

Vancomycin-resistant enterococci, 2019

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 is confirmed as phenotypically vancomycin resistant, by gradient strip on Mueller-Hinton agar and interpreted according to European Committee on Antimicrobial Susceptibility Testing (EUCAST)¹ breakpoints. The *van* gene is identified by PCR^{2,3,4,5,6,7}, susceptibility to a range of antibiotics is determined, and isolates are typed by pulsed-field gel electrophoresis (PFGE). In addition, the index isolate of each new PFGE profile identified among vancomycin-resistant *E. faecium* is typed by multilocus sequence typing (MLST).⁸

A total of 110 vancomycin-resistant enterococci were referred to ESR in 2019: 104 *E. faecium* and six *E. faecalis*. Of these, six *E. faecium* and three *E. faecalis* were not phenotypically vancomycin resistant. Six isolates were excluded from the dataset as they were duplicates, including three isolates that were referred by two different laboratories.

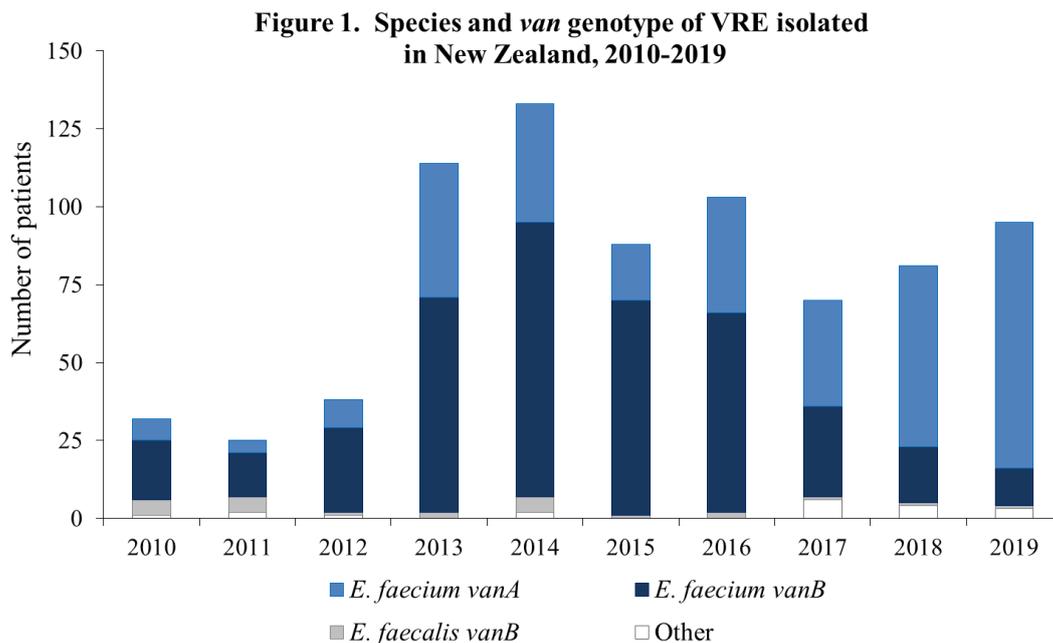
VRE from 88 patients were confirmed in 2019. While 88 patients were identified with VRE, this report includes results for 95 VRE isolates, as two distinct VRE strains were isolated from each of four patients and four distinct VRE strains were isolated from one patient. The site of isolation was reported for all 95 isolates. The majority (92/95, 96.8%) were isolated from screening specimens (i.e., rectal swabs and faecal specimens). The remaining VRE were from blood (1/95, 1.1%), a biopsy sample (1/95, 1.1%) and gallbladder drain fluid (1/95, 1.1%).

The species and *van* genotype distribution of the 95 VRE confirmed in 2019 was as follows:

- 79 *vanA E. faecium*;
- 12 *vanB E. faecium*;
- 1 *vanD E. faecium*;
- 2 *vanA E. faecalis*; and
- 1 *vanB E. faecalis*.

