanjilal, S., Brigl, M., & Pecora, N. D. (2025). Whole-genome sequencing surveillance of vancomycin-resistant Enterococcus faecium (VRE) detects hospital outbreaks and identifies the postanesthesia care unit as a transmission locus. *Infection Control & Hospital Epidemiology*, *46*(8), 797–804. https://doi.org/10.1017/ice.2025.68

29. Seemann, T. (2016). *MLST: scan contig files against PubMLST typing schemes*.

30. Sherry, N. L., Horan, K. A., Ballard, S. A., Gonçalves da Silva, A., Gorrie, C. L., Schultz, M. B., Stevens, K., Valcanis, M., Sait, M. L., Stinear, T. P., Howden, B. P., & Seemann, T. (2023). An ISO-certified genomics workflow for identification and surveillance of antimicrobial resistance. *Nature Communications*, *14*(1), 60. https://doi.org/10.1038/s41467-022-35713-4

31. Hasman, H., Clausen, P. T. L. C., Kaya, H., Hansen, F., Knudsen, J. D., Wang, M., Holzknecht, B. J., Samulioniené, J., Røder, B. L., Frimodt-Møller, N., Lund, O., & Hammerum, A. M. (2019). LRE-Finder, a Web tool for detection of the 23S rRNA mutations and the optrA, cfr, cfr(B) and poxtA genes encoding linezolid resistance in enterococci from whole-genome sequences. *Journal of Antimicrobial Chemotherapy*, *74*(6), 1473–1476. https://doi.org/10.1093/jac/dkz092

32. Lam, M. M., Seemann, T., Tobias, N. J., Chen, H., Haring, V., Moore, R. J., Ballard, S., Grayson, L. M., Johnson, P. D., Howden, B. P., & Stinear, T. P. (2013). Comparative analysis of the complete genome of an epidemic hospital sequence type 203 clone of vancomycin-resistant Enterococcus faecium. *BMC Genomics*, *14*(1), 595. https://doi.org/10.1186/1471-2164-14-595

33. Seemann, T. (2015). *Snippy: Fast bacterial variant calling from NGS reads*.

34. Robertson, J., & Nash, J. H. E. (2018). MOB-suite: Software tools for clustering, reconstruction and typing of plasmids from draft assemblies. *Microbial Genomics*, *4*(8), e000206. https://doi.org/10.1099/mgen.0.000206

35. *Clinical Breakpoint Tables*. (n.d.). Retrieved August 19, 2026, from https://www.eucast.org/bacteria/clinical-breakpoints-and-interpretation/clinical-breakpoint-tables/

36. De Coster, W., & Rademakers, R. (2023). NanoPack2: Population-scale evaluation of long-read sequencing data. *Bioinformatics*, *39*(5), btad311. https://doi.org/10.1093/bioinformatics/btad311

37. Wick, R. R., Howden, B. P., & Stinear, T. P. (2025). Autocycler: Long-read consensus assembly for bacterial genomes. *Bioinformatics*, *41*(9), btaf474. https://doi.org/10.1093/bioinformatics/btaf474

38. Wick, R. R., Schultz, M. B., Zobel, J., & Holt, K. E. (2015). Bandage: Interactive visualization of de novo genome assemblies. *Bioinformatics*, *31*(20), 3350–3352. https://doi.org/10.1093/bioinformatics/btv383

39. Wick, R. (2025). *Wiki/Autocycler-clean*. GitHub. https://github.com/rrwick/Autocycler/wiki/Autocycler-clean

40. Wick, R. R., Judd, L. M., Gorrie, C. L., & Holt, K. E. (2017). Unicycler: Resolving bacterial genome assemblies from short and long sequencing reads. *PLoS Computational Biology*, *13*(6), e1005595. https://doi.org/10.1371/journal.pcbi.1005595

41. Hall, M. B., Xue, Y., Lee, T. S. E., Herring, E., Hume, J., Wick, R. R., Kidd, T., Runnegar, N., Harris, P. N. A., Graves, B., & Roberts, L. W. (2026). *Novel transposon Tn8026 acts as a global driver of transmissible linezolid resistance in Enterococcus via a linear plasmid* (p. 2026.03.04.26347163). medRxiv. https://doi.org/10.64898/2026.03.04.26347163

42. Wick, R. R., & Holt, K. E. (2022). Polypolish: Short-read polishing of long-read bacterial genome assemblies. *PLoS Computational Biology*, *18*(1), e1009802. https://doi.org/10.1371/journal.pcbi.1009802

43. Bouras, G., Judd, L. M., Edwards, R. A., Vreugde, S., Stinear, T. P., & Wick, R. R. (2024). How low can you go? Short-read polishing of Oxford Nanopore bacterial genome assemblies. *Microbial Genomics*, *10*(6), 001254. https://doi.org/10.1099/mgen.0.001254

