

## Vancomycin-resistant enterococci, 2021

Hospital and community diagnostic laboratories are requested to refer all vancomycin-resistant *Enterococcus faecium* and *Enterococcus faecalis* (VRE) isolates to ESR for the national surveillance of these organisms. At ESR, each referred isolate was confirmed as phenotypically vancomycin resistant, by gradient strip on Mueller-Hinton agar. Susceptibility to teicoplanin was also determined by gradient strip. 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. Gradient strip minimum inhibitory concentrations and disc zones of inhibition were interpreted according to European Committee on Antimicrobial Susceptibility Testing (EUCAST)<sup>1</sup> breakpoints, except for tetracycline which was interpreted using Clinical and Laboratory Standards Institute<sup>2</sup> breakpoints.

The *van* gene was identified by PCR<sup>3,4,5,6,7,8</sup>. Isolates were typed by pulsed-field gel electrophoresis (PFGE) and were also characterised using Illumina-based whole genome sequencing (WGS). Genomic DNA was extracted using the Roche High Pure PCR template preparation kit. DNA libraries were created using the Nextera XT DNA preparation kit (Illumina), 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 vancomycin resistance genes, the acquired resistome and the multi-locus sequence type to be determined. Open-source packages used included the Nullarbor2: 'Reads to report' for public health and clinical microbiology pipeline,<sup>9</sup> SKESA,<sup>10</sup> MLST<sup>11</sup>, and ABRicate<sup>12</sup> using ResFinder.<sup>13</sup>

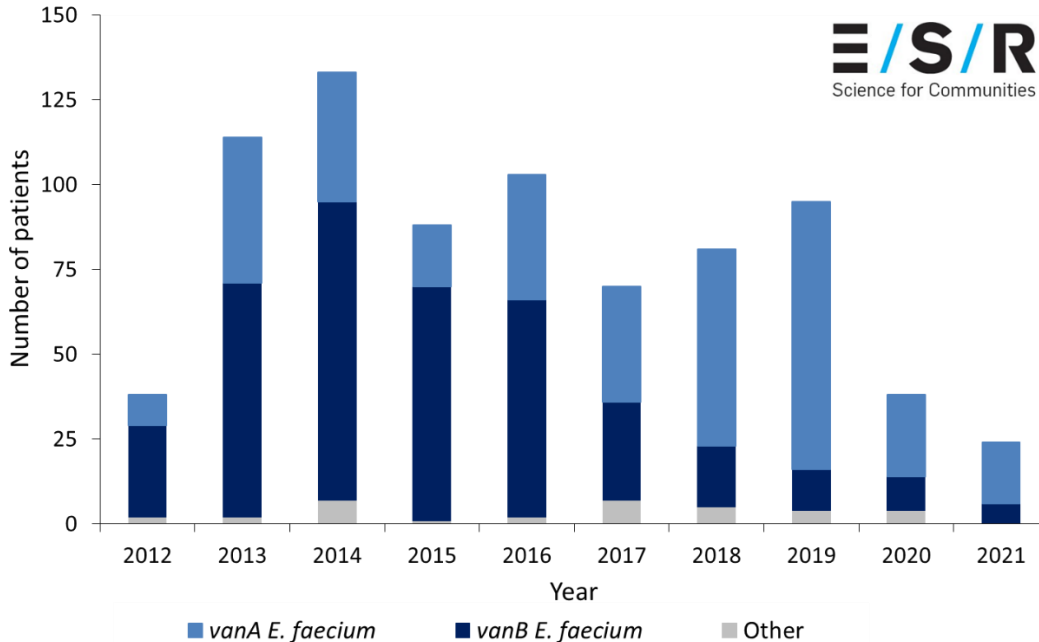
*E. faecium* continue to be the dominant vancomycin-resistant enterococcal species found in New Zealand. A total of 31 viable enterococci were referred to ESR in 2021 as potential vancomycin-resistant enterococci: 26 *E. faecium*, two *E. faecalis*, two *E. avium* and one *E. gallinarum*. Of these, the two *E. faecalis* were excluded from the dataset as they were not confirmed as phenotypically vancomycin resistant. Two *E. faecium* isolates were excluded as they were duplicates. The *E. avium* and *E. gallinarum* isolates were also not included in the analysis below, however all contained *vanA* and the *E. gallinarum* isolate also contained *vanC*.

