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Australian Group on Antimicrobial Resistance (AGAR) Australian Enterococcal Surveillance Outcome Program (AESOP) Bloodstream Infection Annual Report 2023

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on behalf of the Australian Group on Antimicrobial Resistance

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Australian Group on Antimicrobial Resistance (AGAR) Australian Enterococcal Surveillance Outcome Program (AESOP) Bloodstream Infection Annual Report 2023

Geoffrey W Coombs, Denise A Daley, Princy Shoby, Auriane Form, Shakeel Mowlaboccus, on behalf of the Australian Group on Antimicrobial Resistance

Abstract

From 1 January to 31 December 2023, fifty-six institutions across Australia participated in the Australian Enterococcal Surveillance Outcome Program (AESOP). The aim of AESOP 2023 was to determine the proportion of enterococcal bacteraemia isolates in Australia that were antimicrobial resistant, and to determine the *Enterococcus faecium* molecular epidemiology. Of the 1,599 unique episodes of enterococcal bacteraemia investigated, 92.9% were caused by either *E. faecalis* (51.8%) or *E. faecium* (41.1%). Ampicillin and vancomycin resistance were not detected in *E. faecalis* but were detected in 94.2% and 50.8% of *E. faecium* respectively. Two linezolid-resistant *E. faecalis* were identified in 2023. Both isolates had linezolid minimum inhibitory concentrations (MICs) of 6.0 mg/L, were vancomycin susceptible, and harboured the *optrA* gene.

Overall, 53.2% of *E. faecium* harboured either the *vanA* or the *vanB* gene; of these, 27.3% harboured *vanA*, 72.1% harboured *vanB*, and 0.6% harboured *vanA* and *vanB*. The percentage of vancomycin-resistant *E. faecium* bacteraemia isolates in Australia remains substantially higher than that recorded in most European countries. The *E. faecium* isolates consisted of 58 multi-locus sequence types (STs); 85.7% of isolates were classified into seven major STs, each containing ten or more isolates. All major STs belonged to clonal complex (CC) 17, a global hospital-adapted polyclonal *E. faecium* CC. The major STs (ST78, ST1424, ST17, ST80, ST796, ST1421 and ST555) were found across most regions of Australia, with ST78 identified in all regions. Overall, 58.3% of isolates belonging to the seven major STs harboured the *vanA* or *vanB* gene. AESOP 2023 has shown that enterococcal bacteraemia episodes in Australia continues to be frequently caused by polyclonal ampicillin-resistant high-level gentamicin-resistant *vanA*- or *vanB*-positive *E. faecium* which have limited treatment options.

Keywords: Australian Group on Antimicrobial Resistance (AGAR); antimicrobial resistance surveillance; *Enterococcus faecium*; *Enterococcus faecalis*; vancomycin resistant enterococci (VRE); bacteraemia

Background

Globally, *Enterococcus* accounts for approximately 10% of all bacteraemia cases and is the fourth and fifth leading cause of sepsis in North America and Europe, respectively.¹ In the 1970s, healthcare-associated enterococcal infections were primarily due to *Enterococcus faecalis*, but there has been a steady increasing prevalence of *E. faecium* nosocomial infections.^{2,3} Worldwide, the increase in nosocomial *E. faecium* infections has primarily been due to the expansion of polyclonal hospital-adapted clonal complex (CC) 17 strains. While innately resistant to many antimicrobial classes, *E. faecium* has further demonstrated a remarkable capacity to evolve new antimicrobial resistances. In 2009, the Infectious Diseases Society of America highlighted *E. faecium* as one of the key problem bacteria or ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species) requiring new therapies.⁴ In 2024, the World Health Organisation listed vancomycin-resistant *E. faecium* in its bacterial priority list of pathogens.⁵

The Australian Group on Antimicrobial Resistance (AGAR) is a network of laboratories located across Australia that commenced surveillance of antimicrobial resistance in *Enterococcus* species in 1995.⁶ In 2011, AGAR commenced the Australian Enterococcal Sepsis Outcome Program,^{7,8} now known as the Australian Enterococcal Surveillance Outcome Program (AESOP). The objective of AESOP 2023 was to determine the proportion of *E. faecalis* and *E. faecium* bacteraemia isolates demonstrating antimicrobial resistance with particular emphasis on:

1. assessing susceptibility to ampicillin;
2. assessing susceptibility to glycopeptides; and
3. the molecular epidemiology of *E. faecium*.