44. Mikheenko, A., Saveliev, V., Hirsch, P., & Gurevich, A. (2023). WebQUAST: Online evaluation of genome assemblies. *Nucleic Acids Research*, *51*(W1), W601–W606. https://doi.org/10.1093/nar/gkad406

45. Schwengers, O., Jelonek, L., Dieckmann, M. A., Beyvers, S., Blom, J., & Goesmann, A. (2021). Bakta: Rapid and standardized annotation of bacterial genomes via alignment-free sequence identification. *Microbial Genomics*, *7*(11), 000685. https://doi.org/10.1099/mgen.0.000685

46. Frolova, D., Lima, L., Roberts, L. W., Bohnenkämper, L., Wittler, R., Stoye, J., & Iqbal, Z. (2024). Applying rearrangement distances to enable plasmid epidemiology with pling. *Microbial Genomics*, *10*(10), 001300. https://doi.org/10.1099/mgen.0.001300

47. Alhothali, M. (2026). *pELF-Finder* [Python]. https://github.com/Marwa-Alhothali/pELF-Finder

48. Lerminiaux, N., McCracken, M., Bartoszko, J., Grewal, G., Ahmed, S., Johnstone, J., Golding, G. R., & Group, the C. V. working. (2026). *Linear plasmid prevalence and linezolid resistance gene carriage in vancomycin-resistant Enterococcus in Canada from 2009-2024* (p. 2026.05.08.26352429). medRxiv. https://doi.org/10.64898/2026.05.08.26352429

49. Langmead, B., & Salzberg, S. L. (2012). Fast gapped-read alignment with Bowtie 2. *Nature Methods*, *9*(4), 357–359. https://doi.org/10.1038/nmeth.1923

50. Danecek, P., Bonfield, J. K., Liddle, J., Marshall, J., Ohan, V., Pollard, M. O., Whitwham, A., Keane, T., McCarthy, S. A., Davies, R. M., & Li, H. (2021). Twelve years of SAMtools and BCFtools. *GigaScience*, *10*(2), giab008. https://doi.org/10.1093/gigascience/giab008

51. Wickham, H. (2016). *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York. https://ggplot2.tidyverse.org

52. Lex, A., Gehlenborg, N., Strobelt, H., Vuillemot, R., & Pfister, H. (2014). UpSet: Visualization of Intersecting Sets,. *IEEE Transactions on Visualization and Computer Graphics*, *20*(12), 1983–1992. https://doi.org/10.1109/TVCG.2014.2346248

53. Pedersen, T. L. (2025). *patchwork: The Composer of Plots*. https://doi.org/10.32614/CRAN.package.patchwork

54. Shannon, P., Markiel, A., Ozier, O., Baliga, N. S., Wang, J. T., Ramage, D., Amin, N., Schwikowski, B., & Ideker, T. (2003). Cytoscape: A Software Environment for Integrated Models of Biomolecular Interaction Networks. *Genome Research*, *13*(11), 2498–2504. https://doi.org/10.1101/gr.1239303

55. Branger, M., & Leclercq, S. O. (2024). GenoFig: A user-friendly application for the visualization and comparison of genomic regions. *Bioinformatics*, *40*(6), btae372. https://doi.org/10.1093/bioinformatics/btae372

56. Lagadinou, M., Michailides, C., Chatzigrigoriadis, C., Erginousakis, I., Avramidis, P., Amerali, M., Tasouli, F., Chondroleou, A., Skintzi, K., Spiliopoulou, A., Kolonitsiou, F., Leonidou, L., Assimakopoulos, S. F., & Marangos, M. (2025). Impact of the COVID-19 pandemic on vancomycin-resistant Enterococcus bloodstream infections: A 6-year study in Western Greece. *Frontiers in Microbiology*, *16*, 1656334. https://doi.org/10.3389/fmicb.2025.1656334

57. Wyres, K. L., Hawkey, J., Mirčeta, M., Judd, L. M., Wick, R. R., Gorrie, C. L., Pratt, N. F., Garlick, J. S., Watson, K. M., Pilcher, D. V., McGloughlin, S. A., Abbott, I. J., Macesic, N., Spelman, D. W., Jenney, A. W. J., & Holt, K. E. (2021). Genomic surveillance of antimicrobial resistant bacterial colonisation and infection in intensive care patients. *BMC Infectious Diseases*, *21*(1), 683. https://doi.org/10.1186/s12879-021-06386-z

58. O'Toole, R. F., Covalciuc, V., Cooke, A., Thilliez, G., Finlay, E. K., Leong, K. W. C., Farthing, V., & van Hal, S. J. (2025). Genomic characterisation of emerging *Enterococcus faecium vanA* types from 2013 to 2020 in an Australian public hospital. *Journal of Global Antimicrobial Resistance*, *42*, 42–50. https://doi.org/10.1016/j.jgar.2025.02.006