A total of 24 VRE from 23 patients were confirmed in 2021. Two distinct VRE strains were isolated from one patient. All 24 VRE confirmed in 2021 were *E. faecium*. Of these 18 contained *vanA* and six contained *vanB*.

The site of isolation was reported for all 24 isolates. The majority (20/24, 83.3%) were isolated from screening specimens (i.e., rectal swabs and faecal specimens). The remaining VRE were from urine (2/24, 8.3%), a biopsy sample (1/24, 4.2%) or a groin specimen (1/24, 4.2%).

The number of patients with VRE confirmed each year over the last 10 years is shown in Figure 1.

**Figure 1: Species and *van* genotype of VRE isolated in New Zealand, 2012-2021**



In 2021 the lowest number of VRE were isolated in New Zealand since 2006. The low number of VRE isolated in New Zealand is likely to have been affected by the COVID-19 pandemic. Between 2015-2019 the proportion of VRE attributed to *vanA* increased whilst *vanB* decreased, and this trend continued into 2020 and 2021.

In 2021 the majority (15/24, 62.5%) of VRE were isolated from patients in Auckland hospitals: Auckland City Hospital (10/24, 41.7%) and Middlemore Hospital (5/24, 20.8%). Outside the Auckland region, Waikato Hospital had the largest number of isolates (5/24, 20.8%).

No VRE clusters were identified by either PFGE or WGS in 2021. Table 1 shows the various VRE strains identified. Only eight of the VRE (8/24, 33.3%) could be assigned to a PFGE profile, with the remainder having a distinct PFGE profile. By contrast WGS enabled the multilocus sequence type (MLST) of all isolates to be determined. Most VRE (22/24, 91.7%) belonged to the MLST clonal complex 17 (CC17), a hospital-adapted *E. faecium* lineage, although a range of sequence types were found.

**Table 1. Distribution of patients with VRE by healthcare facility, 2021**

Species	<i>van</i> gene	Referred from	PFGE profile/'strain' <sup>1</sup>	MLST/CC <sup>2</sup>	Number of patients
<i>E. faecium</i>	<i>vanA</i>	Auckland City Hospital	EfBA	ST1421/CC17	1
			EfBE	ST761/CC17	1
			EfBF	ST80/CC17	1
			distinct <sup>3</sup>	ST18/CC17	1
			distinct	ST80/CC17	1
			distinct	ST203/CC17	1
			distinct	ST2169/CC22	1
	Waikato Hospital	distinct	ST18/CC17	3	
		distinct	ST80/CC17	1	
		distinct	ST409/undef <sup>4</sup>	1	
	Middlemore Hospital	EfBE	ST761/CC17	1	
		EfBG	ST761/CC17	2	
		distinct	ST80/CC17	1	
	Dunedin Hospital	EfBA	ST1421/CC17	1	
	Whangarei Hospital	distinct	ST1421/CC17	1	
	<i>vanB</i>	Auckland City Hospital	EfAW	ST78/CC17	1
			distinct	ST78/CC17	2
Middlemore Hospital		distinct	ST80/CC17	1	
Wellington Hospital		distinct	ST17/CC17	2	

- 1 In-house pulsed-field gel electrophoresis (PFGE) profile designations. PFGE profiles were analysed with BioNumerics software version 7.6 (Applied Maths, St-Martens-Latem, Belgium) using the Dice coefficient and unweighted-pair group method with arithmetic averages, at settings of 0.5% optimisation and 1.5% position tolerance. The PFGE profiles of isolates designated as the same strain share  $\geq 90\%$  similarity.
- 2 MLST, multilocus sequence type; CC, MLST clonal complex. Derived from whole genome sequencing data, according to the scheme described on the *E. faecium* MLST website at <https://pubmlst.org/efaecium/>.
- 3 PFGE profile distinct (ie,  $< 90\%$  similarity) from any of the profiles designated a strain name.
- 4 Undefined clonal complex. Differs from ST17 at three loci.