Methodology

Participants

Thirty-two laboratories servicing 56 institutions from all Australian states and mainland territories.

Collection period

From 1 January to 31 December 2023, the 32 laboratories collected all enterococcal species isolated from blood cultures. Enterococci of the same species and antimicrobial susceptibility profiles isolated from a patient's blood culture within 14 days of the first positive culture were excluded. A new enterococcal bacteraemia episode in the same patient was recorded if it was confirmed by a further culture of blood taken more than 14 days after the initial positive culture. Data were collected on age, sex, dates of admission and discharge (if admitted), and mortality at seven and 30 days from date of blood culture collection. To avoid interpretive bias, no attempt was made to assign attributable mortality. Each episode of bacteraemia was designated as 'hospital-onset' if the first positive blood culture(s) in an episode was collected > 48 hours after admission.

Laboratory testing

Enterococcal isolates were identified to the species level by the participating laboratories using matrix-assisted laser desorption ionisation (MALDI)—MALDI Biotyper (Bruker Daltonics, USA) or Vitek-MS (bioMérieux, France)—or by the Vitek2® (bioMérieux). Antimicrobial susceptibility testing was performed using the Vitek2® (bioMérieux) or the BD Phoenix™ (Becton Dickinson, USA) automated microbiology systems, according to the manufacturer's instructions. Minimum inhibitory concentration (MIC) data and isolates were referred to the AESOP reference laboratory at Murdoch University. The European Committee on Antimicrobial Susceptibility Testing (EUCAST)⁹ MIC breakpoints were utilised for interpretation. Linezolid and daptomycin non-susceptible isolates and vancomycin-susceptible isolates which harboured the *vanA* or *vanB* genes were retested by Etest® (bioMérieux) using Mueller-Hinton agar as recommended by the manufacturer. The control strain used was *E. faecalis* ATCC® 29212. For all *E. faecium* isolates received, whole genome sequencing (WGS) was performed by the AESOP reference laboratory at Murdoch University on the Illumina NextSeq™ 500 platform.

The multilocus sequence type (ST) was determined using the PubMLST website; *van* genes were identified using nucleotide sequences from the NCBI database and a BLAST interface.

Confidence intervals for proportions, Fisher's exact test for categorical variables, and chi-square test for trend were calculated, if appropriate, using MedCalc for Windows, version 12.7 (MedCalc Software, Belgium).

Approval to conduct the prospective data collection was given by the research ethics committee associated with each participating laboratory.

Results

From 1 January to 31 December 2023, there were 1,599 unique episodes of enterococcal bacteraemia identified. Although twelve different enterococcal species were identified, 828 isolates (51.8%) were *E. faecalis* and 657 isolates (41.1%) were *E. faecium*. One hundred and fourteen enterococci were identified either as *E. lactis* (previously identified as *E. faecium*, 35 isolates), *E. gallinarum* (28 isolates), *E. casseliflavus* (25 isolates), *E. avium* (10 isolates), *E. raffinosus* (6 isolates), *E. durans* (5 isolates), *E. hirae* (2 isolates), *E. gilvus* (1 isolate), *E. mundtii* (1 isolate) or *E. cecorum* (1 isolate).

A significant difference was observed in patient sex ($p < 0.0001$), with 1,039 (65.0%) being male (95% confidence interval [95% CI]: 61.1–69.1). The average age of patients was 63 years, ranging from 0 to 99 years, with a median age of 68 years.

Overall, isolates were evenly divided by place of onset: 801/1,599 (50.1%) were community-onset and 798/1,599 (49.9%) were hospital-onset. However, a significant difference ($p < 0.01$) was observed between *E. faecium* and *E. faecalis* in place of onset: only 26.5% (95% CI: 22.7–30.7) of *E. faecium* episodes were community-onset, compared to 67.3% (95% CI: 61.8–73.1) of *E. faecalis* episodes. All-cause mortality at 30 days, where outcome was known, was 20.4% (95% CI: 18.1–23.0). There was a significant difference in mortality between *E. faecalis* and *E. faecium* episodes (17.0% vs 26.3% respectively, $p < 0.01$). There was no significant difference in mortality between vancomycin susceptible and vancomycin non-susceptible *E. faecium* episodes (23.6% vs 28.7% respectively, $p = 0.17$).

