Australian Enterococcal Sepsis Outcome Programme annual report, 2013

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Abstract

From 1 January to 31 December 2013, 26 institutions around Australia participated in the Australian Enterococcal Sepsis Outcome Programme (AESOP). The aim of AESOP 2013 was to determine the proportion of enterococcal bacteraemia isolates in Australia that are antimicrobial resistant, and to characterise the molecular epidemiology of the *Enterococcus faecium* isolates. Of the 826 unique episodes of bacteraemia investigated, 94.6% were caused by either *E. faecalis* (56.1%) or *E. faecium* (38.5%). Ampicillin resistance was not detected in *E. faecalis* but was detected in over 90% of *E. faecium*. Vancomycin non-susceptibility was reported in 0.2% and 40.9% of *E. faecalis* and *E. faecium* respectively and was predominately due to the acquisition of the *vanB* operon. Overall, 41.6% of *E. faecium* harboured *vanA* or *vanB* genes. The percentage of *E. faecium* bacteraemia isolates resistant to vancomycin in Australia is significantly higher than that seen in most European countries. *E. faecium* isolates consisted of 81 pulsed-field gel electrophoresis pulsotypes of which 72.3% were classified into 14 major pulsotypes containing five or more isolates. Multilocus sequence typing grouped the 14 major pulsotypes into clonal cluster 17, a major hospital-adapted polyclonal *E. faecium* cluster. Of the 2 predominant sequence types, ST203 (80 isolates) was identified across Australia and ST555 (40 isolates) was isolated primarily in the western and central regions (Northern Territory, South Australia and Western Australia) respectively. In conclusion, the AESOP 2013 has shown enterococcal bacteraemias in Australia are frequently caused by polyclonal ampicillin-resistant high-level gentamicin resistant *vanB E. faecium*, which have limited treatment options. *Commun Dis Intell* 2014;38(4):E320–E326.

Keywords: antimicrobial resistance surveillance; *Enterococcus faecium, Enterococcus faecalis,* vancomycin resistant enterococci, bacteraemia

Introduction

Globally, enterococci are thought to account for approximately 10% of all bacteraemias, and in North America and Europe are the 4th and 5th leading cause of sepsis respectively.[1,2] Although in the 1970s healthcare-associated enterococcal infections were primarily due to *Enterococcus faecalis*, there has been a steadily increasing prevalence of *E. faecium* nosocomial infections.[3–5] While innately resistant to many classes of antibiotics, *E. faecium* has 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 (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumanii*, *Pseudomonas aeruginosa*, and *Enterobacter* species) pathogens requiring new therapies.[6]

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.[7] In 2011, AGAR commenced the Australian Enterococcal Sepsis Outcome Programme (AESOP).[8] The objective of AESOP 2013 is 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. molecular epidemiology of *E. faecium*.

Methods

Participants

Twenty-six laboratories from all 8 Australian states and territories participated in the program.

Collection period

From 1 January to 31 December 2013, the 26 laboratories collected all enterococcal species isolated from blood cultures. Enterococci with the same species and antimicrobial susceptibility profiles isolated from a patient's blood culture within 14 days of the 1st positive culture were excluded. A new enterococcal sepsis 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, date of admission and discharge (if admitted), and mortality at 30 days from the 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 1st positive blood culture(s) in an episode was collected more than 48 hours after admission.

Laboratory testing

Enterococcal isolates were identified to the species level by the participating laboratories using one of the following methods: API 20S (bioMérieux), API ID32Strep (bio-Mérieux), Vitek2® (bioMérieux), Phoenix (BD), matrix-assisted laser desorption ionization Biotyper (Bruker Daltonics), Vitek-MS (bioMérieux), polymerase chain reaction (PCR), or conventional biochemical tests. Antimicrobial susceptibility testing was performed by using the Vitek2® (bioMérieux, France) or the Phoenix™ (BD, USA) automated microbiology systems according to the manufacturer's instructions. Minimum inhibitory concentration (MIC) data and isolates were referred to the Australian Collaborating Centre for *Enterococcus* and *Staphylococcus* Species (*ACCESS*) Typing and Research. Clinical and Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints were utilised for interpretation.[9,10] Isolates with either a resistant or an intermediate category were classified as non-susceptible. Molecular testing including *vanA/B* PCR, pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing was performed as previously described.[11–13]

A chi-square test for comparison of 2 proportions was performed and 95% confidence intervals (95%CI) were determined using MedCalc for Windows, version 12.7 (Medcalc Software, Ostend 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 2013, 826 unique episodes of enterococcal bacteraemia were identified. Males comprised a significantly higher proportion of cases than females ($P = 0.02$) with 551 (66.7%) being male (95% CI, 63.4–69.9). The average age of patients was 62 years ranging from 0–99 years with a median age of 67 years. The place of onset was recorded for 804 of the 826 episodes, of which 426 (53.0%) were hospital onset (95% CI, 49.8–56.5). All cause mortality at 30 days was 18.9% (95% CI, 16.1–21.9).