The antimicrobial susceptibility among the 2021 VRE isolates is shown in Table 2. Resistance to ampicillin and ciprofloxacin is common in strains belonging to the CC17 lineage, which was observed in *E. faecium* from New Zealand in 2023 (Table 2). The presence of the *vanA* gene cluster conferred high-level teicoplanin resistance whereas isolates with *vanB* were all teicoplanin-susceptible. All isolates in 2021 were multiresistant.

**Table 2. Resistance among VRE isolates in New Zealand, 2021**

Antimicrobial agent	Percent resistance (%)		
	<i>E. faecium</i>		
	<i>vanA</i> n = 18	<i>vanB</i> n = 6	All n = 24
Ampicillin	94.4	100.0	95.8
Ciprofloxacin	100.0	100.0	100.0
Gentamicin high-level	33.3	16.7	29.2
Linezolid	0.0	0.0	0.0
Quinupristin/dalfopristin	38.9	33.3	37.5
Streptomycin high-level	72.2	50.0	66.7
Teicoplanin	100.0	0.0	75.0
Tetracycline	88.9	83.3	87.5
Multiresistant <sup>1</sup>	100.0	100.0	100.0

1 Resistant to ≥3 classes of antibiotics, in addition to glycopeptides.

## References

- 1 European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 11.0; 2021 Jan. Available from: <https://www.eucast.org>.
- 2 Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. 31<sup>st</sup> ed. Wayne, USA: CLSI; 2021. CLSI supplement M100.
- 3 Clark NC, Cooksey RC, Hill BC, Swenson JM, Tenover FC. Characterization of glycopeptide-resistant enterococci from U.S. hospitals. *Antimicrob Agents Chemother*. 1993 Nov;37(11):2311-7. doi: 10.1128/aac.37.11.2311. PMID: 8285611; PMCID: PMC192384.
- 4 Dahl KH, Simonsen GS, Olsvik O, Sundsfjord A. Heterogeneity in the *vanB* gene cluster of genomically diverse clinical strains of vancomycin-resistant enterococci. *Antimicrob Agents Chemother*. 1999;43(5):1105-1110.
- 5 Boyd DA, Kibsey P, Roscoe D, Mulvey MR. *Enterococcus faecium* N03-0072 carries a new VanD-type vancomycin resistance determinant: characterization of the VanD5 operon. *J Antimicrob Chemother*. 2004 Sep;54(3):680-3.
- 6 Fines M, Perichon B, Reynolds P, Sahm DF, Courvalin P. VanE, a new type of acquired glycopeptide resistance in *Enterococcus faecalis* BM4405. *Antimicrob Agents Chemother*. 1999;43(9):2161-2164.
- 7 McKessar SJ, Berry AM, Bell JM, Turnidge JD, Paton JC. Genetic characterization of vanG, a novel vancomycin resistance locus of *Enterococcus faecalis*. *Antimicrob Agents Chemother*. 2000 Nov;44(11):3224-8.
- 8 Lebreton F, Depardieu F, Bourdon N, et al. D-Ala-d-Ser VanN-type transferable vancomycin resistance in *Enterococcus faecium*. *Antimicrob Agents Chemother*. 2011;55(10):4606-4612. doi:10.1128/AAC.00714-11
- 9 Available at <https://github.com/tseemann/nullarbor>.
- 10 Souvorov, A., Agarwala, R. & Lipman, D. SKESA: strategic k-mer extension for scrupulous assemblies. *Genome Biol* 19, 153 (2018). <https://github.com/ncbi/SKESA>
- 11 Available at <https://github.com/tseemann/mlst>.
- 12 Available at <https://github.com/tseemann/abricate>.
- 13 Bortolaia V, Kaas RS, Ruppe E, et al. ResFinder 4.0 for predictions of phenotypes from genotypes. *J Antimicrob Chemother*. 2020 Dec 1;75(12):3491-3500.