Enterococcus faecalis phenotypic susceptibility results

Apart from erythromycin, high-level gentamicin, and tetracycline, acquired resistance was rare amongst *E. faecalis* isolates (Table 1). Four *E. faecalis* isolates (0.5%) were initially reported as linezolid resistant (EUCAST breakpoint > 4 mg/L). However, by Etest®, two of the four referred isolates had linezolid MICs of 4.0 mg/L and were therefore considered linezolid susceptible. The remaining two isolates had a linezolid MIC of 6.0 mg/L and were classified as linezolid resistant by EUCAST criteria. The two resistant isolates harboured the *optrA* gene.

Table 1: The number and proportion of *E. faecalis* isolates non-susceptible to ampicillin, penicillin, and the non- β -lactam antimicrobials, EUCAST breakpoints,^a AGAR, 2023

Antimicrobial	Isolates (n)	Susceptible, increased exposure % (n)	Resistant % (n)
Ampicillin	818	0.0 (0)	0.0 (0)
Benzylpenicillin	638	— ^b	— ^b
Daptomycin	761	— ^b	— ^b
Linezolid	820	— ^c	0.2 (2)
Teicoplanin	821	— ^c	0.0 (0)
Vancomycin	821	— ^c	0.0 (0)

a EUCAST: European Committee on Antimicrobial Susceptibility Testing.

b No category defined.

c No guidelines for indicated species.

Table 2: The number and proportion of *E. faecium* isolates non-susceptible to ampicillin, penicillin, and the non- β -lactam antimicrobials, EUCAST breakpoints,^a AGAR, 2023

Antimicrobial	Isolates (n)	Susceptible, increased exposure % (n)	Resistant % (n)
Ampicillin	652	0.0 (0)	94.2 (614)
Benzylpenicillin	498	— ^b	— ^b
Daptomycin	82	— ^b	— ^b
Linezolid	653	— ^c	0.0 (0)
Teicoplanin	647	— ^c	12.7 (82)
Vancomycin	656	— ^c	50.8 (333)

a EUCAST: European Committee on Antimicrobial Susceptibility Testing.

b No category defined.

c No guidelines for indicated species.

Enterococcus faecium phenotypic susceptibility results

The majority of *E. faecium* were resistant to multiple antimicrobials including ampicillin, erythromycin, high-level gentamicin, and tetracycline (Table 2). Overall, 333 *E. faecium* isolates (50.8%) were phenotypically vancomycin resistant by EUCAST criteria. Eighty-two isolates (12.7%) were teicoplanin resistant. Three isolates (0.5%) were initially reported as linezolid non-susceptible (EUCAST breakpoint > 4 mg/L). By Etest[®], the three isolates had linezolid MICs of 0.5, 1.5 and 4.0 mg/L and were therefore considered linezolid susceptible.

Genotypic vancomycin susceptibility results

For 349 (42.1%) of the 828 *E. faecalis* isolates, *vanA/vanB* polymerase chain reaction (PCR) testing was performed by the referring laboratories. No *vanA/vanB* genes were detected in *E. faecalis*.

The presence of *vanA* or *vanB* genes was determined by PCR and/or WGS on 639 (97.3%) of the 657 *E. faecium* isolates. Overall, 340 of the 639 isolates (53.2%) harboured a *vanA* and/or *vanB* gene. Of the vancomycin non-susceptible *E. faecium* isolates (Vitek[®] 2 vancomycin MIC > 4 mg/L), 87 harboured *vanA* and 235 harboured *vanB*. Two vancomycin non-susceptible isolates harboured both *vanA* and *vanB*. The *vanA* or *vanB* gene was detected in sixteen vancomycin-susceptible *E. faecium* isolates. Six isolates, with vancomycin MICs ranging from \leq 0.5 to 4.0 mg/L and teicoplanin MICs ranging from \leq 0.5 to 2.0 mg/L, harboured *vanA*. The ten *vanB*-positive isolates had vancomycin MICs ranging from \leq 0.5 mg/L to 4.0 mg/L.