59. Kleinman, D. R., Mitchell, R., McCracken, M., Hota, S. S., Golding, G. R., CNISP VRE Working Group, & Smith, S. W. (2023). Vancomycin-resistant Enterococcus sequence type 1478 spread across hospitals participating in the Canadian Nosocomial Infection Surveillance Program from 2013 to 2018. *Infection Control and Hospital Epidemiology*, *44*(1), 17–23. https://doi.org/10.1017/ice.2022.7

60. McCracken, M., Mitchell, R., Smith, S., Hota, S., Conly, J., Du, T., Embil, J., Johnston, L., Ormiston, D., Parsonage, J., Simor, A., Wong, A., Golding, G., & Canadian Nosocomial Infection Surveillance Program. (2020). Emergence of pstS-Null Vancomycin-Resistant Enterococcus faecium Clone ST1478,

Canada, 2013-2018. *Emerging Infectious Diseases*, *26*(9), 2247–2250. https://doi.org/10.3201/eid2609.201576

61. Maiden, M. C. J., Jansen van Rensburg, M. J., Bray, J. E., Earle, S. G., Ford, S. A., Jolley, K. A., & McCarthy, N. D. (2013). MLST revisited: The gene-by-gene approach to bacterial genomics. *Nature Reviews. Microbiology*, *11*(10), 728–736. https://doi.org/10.1038/nrmicro3093

62. Gorrie, C., Higgs, C., Carter, G., Stinear, T. P., & Howden, B. (2019). Genomics of vancomycin-resistant Enterococcus faecium. *Microbial Genomics*, *5*(7), e000283. https://doi.org/10.1099/mgen.0.000283

63. Higgs, C., Sherry, N. L., Seemann, T., Horan, K., Walpola, H., Kinsella, P., Bond, K., Williamson, D. A., Marshall, C., Kwong, J. C., Grayson, M. L., Stinear, T. P., Gorrie, C. L., & Howden, B. P. (2022). Optimising genomic approaches for identifying vancomycin-resistant Enterococcus faecium transmission in healthcare settings. *Nature Communications*, *13*(1), 509. https://doi.org/10.1038/s41467-022-28156-4

64. Bush, S. J., Foster, D., Eyre, D. W., Clark, E. L., De Maio, N., Shaw, L. P., Stoesser, N., Peto, T. E. A., Crook, D. W., & Walker, A. S. (2020). Genomic diversity affects the accuracy of bacterial single-nucleotide polymorphism–calling pipelines. *GigaScience*, *9*(2), giaa007. https://doi.org/10.1093/gigascience/giaa007

65. Mengesha, T. H., Ali, M. M., Mengistu, M., & Assegu Fenta, D. (2024). High Gastrointestinal Colonization Rate of Vancomycin-Resistant Enterococci among Hospitalized Patients: Potential Source for Resistant Gene. *International Journal of Microbiology*, *2024*(1), 6430026. https://doi.org/10.1155/2024/6430026

66. Tacconelli, E., & Cataldo, M. A. (2008). Vancomycin-resistant enterococci (VRE): Transmission and control. *International Journal of Antimicrobial Agents*, *31*(2), 99–106. https://doi.org/10.1016/j.ijantimicag.2007.08.026

67. Mahony, A. A., Buultjens, A. H., Ballard, S. A., Grabsch, E. A., Xie, S., Seemann, T., Stuart, R. L., Kotsanas, D., Cheng, A., Heffernan, H., Roberts, S. A., Coombs, G. W., Bak, N., Ferguson, J. K., Carter, G. C., Howden, B. P., Stinear, T. P., & Johnson, P. D. R. (2018). Vancomycin-resistant Enterococcus faecium sequence type 796—Rapid international dissemination of a new epidemic clone. *Antimicrobial Resistance and Infection Control*, *7*, 44. https://doi.org/10.1186/s13756-018-0335-z

68. Gast, K. B., van Oudheusden, A. J. G., Murk, J. L., Stohr, J. J. J. M., Buiting, A. G., & Verweij, J. J. (2021). Successful containment of two vancomycin-resistant *Enterococcus faecium* (VRE) outbreaks in a Dutch teaching hospital using environmental sampling and whole-genome sequencing. *Journal of Hospital Infection*, *111*, 132–139. https://doi.org/10.1016/j.jhin.2021.02.007

69. Correa-Martinez, C. L., Tönnies, H., Froböse, N. J., Mellmann, A., Kampmeier, S., Correa-Martinez, C. L., Tönnies, H., Froböse, N. J., Mellmann, A., & Kampmeier, S. (2020). Transmission of Vancomycin-

Resistant Enterococci in the Hospital Setting: Uncovering the Patient–Environment Interplay. *Microorganisms*, *8*(2). https://doi.org/10.3390/microorganisms8020203

70. Lee, A. S., White, E., Monahan, L. G., Jensen, S. O., Chan, R., & Hal, S. J. van. (2018). Defining the Role of the Environment in the Emergence and Persistence of vanA Vancomycin-Resistant Enterococcus (VRE) in an Intensive Care Unit: A Molecular Epidemiological Study. *Infection Control & Hospital Epidemiology*, *39*(6), 668–675. https://doi.org/10.1017/ice.2018.29

