Australian Group on Antimicrobial Resistance (AGAR) Australian Enterococcal Sepsis Outcome Programme (AESOP) Annual Report 2013

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Abstract

From 1st January to 31st 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 *E. 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 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* consisted of 81 PFGE pulsotypes of which 72.3% of isolates 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 two predominant sequence types, ST203 (80 isolates) was identified across Australia and ST555 (40 isolates) was isolated primarily in the western/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.

Background

Globally enterococci are thought to account for approximately 10% of all bacteraemias, and in North America and Europe are the fourth and fifth 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 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
3. Molecular epidemiology of *E. faecium*

Methodology

Participants: Twenty-six laboratories from all eight Australian states and territories.

Collection Period: From 1st January to 31st 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 first 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 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 were collected >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 (MALDI) Biotyper (Bruker Daltonics), Vitek-MS (bioMérieux), 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 (MLST) was performed as previously described.[11-13]

Results

From 1 January to 31 December 2013, 826 unique episodes of enterococcal bacteraemia were identified. A difference was seen in patient sex (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. 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 nine *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.

E. faecalis **Phenotypic Susceptibility Results:** Apart from erythromycin, tetracycline, ciprofloxacin and high-level gentamicin, resistance was rare amongst *E. faecalis* (Table 1). Ampicillin resistance was not detected and only one 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 one per cent of isolates were non-susceptible to daptomycin and teicoplanin.

E. faecium **Phenotypic Susceptibility Results:** 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 eight (2.5%) isolates were teicoplanin and linezolid non-susceptible respectively.

Genotypic Vancomycin Susceptibility Results: 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 eight isolates (vancomycin and

teicoplanin MICs ≥32 mg/L) and *vanB* in 121 isolates (vancomycin MICs 8 [four isolates] and ≥32 mg/L [117 isolates]). Seven of the eight *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.

E. 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 <5 isolates, 58 had only one isolate. Overall 219 (72.8%) of the 301 isolates were grouped into the 14 major pulsotypes from which eight multilocus sequence types (STs) were identified. Using eBURST, the eight STs were grouped into clonal complex (CC) 17.

Of the two predominant sequence types, ST203 (80 isolates) was identified across Australia and ST555 (40 isolates), was isolated primarily in the western/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.

vanA or *vanB* genes were identified in two (five isolates) and ten (113 isolates) major pulsotypes respectively (Table 4). Efm22 (ST18) harboured *vanA* and *vanB* genes. Twelve minor pulsotypes (13 isolates) and one non typed *E. faecium* isolates also harboured *vanB* genes. In addition *vanA* genes were detected in three minor pulsotypes (three isolates). Over 90% of Efm2 (ST203), Efm76 (ST203), Efm77 (ST555), Efm74 (ST796) and Efm3 (ST341) harboured *vanB* genes. In contrast at least 90% of Efm 1 (ST203), Efm75 (ST203), Efm6 (ST203), Efm4 (ST555) and Efm78 (ST761) did not harbour *van* genes. Four of the eight *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. By 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 (http://www.ecdc.europa.eu/en/healthtopics/antimicrobial_resistance/database/Pages/database.aspx) comparison of Australia antimicrobial resistance data with other countries is possible.

In the 2012 European Centre for Disease Prevention and Control and Prevention (ECDC) Enterococci surveillance program the European Union/European Economic Area (EU/EEA) population-weighted mean percentage of *E. faecium* resistant to vancomycin was 8.1%, ranging 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 above 15%. (http://ecdc.europa.eu/en/publications/Publications/antimicrobial-resistance-surveillance-europe-2012.pdf).

In AESOP 2013 approximately 40% of enterococcal bacteraemia 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 been 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 multidrug-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 VRE with pandemic potential. In AESOP 2013 five 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.

Conclusions

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.

AGAR Participants

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

Antimicrobial	Tested	Breakpoint (mg/L)	Non-Susceptible (%)
Ampicillin	463	>8[a]	0
		>4[b]	0
Vancomycin	463	>4[c]	1(0.2)
Erythromycin	451	>0.5[c]	375 (83.2)
Tetracycline	419	>4[c]	314 (74.9)
Ciprofloxacin	424	>1[c]	91(21.5)
Daptomycin	397	>4[c]	1 (0.3)
Teicoplanin	462	>8[a]	3 (0.6)
		>2[b]	4 (0.9)
Linezolid	462	>2[c]	29 (6.3)
Nitrofurantoin	454	>32[a]	8 (1.8)
		>64[b]	4 (0.9)
High Level Gentamicin	463	>128[a]	150 (32.4)

[a]CLSI non-susceptible breakpoint
[b]EUCAST non-susceptible breakpoint
[c]CLSI and EUCAST non-susceptible breakpoint

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

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

[a]CLSI non-susceptible breakpoint
[c]EUCAST non-susceptible breakpoint
[c]CLSI and EUCAST non-susceptible breakpoint

Table 3: The number and proportion of major *Enterococcus faecium* (Efm) pulsed-field gel electrophoresis pulsotypes, Australia, 2013, by region

Type	ST	ACT	NSW	NT	Qld	SA	Tas	Vic	WA	Aus
Efm1	ST203	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		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	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

ACT = Australian Capital Territory; NSW = New South Wales; NT = Northern Territory; Qld = Queensland; SA = South Australia; Tas = Tasmania; Vic = Victoria; WA = Western Australia; Aus = Australia, ND = Not done

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

Pulsotypes	ST	#	vanA	vanB	Not Detected
Efm1	ST203	10	0	1 (10.0)	9 (90)
Efm2		31	0	31 (100)	0
Efm75		20	0	2 (10)	18 (90)
Efm76		13	0	12 (92.3)	1 (7.7)
Efm6		6	0	0	6 (100)
Efm4	ST555	31	0	0	31 (100)
Efm77		9	0	9 (100)	0
Efm74	ST796	32	0	32 (100)	0
Efm5	ST17	18	0	3 (16.7)	15 (83.3)
Efm18		5	4 (80)	0	1 (20)
Efm3	ST341	19	0	19 (100)	0
Efm24	ST192	12	0	3 (25)	9 (75)
Efm22	ST18	8	1 (12.5)	2 (25)	5 (62.5)
Efm78	ST761	5	0	0	5 (100)
Total		219	5 (2.3)	114 (52)	100 (45.7)

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