Australian Enterococcal Sepsis Outcome Progamme, 2011

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Abstract

From 1 January to 31 December 2011, 29 institutions around Australia participated in the Australian Enterococcal Sepsis Outcome Programme (AESOP). The aim of AESOP 2011 was to determine the proportion of enterococcal bacteraemia isolates in Australia that are antimicrobial resistant, with particular emphasis on susceptibility to ampicillin and the glycopeptides, and to characterise the molecular epidemiology of the *Enterococcus faecalis* and *E. faecium* isolates. Of the 1,079 unique episodes of bacteraemia investigated, 95.8% were caused by either *E. faecalis* (61.0%) or *E. faecium* (34.8%). Ampicillin resistance was detected in 90.4% of *E. faecium* but not detected in *E. faecalis*. Using Clinical and Laboratory Standards Institute breakpoints (CLSI), vancomycin non-susceptibility was reported in 0.6% and 31.4% of *E. faecalis* and *E. faecium* respectively and was predominately due to the acquisition of the *vanB* operon. Approximately 1 in 6 *vanB E. faecium* isolates however, had an minimum inhibitory concentration at or below the CLSI vancomycin susceptible breakpoint of ≤ 4 mg/L. Overall, 37% of *E. faecium* harboured *vanA* or *vanB* genes. Although molecular typing identified 126 *E. faecalis* pulsed-field gel electrophoresis (PFGE) pulsotypes, more than 50% belonged to 2 pulsotypes that were isolated across Australia. *E. faecium* consisted of 73 PFGE pulsotypes from which 43 multilocus sequence types were identified. Almost 90% of the *E. faecium* were identified as clonal complex 17 clones, of which approximately half were characterised as sequence type 203, which was isolated Australia-wide. In conclusion, the AESOP 2011 has shown that although polyclonal, enterococcal bacteraemias in Australia are frequently caused by ampicillin-resistant *vanB E. faecium*. *Commun Dis Intell* 2014;38(3):E247–E252.

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

Introduction

Globally, enterococci are thought to account for approximately 10% of all bacteraemias,[1] and in North America and Europe are the 4th and 5th leading cause of sepsis, respectively.[2] Although in the 1970s, healthcare-associated enterococcal infections were primarily due to *Enterococcus faecalis*,[3] there has been a steadily increasing prevalence of *E. faecium* nosocomial infections.[4,5] While innately resistant to many classes of antibiotics, *E. faecium* has demonstrated a remarkable capacity to evolve new antimicrobial resistances. By the early 1990s vancomycin resistant *E. faecium* had become the 2nd most common nosocomial pathogen in the United States of America (USA),[6] and was endemic in many North American hospitals.[7] Vancomycin resistance in *E. faecium* bacteraemia isolates ranges from 5%–35% in Europe to 60% in North America.[8,9] 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.[10]

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.[11] In 2011, AGAR commenced the Australian Enterococcal Sepsis Outcome Programme (AESOP). The objective of the AESOP 2011 was to determine the proportion of *E. faecalis* and *E. faecium* bacteraemia isolates demonstrating antimicrobial resistance with particular emphasis on:

- assessing susceptibility to ampicillin;
- assessing susceptibility to glycopeptides; and
- molecular epidemiology of *E. faecalis* and *E. faecium*.

Methodology

Participants

Twenty-nine laboratories including 26 public and 3 private laboratories from all 8 Australian states and territories participated in the AESOP 2011 study.

Collection period

From 1 January to 31 December 2011, 29 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.