71. Grabsch, E. A., Mahony, A. A., Cameron, D. R. M., Martin, R. D., Heland, M., Davey, P., Petty, M., Xie, S., & Grayson, M. L. (2012). Significant reduction in vancomycin-resistant enterococcus colonization and bacteraemia after introduction of a bleach-based cleaning–disinfection programme. *Journal of Hospital Infection*, *82*(4), 234–242. https://doi.org/10.1016/j.jhin.2012.08.010

72. Frakking, F. N. J., Bril, W. S., Sinnige, J. C., Klooster, J. E. V., De Jong, B. A. W., Van Hannen, E. J., & Tersmette, M. (2018). Recommendations for the successful control of a large outbreak of vancomycin-resistant Enterococcus faecium in a non-endemic hospital setting. *Journal of Hospital Infection*, *100*(4), e216–e225. https://doi.org/10.1016/j.jhin.2018.02.016

73. Fang, H., Fröding, I., Ullberg, M., & Giske, C. G. (2021). Genomic analysis revealed distinct transmission clusters of vancomycin-resistant *Enterococcus faecium* ST80 in Stockholm, Sweden. *Journal of Hospital Infection*, *107*, 12–15. https://doi.org/10.1016/j.jhin.2020.10.019

74. Hashimoto, Y., Kita, I., Suzuki, M., Hirakawa, H., Ohtaki, H., & Tomita, H. (2020). First Report of the Local Spread of Vancomycin-Resistant Enterococci Ascribed to the Interspecies Transmission of a vanA Gene Cluster-Carrying Linear Plasmid. *mSphere*. (1752 N St., N.W., Washington, DC). https://doi.org/10.1128/msphere.00102-20

75. Egan, S. A., Corcoran, S., McDermott, H., Fitzpatrick, M., Hoyne, A., McCormack, O., Cullen, A., Brennan, G. I., O'Connell, B., & Coleman, D. C. (2020). Hospital outbreak of linezolid-resistant and vancomycin-resistant ST80 *Enterococcus faecium* harbouring an *optrA*-encoding conjugative plasmid investigated by whole-genome sequencing. *Journal of Hospital Infection*, *105*(4), 726–735. https://doi.org/10.1016/j.jhin.2020.05.013

76. Kent, A. G., Spicer, L. M., Campbell, D., Breaker, E., McAllister, G. A., Ewing, T. O., Longo, C., Balbuena, R., Burroughs, M., Burgin, A., Padilla, J., Johnson, J. K., Halpin, A. L., McKay, S. L., Rasheed, J. K., Elkins, C. A., Karlsson, M., Lutgring, J. D., & Gargis, A. S. (2024). Sentinel Surveillance reveals phylogenetic diversity and detection of linear plasmids harboring vanA and optrA among enterococci collected in the United States. *Antimicrobial Agents and Chemotherapy*, *68*(11), e00591-24. https://doi.org/10.1128/aac.00591-24

77. Bakthavatchalam, Y. D., Puraswani, M., Livingston, A., Priya, M., Venkatesan, D., Sharma, D., Iyadurai, R., Pichamuthu, K., Veeraraghavan, B., & Mathur, P. (2022). Novel linear plasmids carrying vanA

cluster drives the spread of vancomycin resistance in Enterococcus faecium in India. *Journal of Global Antimicrobial Resistance*, *29*, 168–172. https://doi.org/10.1016/j.jgar.2022.03.013

78. Cinthi, M., Coccitto, S. N., Simoni, S., Gherardi, G., Palamara, A. T., Di Lodovico, S., Di Giulio, M., Du, X.-D., Vignaroli, C., Brenciani, A., & Giovanetti, E. (2025). The optrA, cfr(D) and vanA genes are co-located on linear plasmids in linezolid- and vancomycin-resistant enterococcal clinical isolates in Italy. *Journal of Antimicrobial Chemotherapy*, *80*(5), 1362–1370. https://doi.org/10.1093/jac/dkaf082

79. Hashimoto, Y., Suzuki, M., Kobayashi, S., Hirahara, Y., Kurushima, J., Hirakawa, H., Nomura, T., Tanimoto, K., & Tomita, H. (2023). Enterococcal Linear Plasmids Adapt to Enterococcus faecium and Spread within Multidrug-Resistant Clades. *Antimicrobial Agents and Chemotherapy*, *67*(4), e01619-22. https://doi.org/10.1128/aac.01619-22

80. Boumasmoud, M., Dengler Haunreiter, V., Schweizer, T. A., Meyer, L., Chakrakodi, B., Schreiber, P. W., Seidl, K., Kühnert, D., Kouyos, R. D., & Zinkernagel, A. S. (2022). Genomic Surveillance of Vancomycin-Resistant Enterococcus faecium Reveals Spread of a Linear Plasmid Conferring a Nutrient Utilization Advantage. *mBio*, *13*(2), e03771-21. https://doi.org/10.1128/mbio.03771-21