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

There were slightly more VRE isolates in 2019 compared to 2017 and 2018. The prevalence of *vanA E. faecium* continued to increase, accounting for 83.2% of all VRE in New Zealand in 2019. This contrasts with the prevalence of *vanB E. faecium* between 2010 and 2016. Australia has also reported an increased proportion of *vanA* in recent years.⁹



In 2019 the majority (64/95, 65.3%) of VRE were isolated from patients in Auckland hospitals: Middlemore Hospital (32/95, 32.7%), Auckland City Hospital (25/95, 26.5%) and North Shore Hospital (6/95, 6.1%). Waikato Hospital accounted for the next largest number of isolates (11/95, 11.2%). Patients who were in more than one hospital were only counted in the first hospital.

Table 1 also shows the various VRE strains identified in 2019. Most *vanA E. faecium* (36/95, 45.6%) had distinct pulsed field gel electrophoresis (PFGE) profiles. Of those that could be assigned to a strain, the most common strains were EfBF and EfBE, both of which belong to the MLST clonal complex (CC) 17 – a hospital-adapted *E. faecium* lineage. Strain EfBF (ST80) and strain EfBG (ST761) were newly identified in 2019. The 16 isolates of strain EfBF were found only in the Auckland region whereas the six EfBG isolates were found in both Auckland (5) and Christchurch (1).

Most *vanB E. faecium* isolates had distinct PFGE profiles (Table 1). There were a small number of isolates assigned to each of EfAW, EfAU and EfAP, which belong to CC17. All strains had been found in New Zealand in previous years.

We are currently undertaking a project to assess the ability of Illumina-based whole genome sequencing to type New Zealand VRE. It is hoped that the 2021 annual report will contain typing information from this new platform.

Table 1. Distribution of patients with VRE by healthcare facility, 2019

Species	van gene	Referred from	PFGE profile / ¹ strain ¹	MLST/CC ²	Number of patients	
<i>E. faecium</i>	A	Middlemore Hospital	EfBF	ST80/CC17	8	
			EfBE	ST761/CC17	8	
			EfBG	ST761/CC17	1	
			EfBA	ST1421/CC17	1	
			EfAX	ST80/CC17	1	
			distinct ³		8	
		Auckland City Hospital	EfBF	ST80/CC17	7	
			EfBE	ST761/CC17	4	
			EfBG	ST761/CC17	2	
			EfBA	ST1421/CC17	1	
		distinct			8	
		Waikato Hospital	EfBA	ST1421/CC17	1	
			distinct		7	
		Auckland community	EfBG	ST761/CC17	1	
			EfBF	ST80/CC17	1	
			EfBE	ST761/CC17	1	
			distinct		2	
		North Shore Hospital	EfBG	ST761/CC17	1	
			distinct		4	
		Christchurch Hospital	EfBE	ST80/CC17	1	
			EfBG	ST761/CC17	1	
			distinct		3	
		Hawke's Bay Hospital	distinct		2	
		Wellington Hospital	EfBA	ST1421/CC17	2	
		Dunedin Hospital	EfBA	ST1421/CC17	1	
			distinct		1	
		Nelson Hospital	distinct		1	
		B	Middlemore Hospital	EfAP	ST796/CC17	2
				EfAU	ST203/CC17	1
			Auckland City Hospital	EfAW	ST78/CC17	1
				distinct		1
			Waikato Hospital	distinct		2
North Shore Hospital	EfAP		ST796/CC17	1		
Hawke's Bay Hospital	EfAW		ST78/CC17	1		
Wellington Hospital	distinct			1		
Christchurch Hospital	distinct			1		
Dunedin Hospital	EfAW		ST78/CC17	1		
D	Waikato Hospital	- ⁴		1		
<i>E. faecalis</i>	A	Auckland City Hospital	distinct		1	
		Middlemore Hospital	distinct		1	
	B	Middlemore Hospital	distinct		1	

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Footnotes for Table 1:

- 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. PFGE profile designations in boldface are profiles of strains newly identified in 2019.
- 2 MLST, multilocus sequence type; CC, MLST clonal complex. MLST only determined for vancomycin-resistant *E. faecium*. MLST performed according to the scheme described on the *E. faecium* MLST website at <https://pubmlst.org/efaecium/>, by either PCR and Sanger sequencing or whole genome sequencing.
- 3 PFGE profile distinct (ie, $<90\%$ similarity) from any of the profiles designated a strain name.
- 4 The *vanD E. faecium* isolate was not typed.