E. faecium molecular epidemiology

Of the 657 episodes, 610 *E. faecium* isolates (92.8%) were available for WGS. The 610 isolates were classified into 58 STs, including seven STs with ten or more isolates (Table 3). Of the 51 STs with fewer than 10 isolates each, 37 were each represented by only one isolate. Overall, 523 (85.7%) of the 610 isolates were grouped into the seven major STs (greater than ten isolates). Using eBURST, all major STs were grouped into CC17.

Geographical distribution of the STs varied (Table 3). Amongst the seven major STs, ST78 (141 isolates) was identified in all regions; ST1424 (113 isolates) was identified in all regions except Western Australia and the Northern Territory; ST17 (96 isolates) and ST80 (70) were identified in all regions except the Northern Territory; ST796 (47 isolates) was only identified in Victoria, South Australia, Western Australia and the Northern Territory; ST1421 (37 isolates) was identified in all regions except the Northern Territory and the Australian Capital Territory; and ST555 (19 isolates) was identified only in New South Wales, Victoria, South Australia, Western Australia and the Northern Territory.

The *vanA* gene was detected in four major STs (79 isolates from ST1424, ST17, ST80 and ST1421) (Table 4). The *vanB* gene was detected in all seven major STs (224 isolates). Four minor STs (ST817, ST2220, ST761 and ST375) harboured at least one *vanA*-positive isolate; six minor STs (ST2439, ST2682, ST192, ST203, ST2693 and ST2690) harboured at least one *vanB*-positive isolate.

Table 3: The number and proportion of major *Enterococcus faecium* sequence types, AGAR, 2023, by state and territory

MLST ^a	Percentage, % (n) ^b								
	ACT	NSW	NT	Qld	SA	Tas.	Vic.	WA	Australia
ST78	48.0 (12)	28.5 (49)	5.9 (1)	4.7 (3)	22.2 (12)	7.1 (2)	27.5 (47)	19.0 (15)	23.1 (141)
ST1424	16.0 (4)	26.7 (46)	0.0 (0)	32.8 (21)	9.3 (5)	53.6 (15)	12.9 (22)	0.0 (0)	18.5 (113)
ST17	4.0 (1)	7.6 (13)	0.0 (0)	31.3 (20)	9.3 (5)	25.0 (7)	9.4 (16)	43.0 (34)	15.7 (96)
ST80	20.0 (5)	6.4 (11)	0.0 (0)	20.3 (13)	3.7 (2)	3.6 (1)	12.9 (22)	20.3 (16)	11.5 (70)
ST796	0.0 (0)	0.0 (0)	11.8 (2)	0.0 (0)	3.7 (2)	0.0 (0)	23.4 (40)	3.8 (3)	7.7 (47)
ST1421	4.0 (1)	12.8 (22)	41.2 (7)	3.1 (2)	3.7 (2)	0.0 (0)	0.6 (1)	2.5 (2)	6.1 (37)
ST555	0.0 (0)	0.6 (1)	17.6 (3)	0.0 (0)	24.1 (13)	0.0 (0)	0.6 (1)	1.3 (1)	3.1 (19)
Other types (n = 58)	8.0 (2)	17.4 (30)	23.5 (4)	7.8 (5)	24.1 (13)	10.7 (3)	12.9 (22)	10.1 (8)	14.3 (87)
Total	25	172	17	64	54	28	171	79	610

^a MLST: multi-locus sequence type.

^b ACT: Australian Capital Territory; NSW: New South Wales; NT: Northern Territory; Qld: Queensland; SA: South Australia; Tas.: Tasmania; Vic.: Victoria; WA: Western Australia.

Table 4: The number and proportion of major *Enterococcus faecium* sequence types harbouring *vanA/vanB* genes, AGAR, 2023

MLST ^b	Percentage ^a (n)				Total, n
	<i>vanA</i>	<i>vanB</i>	<i>vanA</i> and <i>vanB</i>	<i>vanA</i> or <i>vanB</i> not detected	
ST78	0.0 (0)	100.0 (141)	0.0 (0)	0.0 (0)	141
ST1424	39.8 (45)	3.5 (4)	0.0 (0)	56.6 (64)	113
ST17	2.1 (2)	8.3 (8)	0.0 (0)	89.6 (86)	96
ST80	1.4 (1)	10.0 (7)	1.4 (1)	87.1 (61)	70
ST796	0.0 (0)	97.9 (46)	2.1 (1)	0.0 (0)	47
ST1421	83.8 (31)	2.7 (1)	0.0 (0)	13.5 (5)	37
ST555	0.0 (0)	89.5 (17)	0.0 (0)	10.5 (2)	19
Other types	6.9 (6)	10.3 (9)	0.0 (0)	82.8 (72)	87
Total	13.9 (85)	38.2 (233)	0.3 (2)	47.5 (290)	610

^a Percentage of total with *van* genes.