81. *AusTrakka*. (n.d.). Retrieved August 9, 2026, from https://austrakka.net/

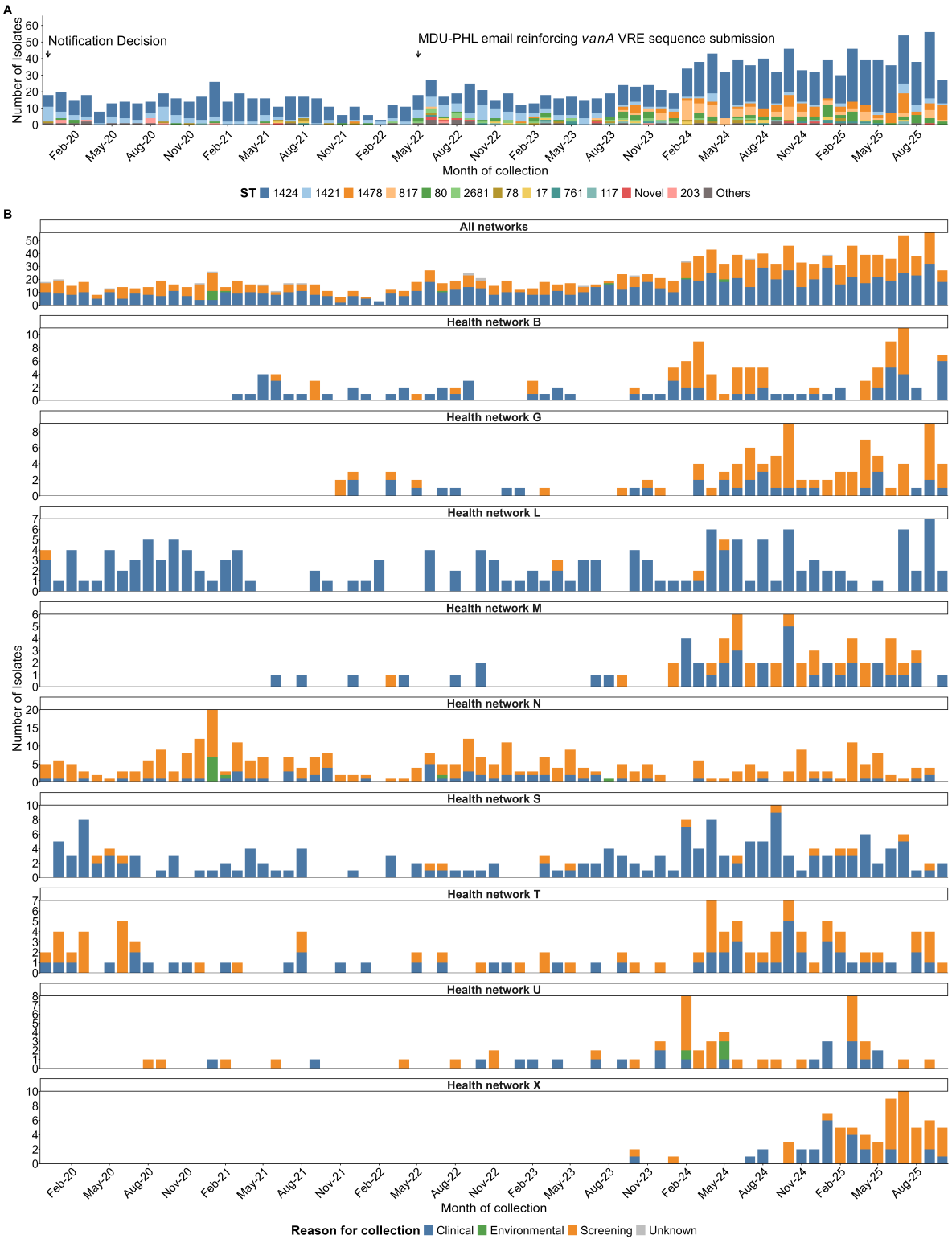
82. *AusTrakka: Fast-tracking nationalized genomics surveillance in response to the COVID-19 pandemic \textbar Nature Communications*. (n.d.). Retrieved December 29, 2025, from https://www.nature.com/articles/s41467-022-28529-9

83. Lane, C. R., Brett, J., Schultz, M., Gorrie, C. L., Stevens, K., Cameron, D. R. M., St George, S., van Diemen, A., Easton, M., Stuart, R. L., Sait, M., Peleg, A. Y., Stewardson, A. J., Cheng, A. C., Spelman, D. W., Waters, M. J., Ballard, S. A., Sherry, N. L., Williamson, D. A., … Howden, B. P. (2020). Search and Contain: Impact of an Integrated Genomic and Epidemiological Surveillance and Response Program for Control of Carbapenemase-producing Enterobacterales. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, *73*(11), e3912–e3920. https://doi.org/10.1093/cid/ciaa972