The antimicrobial susceptibility among the 2019 VRE is shown in Table 2.

Table 2. Resistance among VRE in New Zealand, 2019

Antimicrobial agent ¹	Percent resistance (%)		
	<i>E. faecium</i> ²		All ³ n = 95
	<i>vanA</i> n = 79	<i>vanB</i> n = 12	
ampicillin	98.7	100.0	95.8
ciprofloxacin	91.4	100.0	91.6
gentamicin high-level	19.0	58.3	26.3
linezolid	1.3	0.0	2.1
nitrofurantoin ⁴	-	-	0.0 ⁴
quinupristin/dalfopristin ⁵	43.0	8.3	38.0
streptomycin high-level	54.4	8.3	48.4
teicoplanin	94.9	0.0	82.1
tetracycline	86.1	91.7	86.3
multiresistant ⁶	91.1	91.7	89.5

- 1 Teicoplanin susceptibilities were determined by gradient strip minimum inhibitory concentrations (MICs). Ampicillin, ciprofloxacin, gentamicin, linezolid, nitrofurantoin (*E. faecalis* only), quinupristin/dalfopristin (*E. faecium* only), streptomycin and tetracycline susceptibilities were determined by disc testing. MICs and zones of inhibition were interpreted according to the current EUCAST clinical breakpoints, except for tetracycline zones which were interpreted according to the current CLSI breakpoints.
- 2 Susceptibility data not shown separately for the *E. faecium* with *vanD*, but this data is included in the results for all VRE. The isolate was resistant to ampicillin, ciprofloxacin and teicoplanin.
- 3 Susceptibility data not shown separately for the *E. faecalis* isolates, but this data is included in the results for all VRE. The two *vanA E. faecalis* were resistant to ciprofloxacin, high-level gentamicin, tetracycline and teicoplanin. The *vanB E. faecalis* was resistant to high-level gentamicin, tetracycline and teicoplanin.
- 4 The overall rate of resistance is for the three *E. faecalis* isolates only, as the EUCAST nitrofurantoin breakpoints are specifically for *E. faecalis*.
- 5 *E. faecalis* are considered intrinsically resistant to quinupristin/dalfopristin, so the overall rate of resistance is only for the 92 *E. faecium* isolates.
- 6 Resistant to ≥ 3 classes of antibiotics in addition to glycopeptides (quinupristin/dalfopristin resistance not included for *E. faecalis* and nitrofurantoin resistance not included for *E. faecium*).

References

- ¹ European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 9.0; 2019 Jan. Available from: URL: https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_9.0_Breakpoint_Tables.pdf.
- ² 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.
- ³ 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. doi:10.1128/AAC.43.5.1105
- ⁴ 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. doi: 10.1093/jac/dkh391. Epub 2004 Aug 12. PMID: 15308604.
- ⁵ 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. doi:10.1128/AAC.43.9.2161
- ⁶ 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. doi: 10.1128/aac.44.11.3224-3228.2000. PMID: 11036060; PMCID: PMC101640.
- ⁷ 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
- ⁸ Homan WL, Tribe D, Poznanski S, Li M, Hogg G, Spalburg E, Van Embden JD, Willems RJ. Multilocus sequence typing scheme for *Enterococcus faecium*. *J Clin Microbiol*. 2002 Jun;40(6):1963-71. doi: 10.1128/jcm.40.6.1963-1971.2002. PMID: 12037049; PMCID: PMC130786.
- ⁹ Lee RS, Gonçalves da Silva A, Baines SL, Strachan J, Ballard S, Carter GP, Kwong JC, Schultz MB, Bulach DM, Seemann T, Stinear TP, Howden BP. The changing landscape of vancomycin-resistant *Enterococcus faecium* in Australia: a population-level genomic study. *J Antimicrob Chemother*. 2018 Dec 1;73(12):3268-3278. doi: 10.1093/jac/dky331. PMID: 30189014.