^b MLST: multi-locus sequence type.

Discussion

Enterococci are intrinsically resistant to a broad range of antimicrobials including the cephalosporins and sulfonamides. Because of their ability to acquire additional resistance through the transfer of plasmids and transposons and to disseminate easily in the hospital environment, enterococci have become difficult to treat and provide major infection control challenges.

In AESOP 2023, a total of 41.1% of enterococcal bacteraemia were due to *E. faecium*, of which 50.8% (95% CI: 45.5–56.5) were phenotypically vancomycin non-susceptible by Vitek2[®] or BD Phoenix[™]. However, 53.2% of *E. faecium* isolates tested (340/639) harboured a *vanA* and/or *vanB* gene, of which 27.3% were *vanA*-positive. Overall, 93 *E. faecium* isolates (14.6%) harboured the *vanA* gene. Over the last five years (2019–2023), there has been a significant decreasing trend in *vanA*-positive *E. faecium* in Australia (χ^2 for linear trend = 12.61, $p < 0.01$).^{10–14} This is primarily due to a decrease in the number of ST1424 isolates. The majority of *E. faecium* isolates were non-susceptible to multiple antimicrobials including ampicillin, erythromycin, high-level gentamicin, and tetracycline.

As the AGAR programs are similar to the antimicrobial surveillance programs conducted in Europe, comparison of Australian antimicrobial resistance data with other countries is possible.

In the 2022 European Antimicrobial Resistance Surveillance Network (EARS-Net) program, the national percentages of vancomycin-resistant *E. faecium* ranged from 0.0% in Iceland to 67.7% in Lithuania.^{15,16} The AESOP 2023 survey confirms that the incidence of vancomycin-resistant *E. faecium* bacteraemia in Australia continues to be a significant problem.

Ten (4.1%) of the 245 *vanB*-positive *E. faecium* and six (6.5%) of the 93 *vanA*-positive *E. faecium* isolates had a vancomycin MIC at or below the EUCAST susceptible breakpoint (≤ 4 mg/L) and therefore would not have been identified using routine phenotypic antimicrobial susceptibility methods.

By WGS, *E. faecium* was shown to be polyclonal, consistent with the known plasticity of the enterococcal genome. The seven major *E. faecium* STs identified form part of CC17, a global hospital-derived lineage that has successfully adapted to hospital environments. CC17 is characteristically ampicillin and quinolone resistant and subsequent acquisition of *vanA*- or *vanB*-containing transposons by horizontal transfer has resulted in multi-resistant CC17 enterococci with pandemic potential.

In AESOP 2023, seven *E. faecium* STs predominated: ST78 (100% harboured *vanB*); ST1424 (39.8% *vanA*, 3.5% *vanB*); ST17 (2.1% *vanA*, 8.3% *vanB*); ST80 (1.4% *vanA*, 10.0% *vanB*, 1.4% *vanA* and *vanB*); ST796 (97.9% *vanB*, 2.1% *vanA* and *vanB*); ST1421 (83.8% *vanA*, 2.7% *vanB*) and ST555 (0% *vanA*, 89.5% *vanB*).

Conclusions

The AESOP 2023 study has shown that, although predominately caused by *E. faecalis*, enterococcal bacteraemia in Australia is frequently caused by ampicillin-resistant, high-level gentamicin-resistant and vancomycin-resistant *E. faecium*. Furthermore, the percentage of *E. faecium* bacteraemia isolates resistant to vancomycin in Australia (50.8%) remains significantly higher than that seen in most European countries. In addition to being a significant cause of healthcare-associated bacteraemia, the emergence of multiple multi-resistant hospital-adapted *E. faecium* strains has become a major infection control issue in Australian hospitals.

Ongoing studies on the enterococcal genome will contribute to our understanding of the rapid and ongoing evolution of enterococci in the hospital environment and will assist in preventing their nosocomial transmission.

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