84. *Studies—Australian Pathogen Genomics Program*. (n.d.). Retrieved June 5, 2026, from https://www.auspathogen.org.au/studies/candida-auris

85. Department of Health. Victoria, A. (2026, July 29). *Victorian Admitted Episodes Dataset*. State Government of Victoria, Australia. https://content.health.vic.gov.au/data-reporting/victorian-admitted-episodes-dataset

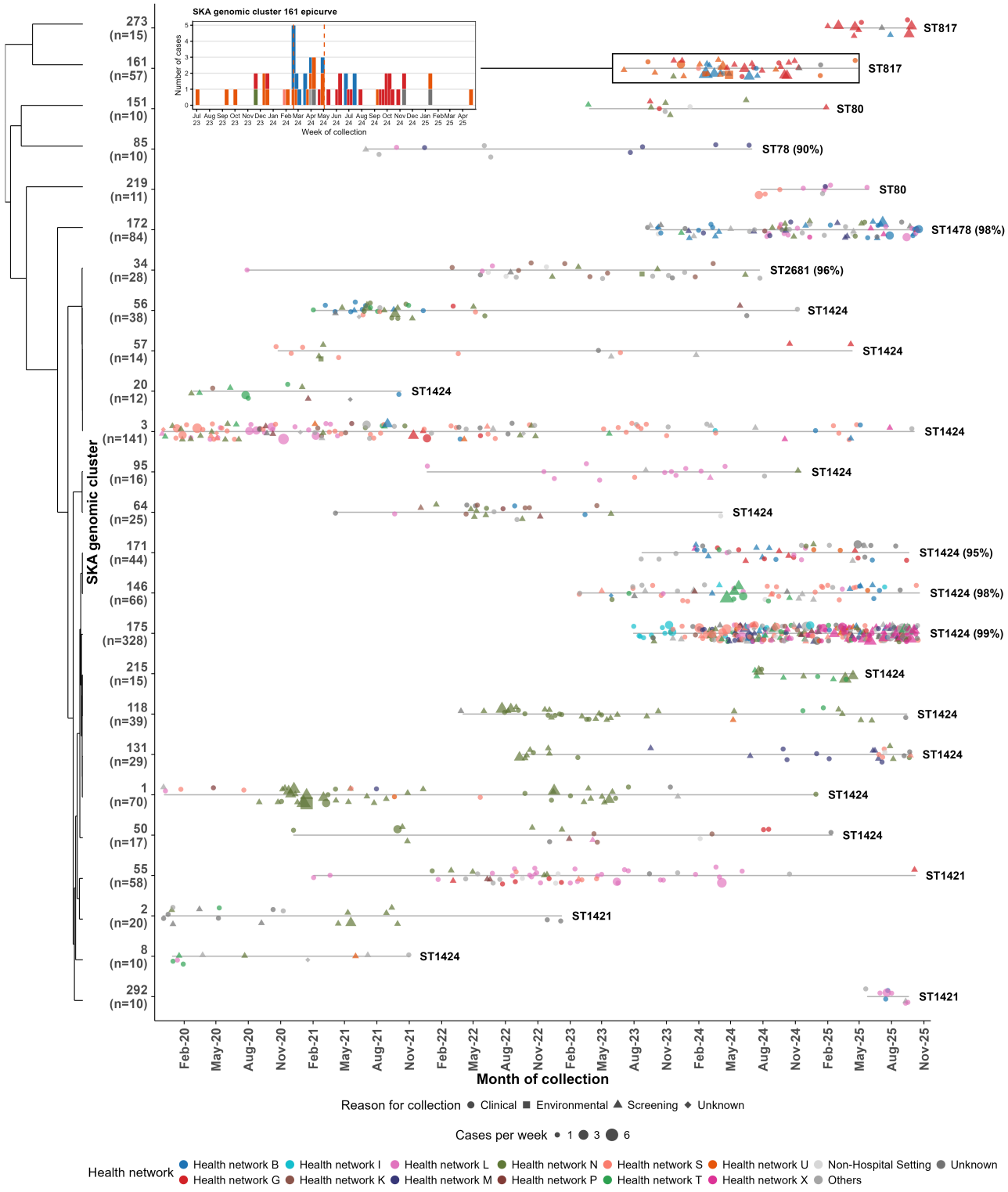
# Figures




## Figure 1

Temporal distribution of Victorian *vanA E. faecium* isolates collected between Dec 2019 and Oct 2025. A) Monthly number of isolates stratified by ST. Arrows indicated major surveillance and public health events, including the Victorian notification decision and MDU-PHL communication reinforcing *vanA* VRE isolate submission. B) Monthly distribution of isolates by reason for collection. Bars are coloured according to reason for collection (clinical, screening, environmental or unknown). The upper plot

represents all healthcare networks combined, while subsequent plots represent healthcare networks with more than 45 isolates submitted during the surveillance period.




