Science for Communities
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Vancomycin-resistant enterococci, 2022

Summary

Vancomycin-resistant enterococci are under passive surveillance in New Zealand, with voluntary participation by all diagnostic laboratories in New Zealand.

In 2022, a total of 29 vancomycin-resistant *Enterococcus faecium* and *Enterococcus faecalis* (VRE) from 29 patients were confirmed. This is higher than 2021, but lower than the number of VRE isolates identified annually between 2013 and 2020.

Enterococcus faecium remains the dominant vancomycin-resistant enterococcus in New Zealand. The VRE in 2022 had a mix of resistance genes; 34.4% (10/29) *vanA*, 62.1% (18/29) *vanB* and (3.4%) 1/29 *vanN*. The majority of VRE samples in 2022 were from screening samples, with only 2 of 29 isolates obtained from a clinical specimen.

VRE were predominantly referred from diagnostic laboratories in the Auckland region (62.1%, 18/29), and with all isolates referred from the Auckland region, Northland or Waikato.

Whole genome sequencing (WGS) identified a range of sequence types. The largest cluster was found in isolates referred from Waikato hospital, with a total of seven ST17 isolates, all identified in the last four months of 2022.

Methods

Hospital and community diagnostic laboratories are requested to refer all vancomycin-resistant *Enterococcus faecium* and *Enterococcus faecalis* to ESR for the national surveillance of these organisms.

At ESR, the vancomycin and teicoplanin minimum inhibitory concentrations (MICs) were confirmed by gradient strip on Mueller-Hinton agar. Susceptibility to ampicillin, ciprofloxacin, high-level gentamicin, linezolid, nitrofurantoin (*E. faecalis* only), quinupristin-dalfopristin (*E. faecium* only), high-level streptomycin and tetracycline were determined by disc testing. Susceptibility to daptomycin was determined by broth microdilution using ISO 20776-1 (2006). Susceptibility testing results were interpreted according to European Committee on Antimicrobial Susceptibility Testing (EUCAST)[1] breakpoints, except for tetracycline and daptomycin, which were interpreted using Clinical and Laboratory Standards Institute[2] breakpoints.

The *van* genes were identified by PCR[3,4,5,6,7,8] or determined from WGS data. Results were compared to *van* gene data supplied by diagnostic laboratories.

Isolates were typed by pulsed-field gel electrophoresis (PFGE) and Illumina-based WGS. DNA macrorestriction by PFGE of *SmaI*-digested genomic DNA was performed

as previously described[9]. PFGE profiles were analysed with BioNumerics software version 8.1 (bioMérieux, France) using the Dice coefficient and unweighted-pair group method with arithmetic averages, 0.5% optimisation and 1.5% position tolerance. In-house PFGE profiles of isolates designated as the same strain share >90% similarity. DNA libraries were created using the plexWell Library Preparation kit (SeqWell), and sequencing was performed using Illumina technology. Data were analysed using an in-house developed pipeline linking together open-source packages and in-house scripts, which enables the resistome, the multi-locus sequence type (MLST) and the genetic relatedness of isolates to be determined. Open-source packages used included SKESA[10] , MLST[11], AMRFinderPlus[12], core genome MLST (cgMLST) using Chewbacca[13], FastANI[14] to assess average nucleotide identity between genomes, and reference-free (kmer based) single nucleotide polymorphism (SNP)-clustering using split kmer analysis (SKA)[15]. A cgMLST pairwise allelic difference threshold of ≤25 was used to investigate possible clusters[16]. A threshold of 7 SKA SNPs was used to define clusters involving probable transmission events[16].

Results

E. faecium continue to be the dominant vancomycin-resistant enterococcal species in New Zealand (Figure 1). A total of 34 viable enterococci were referred to ESR in 2022 as potential vancomycin-resistant enterococci: 31 *E. faecium*, two *E. avium* and one *E. casseliflavus*. Of these, two *E. faecium* isolates were excluded as they were duplicates. The two *E. avium* and one *E. casseliflavus* were also not included in the analysis below, however all contained *vanA.*