Although 9 *Enterococcus* species were identified, 56.1% (463 isolates) were *E. faecalis* and 38.5% (318) were *E. faecium*. Forty-five enterococci were identified either as *Enterococcus casseliflavus* (16 isolates), *E. gallinarum* (10), *E. avium* (5), *E. hirae* (5) *E. raffinosus* (3), *E. durans* (3) or *E. gilvus* (1). Two isolates could not be identified to the species level.

Enterococcus faecalis phenotypic susceptibility

Apart from erythromycin, tetracycline, ciprofloxacin and high-level gentamicin, resistance was rare among *E. faecalis* (Table 1). Ampicillin resistance was not detected and only 1 isolate was vancomycin non-susceptible. Of concern, 29 (6.3%) *E. faecalis*, isolated across Australia, were linezolid non-susceptible (MIC = 4 mg/L). Less than 1% of isolates were non-susceptible to daptomycin and teicoplanin.

Enterococcus faecium phenotypic susceptibility

The majority of *E. faecium* were non-susceptible to multiple antimicrobials (Table 2). Most isolates were non-susceptible to ampicillin, erythromycin, tetracycline, ciprofloxacin, nitrofurantoin and high-level gentamicin. Overall, 130 (40.9%) of the 318 *E. faecium* were phenotypically vancomycin non-susceptible (MIC > 4 mg/L). Fifteen (4.7%) and 8 (2.5%) isolates were teicoplanin and linezolid non-susceptible respectively.

Genotypic vancomycin susceptibility

The vancomycin non-susceptible *E. faecalis* isolate (MIC ≥ 32 mg/L) harboured a *vanB* gene. *VanA/B* PCR was performed on 129 isolates of the 130 vancomycin non-susceptible *E. faecium* isolates. *VanA* was detected in 8 isolates (vancomycin and teicoplanin MICs ≥ 32 mg/L) and *vanB* in 121 isolates (vancomycin MICs 8 [4 isolates] and ≥ 32 mg/L [117 isolates]). Seven of the 8 *vanA E. faecium* isolates were from New South Wales. Of the 121 *vanB E. faecium* isolates, seven were teicoplanin resistant by EUCAST criteria (MIC > 32 mg/L). *VanA/B* PCR was performed on 181 of the 188 vancomycin susceptible *E. faecium* isolates of which eight (4.4%) harboured a *vanB* gene.

Enterocoocus faecium molecular epidemiology

By PFGE, 301 of the 318 *E. faecium* were classified into 81 pulsotypes, including 14 major pulsotypes with five or more isolates (Table 3). Of the 67 pulsotypes with less than 5 isolates, 58 had only 1 isolate. Overall, 219 (72.8%) of the 301 isolates were grouped into the 14 major pulsotypes from which

Table 1: The number and proportion of *Enterococcus faecalis* non-susceptible to ampicillin and the non-ß-lactam antimicrobials, Australia, 2013

Antimicrobial	Tested	Breakpoint (mg/L)	Non-susceptible	
			n	%
Ampicillin	463	>8*	0	
		>4†	0	
Vancomycin	463	>4‡	1	0.2
Erythromycin	451	>0.5‡	375	83.2
Tetracycline	419	>4‡	314	74.9
Ciprofloxacin	424	>1‡	91	21.5
Daptomycin	397	>4‡	1	0.3
Teicoplanin	462	>8*	3	0.6
		>2†	4	0.9
Linezolid	462	>2‡	29	6.3
Nitrofurantoin	454	>32*	8	1.8
		>64†	4	0.9
High level gentamicin	463	>128*	150	32.4

* Clinical and Laboratory Standards Institute (CLSI) non-susceptible breakpoint.

† European Committee on Antimicrobial Susceptibility Testing (EUCAST) non-susceptible breakpoint.

‡ CLSI and EUCAST non-susceptible breakpoint.