## Figure 2

Timeline of SKA genomic clusters among Victorian *vanA* gene-carrying *E. faecium*isolates identified between Dec 2019 and Oct 2025. Left, a hierarchical clustering dendrogram provided to illustrate the

relative genetic similarity among major genomic clusters. The dendrogram is intended as a visual representation of the relative similarity between clusters rather than a phylogenetic reconstruction. Right, genomic clusters were defined using ≤15 SNP threshold. Each data point represents the number of isolates per week of collection, coloured by health network and shaped by reason for collection: clinical isolates are shown as circles, screening isolates as triangles, environmental isolates are shown as squares and unknown collection reason as diamonds. Only major clusters containing 10 or more isolates are shown. Singletons were excluded from this figure. The dominant ST for each cluster is highlighted. The inset plot shows the epidemic curve of SKA cluster 161, with bars representing the weekly number of isolates coloured by health network. Dashed orange lines indicate the dates on which health network U conducted environmental screening.

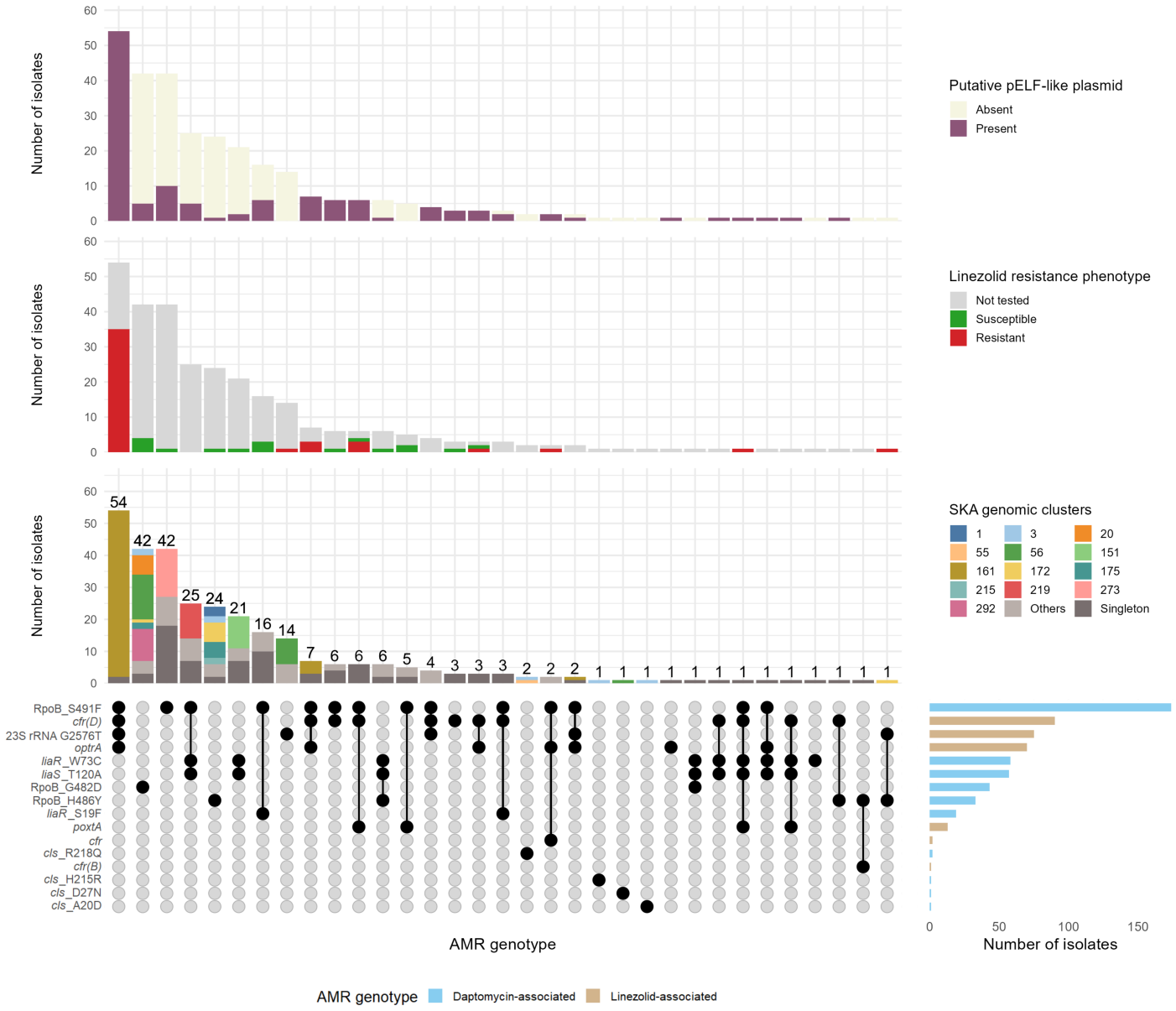
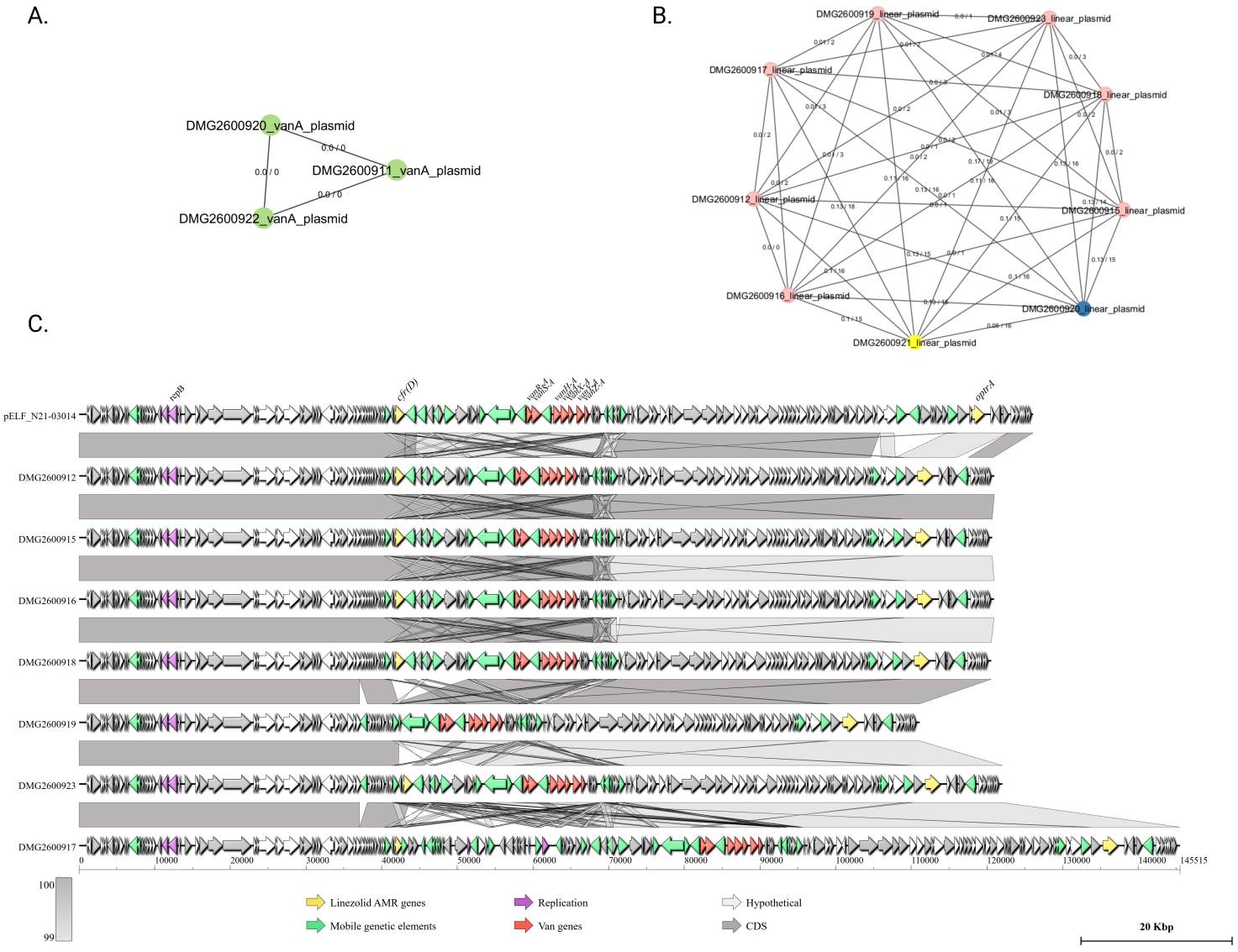



**Figure 3**

Distribution of AMR genotypes associated with daptomycin and linezolid resistance among *vanA* VREfm isolates. Vertical bars represent the number of isolates associated with each AMR genotype combination while horizontal bars show the total frequency of each AMR genotype. From bottom to top, stacked annotation bars indicate the distribution of SKA genomic clusters, linezolid susceptibility phenotypes, and the presence of putative pELF-like plasmids for each AMR genotype combination. Linezolid susceptibility phenotypes are coloured according to resistance status, with isolates not tested shown in grey.




## Figure 4

Pling clusters of *vanA*-carrying plasmids with containment /DCJ-indel distances shown at edges, **A)** showing pling (community 1) with nodes representing circular plasmids while **B)** is showing clusters of linear plasmids communities where nodes sharing the same colour (pink) belong to the same (community 2) while nodes coloured blue and yellow represents singletons. **C)** Comparison of pELF-plasmids within pling community 2 with the pELF_N21-03014 (Accession no. CP197457 from Canada).

Coding DNA sequences (CDS) are indicated by arrows. Shared regions with a length of 500bp and >99% similarity are shown with the vertical gray synteny blocks.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementaryfigure1.png
- Supplementaryfigure2.png
- Supplementarytables.xlsx