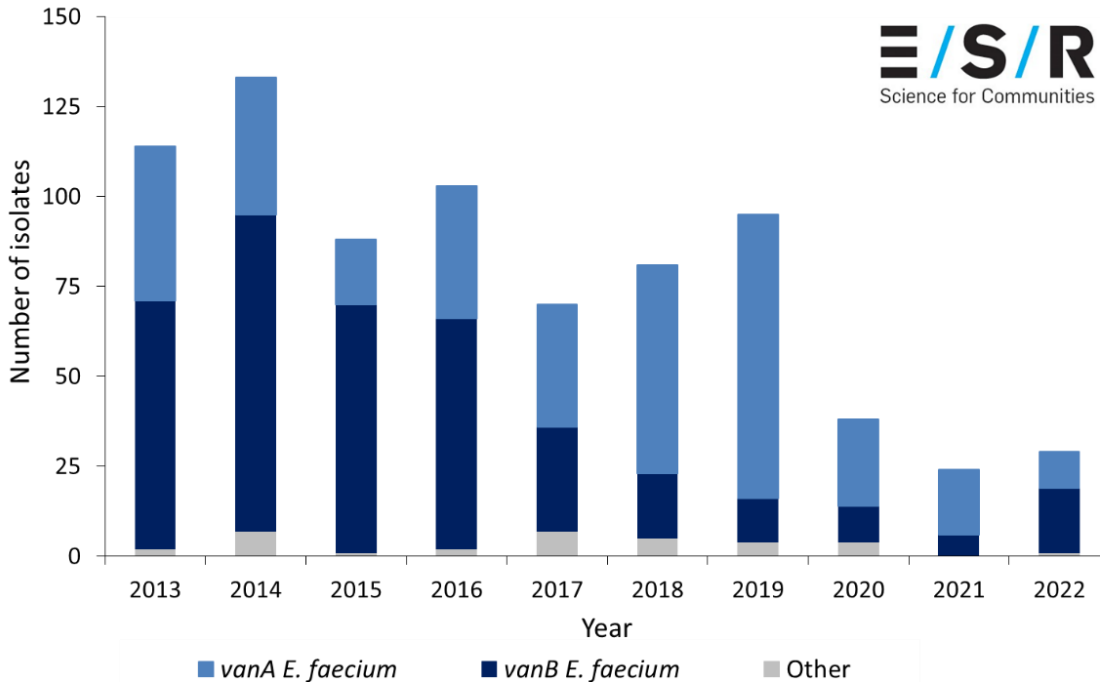

Figure 1: Species and *van* genotype of VRE isolated in New Zealand, 2013-2022

A total of 29 VRE from 29 patients were confirmed in 2022, and all were *E. faecium*. Of these 10 contained *vanA*, 18 contained *vanB* and one contained *vanN*. The number of VRE confirmed each year over the last 10 years is shown in Figure 1. Between 2015-2019 the percentage of VRE attributed to *vanA* increased, with the highest percentage (83.2%) found in 2019. In 2020-2021 the percentage of VRE attributed to *van*A remained higher than *vanB,* although in 2022 most VRE were attributed to *vanB*, accounting for 62.1% of all isolates.

The site of isolation was reported for all 29 isolates. The majority (27/29, 93.1%) were isolated from screening specimens (i.e. rectal swabs and faecal specimens). The remaining VRE were from blood (2/29, 6.9%).

In 2022 the majority (18/29, 62.1%) of VRE were isolated from patients in Auckland hospitals (Table 1): Auckland City Hospital (13/29, 44.8%) and Middlemore Hospital (5/29, 17.2%), with a further two isolates from Auckland community patients. Outside the Auckland region, Waikato Hospital had the largest number of isolates (7/29, 24.1%).

Table 1. Distribution of patients with VRE by healthcare facility and VRE profile, 2022

Species	*van* gene	Referred from	MLST/CC	PFGE profile	Number of patients
E. faecium	*vanA*	Auckland City Hospital	ST761/CC17	EfBE	1
			ST761/CC17	Distinct	3
			ST80/CC17	Distinct	1
			ST1421/CC17	Distinct	1
		Middlemore Hospital	ST80/CC17	EfBD	1
			ST761/CC17	EfBE	2
		Auckland community	ST761/CC17	EfBE	1
	vanB	Waikato Hospital	ST17/CC17	EfBH	7
		Auckland City Hospital	ST80/CC17	Distinct	2
			ST17/CC17	Distinct	4
		Auckland community	ST78/CC17	EfAW	1
			ST80/CC17	Distinct	1
		Middlemore Hospital	ST17/CC17	Distinct	2
		Whangarei Hospital	ST78/CC17	Distinct	1
	vanN	Auckland City Hospital	ST2426	Not tested	1

WGS-based typing methods enabled all isolates to be characterised. By contrast, using PFGE over half the isolates (15/28, 53.6%) were reported as 'distinct' (Table 1). Six sequence types were identified, with most (28/29, 96.6%) belonging to clonal complex 17 (CC17), a hospital-adapted *E. faecium* lineage. The availability of WGS data enables more discriminatory methods, that characterise a larger portion of the genome to be used to investigate the genetic relatedness of isolates.

A total of six VRE clusters, with a total of 21 isolates, were identified (Figure 2). The largest cluster had seven ST17 *E. faecium* with *vanB*, referred by Waikato Hospital (Table 1). All were identified in the last four months of 2022. There were two other clusters of ST17 *vanB* VRE in 2022 from LabPlus (4 isolates) and Middlemore Hospital (2 isolates), that were distinct Waikato Hospital ST17 isolates and distinct from each other. The fourth cluster had four ST761 *vanA E. faecium* with the EfBE PFGE profile, referred by three laboratories in the Auckland region (Table 1). The other two clusters involved two ST761 *vanA E. faecium* isolates and two ST78 *vanB E. faecium* from the Northland/Auckland community.