Table 2: The number and proportion of *Enterococcus faecium* non-susceptible to ampicillin and the non-ß-lactam antimicrobials, Australia, 2013

Antimicrobial	Tested	Breakpoint (mg/L)	Non-susceptible	
			n	%
Ampicillin	318	>8*	295	92.8
		>4†	296	93.1
Vancomycin	318	>4c‡	130	40.9
Erythromycin	309	>0.5‡	296	95.8
Tetracycline	294	>4‡	146	49.7
Ciprofloxacin	305	>1‡	290	95.1
Teicoplanin	318	>8*	15	4.7
		>2†	15	4.7
Linezolid	315	>2‡	8	2.5
Nitrofurantoin	315	>32*	259	82.2
		>64†	114	36.2
High level gentamicin	317	>128*	196	61.8

* Clinical and Laboratory Standards Institute (CLSI) non-susceptible breakpoint.

† European Committee on Antimicrobial Susceptibility Testing (EUCAST) non-susceptible breakpoint.

‡ CLSI and EUCAST non-susceptible breakpoint.

8 multilocus sequence types (STs) were identified. Using eBURST, the 8 STs were grouped into clonal complex (CC) 17.

Of the 2 predominant sequence types, ST203 (80 isolates) was identified across Australia and ST555 (40 isolates), was isolated primarily in the western and central regions (Northern Territory, South Australia and Western Australia). ST796 (32 isolates) was only identified in Victoria while ST17 (23 isolates) was identified on the eastern coast (Queensland, New South Wales, Victoria) and in Western Australia. ST341 (19 isolates), ST192 (12 isolates) and ST18 (8 isolates) were

primarily identified in New South Wales, Victoria and Queensland respectively. ST761 (5 isolates) was identified only in New South Wales.

*Van*A or *vanB* genes were identified in 2 (5 isolates) and 10 (113 isolates) major pulsotypes respectively (Table 4). Efm22 (ST18) harboured *vanA* and *vanB* genes. Twelve minor pulsotypes (13 isolates) and 1 non-typed *E. faecium* isolates also harboured

Table 3: The number and proportion of major *Enterococcus faecium* pulsed-field gel electrophoresis pulsotypes, Australia, 2013, by state or territory

		ACT		NSW		NT		Qld		SA		Tas.		Vic.		WA		Aus.	
Type	ST	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Efm1		1	5.5	3	3.0	0		3	8.1	0		1	20.0	0		2	4.8	10	3.1
Efm2		0		0		0		11	29.7	15	46.9	0		5	6.3	0		31	9.8
Efm75	ST203	7	38.9	4	4.0	0		7	18.9	0		0		2	2.5	0		20	6.3
Efm76		0		13	12.9	0		0		0		0		0		0		13	4.1
Efm6		0		3	3.0	0		0		0		0		3	3.8	0		6	1.9
Efm4	ST555	0		0		0		0		8	25.0	0		1	1.3	22	52.4	31	9.8
Efm77		0		1	1.0	3	100	0		4	12.5	0		0		1	2.4	9	2.8
Efm74	ST796	0		0		0		0		0		0		32	40.0	0		32	10.1
Efm5	ST17	1	5.5	8	7.9	0		2	5.4	0		0		2	2.5	5	11.9	18	5.7
Efm18		0		5	5.0	0		0		0		0		0		0		5	1.6
Efm3	ST341	3	16.7	14	13.9	0		2	5.4	0		0		0		0		19	6.0
Efm24	ST192	0		2	1.9	0		0		0		0		10	12.7	0		12	3.8
Efm22	ST18	0		2	2.0	0		5	13.5	1	3.1	0		0		0		8	2.5
Efm78	ST761	0		5	5.0	0		0		0		0		0		0		5	1.6
Other	ND	6	33.3	29	28.7	0		6	16.2	4	12.5	4	80.0	22	27.9	11	26.2	82	25.9
ND	ND	0		12	11.9	0		1	2.7	0		0		3	3.8	1	2.4	17	5.4
Total		18		101		3		37		32		5		80		42		318	

ND = Not done

Table 4: Number and proportion of major *Enterococcus faecium* pulsed-field gel electrophoresis pulsotypes harbouring *vanA/B* genes, Australia, 2013

			vanA		vanB		Not detected	
Pulsotypes	ST	Number	n	%	n	%	n	%
Efm1		10	0		1	10.0	9	90.0
Efm2		31	0		31	100.0	0	
Efm75	ST203	20	0		2	10.0	18	90.0
Efm76		13	0		12	92.3	1	7.7
Efm6		6	0		0		6	100.0
Efm4	ST555	31	0		0		31	100.0
Efm77		9	0		9	100.0	0	
Efm74	ST796	32	0		32	100.0	0	
Efm5	ST17	18	0		3	16.7	15	83.3
Efm18		5	4	80.0	0		1	20.0
Efm3	ST341	19	0		19	100.0	0	
Efm24	ST192	12	0		3	25.0	9	75.0
Efm22	ST18	8	1	12.5	2	25.0	5	62.5
Efm78	ST761	5	0		0		5	100.0
Total		219	5	2.3	114	52.0	100	45.7