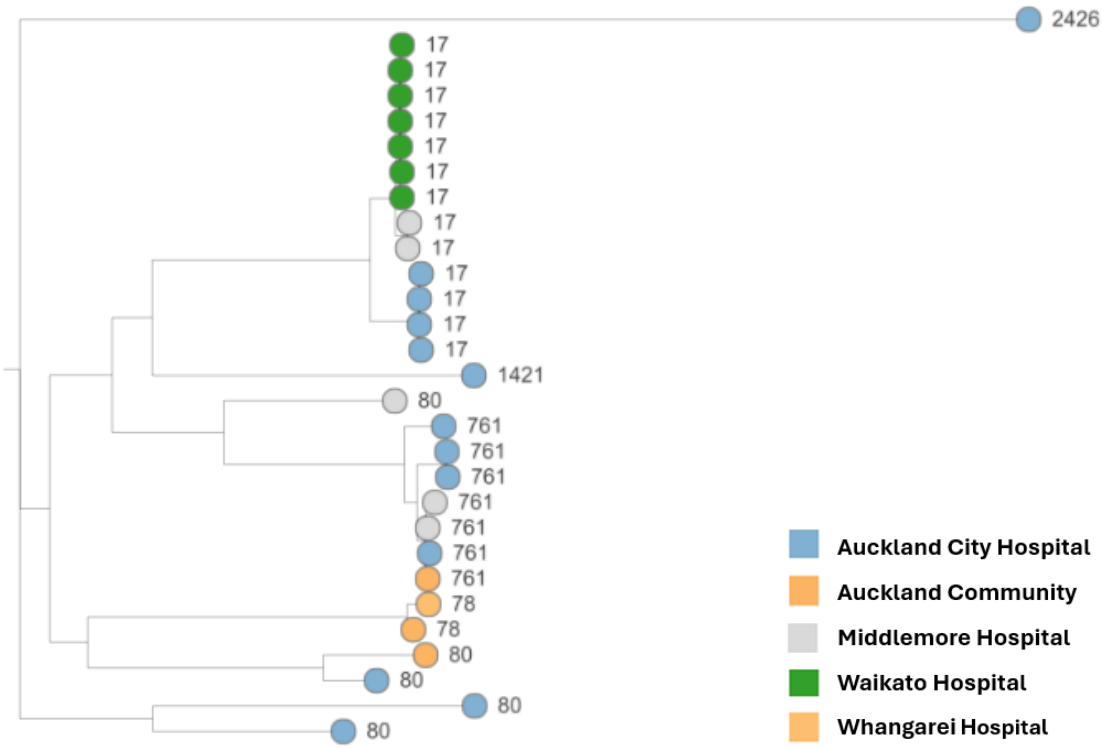

Figure 2: Genetic relatedness of VRE found in New Zealand in 2022, by cgMLST, labelled with 7-gene MLST sequence type.

Antimicrobial susceptibility among the 2022 VRE isolates is shown in Table 2. Resistance to ampicillin and ciprofloxacin is common in the CC17 lineage, which was observed in New Zealand VRE in 2022 (Table 2). The presence of the *vanA* gene cluster conferred high-level teicoplanin resistance whereas isolates with *vanB* were all teicoplanin-susceptible. All VRE with *vanA* or *vanB* in 2022 were multiresistant.

Table 2. Percent resistance among VRE isolates in New Zealand, 2022

	Percent resistance (%) in *E. faecium*		
	vanA	***vanB***	**All[1]**
Antimicrobial	**n = 10**	**n = 18**	**n = 29**
Ampicillin	100.0	100.0	96.6
Ciprofloxacin	100.0	100.0	96.6
Daptomycin	0.0	0.0	0.0
Gentamicin high-level	30.0	11.1	17.2
Linezolid	10.0	0.0	3.5
Quinupristin/dalfopristin	80.0	77.8	75.9
Streptomycin high-level	80.0	61.1	65.5
Teicoplanin	100.0	0.0	34.5
Tetracycline	100.0	88.9	89.7
Multiresistant[2]	100.0	100.0	96.6

1 Includes the isolate containing *vanN*, as well as all *vanA* and *vanB* positive isolates.
2 Resistant to three classes of antibiotics, in addition to glycopeptides.

Discussion

The numbers of VRE isolates identified in New Zealand remained low in 2022. This number of VRE isolates is likely to have been affected by the COVID-19 pandemic and response measures, including border closures and reduced international travel.

In 2022 the burden of VRE currently sat predominantly in Waikato, Auckland and Northland, with no VRE isolates identified outside of these regions. Current surveillance of VRE in New Zealand indicates that clinical infections are uncommon.

The availability of WGS data enables more discriminatory typing methods to be used, than traditional PFGE. This has allowed characterisation of a larger portion of the genome to investigate the genetic relatedness of isolates and support surveillance and outbreak investigation and control. Ongoing utilisation of WGS-based methods will improve VRE surveillance in New Zealand

The majority of VRE in New Zealand in 2022 were closely related to VRE from other New Zealand patients, suggesting that transmission is occurring both within and between New Zealand health care facilities. This highlights the need to maintain high standards of infection, prevention and control to minimise future transmission events and the ensuing adverse patient impact.

In 2022, New Zealand hospitals continue to implement a ‘stamp it out’ approach to VRE, with ongoing screening and infection control practices to eliminate VRE and prevent it becoming endemic in New Zealand.

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