vanB genes. In addition, *vanA* genes were detected in 3 minor pulsotypes (3 isolates). Over 90% of Efm2 (ST203), Efm76 (ST203), Efm77 (ST555), Efm74 (ST796) and Efm3 (ST341) harboured *vanB* genes. In contrast, at least 90% of Efm1 (ST203), Efm75 (ST203), Efm6 (ST203), Efm4 (ST555) and Efm78 (ST761) did not harbour *van* genes. Four of the 8 *vanA E. faecium* isolates were characterised as Efm18 pulsotype (ST17).

Discussion

Enterococci are intrinsically resistant to a broad range of antimicrobials including the cephalosporins and sulphonamides. Through 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.

All data being collected in the AGAR sepsis programs are generated as part of routine patient care in Australia with most being available through laboratory and hospital bed management information systems. Isolates are referred to a central laboratory where strain and antimicrobial resistance determinant characterisation is performed. As the programs are similar to those conducted in Europe comparison of Australian antimicrobial resistance data with other countries is possible.[14]

In the 2012 European Centre for Disease Prevention and Control and Prevention Enterococci surveillance program the European Union/European Economic Area (EU/EEA) population-weighted mean percentage of *E. faecium* resistant to vancomycin was 8.1%. This ranged from 0.0% in Bulgaria, Croatia, Estonia, Iceland, Luxembourg, Netherlands, Slovenia and Sweden to 44.0% in Ireland. Germany (16.2%), Greece (17.2%) and Portugal (23.3%) were the only other EU/EEA countries to report levels above 15%.[15]

In AESOP 2013, approximately 40% of enterococcal bacteraemias were due to *E. faecium* of which 40.9% (95% CI, 35.4–46.5) were vancomycin non-susceptible. Unlike Europe, where vancomycin resistance has predominately been due to the acquisition of the *vanA* operon, almost all AESOP 2013 *E. faecium* isolates harbouring *van* genes carried the *vanB* operon. In addition to vancomycin resistance, the majority of *E. faecium* isolates were non-susceptible to multiple antimicrobials including ampicillin (92.8%, 95% CI, 89.4–95.4), and high level gentamicin (61.8%, 95%CI 56.2–67.2). In the previous AGAR enterococcal sepsis study, AESOP 2011, 37% and 90% of *E. faecium* harboured *vanA/B* genes and were ampicillin resistant respectively; suggesting the incidence of multi-drug-resistant *E. faecium* bacteraemia in Australia is increasing.

Eight (6.2%) of the 129 *vanB E. faecium* isolates had a vancomycin MIC at or below the CLSI and the EUCAST susceptible breakpoint (≤ 4 mg/L) and would not have been identified using routine phenotypic antimicrobial susceptibility methods.

With the use of PFGE, *E. faecium* was shown to be very polyclonal, consistent with the known plasticity of the enterococcal genome. The 14 major *E. faecium* pulsotypes formed 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 in CC17 clones has resulted in vancomycin resistant enterococci with pandemic potential. In AESOP 2013, 5 major pulsotypes not characterised in AESOP 2011 were identified, including: Efm74 (32 isolates), Efm75 (20 isolates), Efm76 (13 isolates), Efm77 (9 isolates) and Efm78 (5 isolates). Pulsotypes Efm 76 and Efm78 were identified in New South Wales and Efm74 in Victoria. Efm75 was identified in several regions on the east coast of Australia, while Efm77 was primarily in the central regions.

Conclusion

The AESOP 2013 study has shown that although predominately caused by *E. faecalis,* enterococcal bacteraemia in Australia is frequently caused by ampicillin-resistant high-level gentamicin-resistant *vanB E. faecium*. Furthermore the percentage of *E. faecium* bacteraemia isolates resistant to vancomycin in Australia is significantly higher than that seen in almost all European countries. In addition to being a significant cause of healthcare-associated sepsis, the emergence of multiple multi-resistant hospital-adapted *E. faecium* strains has become a major infection control issue in Australian hospitals. Further studies on the enterococcal genome will contribute to our understanding of the rapid and ongoing evolution of enterococci in the hospital environment and assist in preventing their nosocomial transmission.

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