Laboratory testing

Ampicillin susceptibility testing was performed according to each laboratory's routine standardised methodology. Clinical and Laboratory Standards Institute (CLSI) breakpoints were utilised for interpretation.[12] Of the 1,033 *E. faecalis* and *E. faecium* sepsis isolates, 963 (93.2%) were referred to the Australian Collaborating Centre for Enterococcus and Staphylococcus Species (ACCESS) Typing and Research for vancomycin and teicoplanin minimum inhibitory concentration (MIC) estimation by Etest (bioMérieux) according to the manufacturer's guidelines. Isolates with a CLSI intermediate or resistant category were classified as non-susceptible. Molecular testing including *vanA/B* polymerase chain reaction, pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST) was performed as previously described.[13–15]

Results

From 1 January to 31 December 2011, 1,079 unique episodes of enterococcal bacteraemia were identified. Although 8 *Enterococcus* species were identified, 61.0% (658 isolates) were *E. faecalis* and 34.8% (375) were *E. faecium*. Forty-five enterococci were identified either as *E. casseliflavus* (15 isolates), *E. gallinarum* (14), *E. avium* (8), *E. raffinosus* (4), *E. durans* (2) or *E. hirae* (2). One isolate could not be identified to the species level.

Phenotypic susceptibility results

Overall 90.4% (339) of the 375 isolates of *E. faecium* were ampicillin resistant. Ampicillin resistance was not detected in the 658 *E. faecalis* isolates. Vancomycin and teicoplanin MICs were performed on the 622 *E. faecalis* and 341 *E. faecium* referred to ACCESS Typing and Research. The vancomycin MICs for the *E. faecalis* isolates ranged from 0.25–>256 mg/L with a mode of 2 mg/L. The 3 vancomycin non-susceptible isolates (MIC >4 mg/L) had MICs of 16, 32 and >256 mg/L, respectively. The *E. faecalis* teicoplanin MICs ranged from 0.064–2 mg/L with a mode of 0.25 mg/L. None of the *E. faecalis* isolates had a teicoplanin MIC above the CLSI susceptible breakpoint of ≤8 mg/L. The vancomycin MICs for the *E. faecium* isolates ranged from 0.25–>256 mg/L with a mode of 1.0 mg/L. Overall, 31.4% (107 isolates) of *E. faecium* had a vancomycin MIC >4 mg/L. The *E. faecium* teicoplanin MICs ranged from 0.047–>256 mg/L, with a mode of 0.5 mg/L. Five isolates had a teicoplanin MIC >8 mg/L.

Genotypic vancomycin susceptibility results

The 3 vancomycin non-susceptible *E. faecalis* isolates harboured a *vanB* gene. Two and 104 of the 107 *E. faecium* vancomycin non-susceptible isolates harboured *vanA* and *vanB* genes respectively. The *vanA/vanB* negative vancomycin non-susceptible *E. faecium* isolate had a MIC of 6 mg/L. Twenty of the 234 vancomycin susceptible *E. faecium* isolates (MIC ≤4 mg/L) also harboured *vanB* genes. The 2 *vanA E. faecium* isolates had a vancomycin MIC >256 mg/L. Thirty-three vancomycin non-susceptible *E. faecium* isolates had a vancomycin MIC within the CLSI intermediate category of 8–16 mg/L. Only 71 (57.2%) of the 124 *vanB E. faecium* isolates had a MIC above the CLSI vancomycin resistant breakpoint (≥32 mg/L). The 2 *vanA E. faecium* isolates had teicoplanin MICs of 32 and 64 mg/L. Of the 124 *vanB E. faecium* isolates one was teicoplanin intermediate (MIC 16 mg/L) and three were resistant (MIC >32 mg/L) by CLSI criteria.

Molecular epidemiology

By PFGE, 618 of the 622 *E. faecalis* were classified into 126 pulsotypes of which 9 pulsotypes (Efs1 to Efs9) had 10 or more isolates. Four isolates could not be typed by PFGE. Of the 117 pulsotypes that have less than 10 isolates, 66 pulsotypes were represented by only 1 isolate. Geographically, the 9 major pulsotypes were widely distributed, with the 2 predominant pulsotypes EFs1 (191 isolates) and Efs2 (103 isolates), isolated across Australia (Table 1). The 3 *vanB E. faecalis* were detected in pulsotype Efs2.

By PFGE, the 341 *E. faecium* isolates were classified into 73 pulsotypes from which 43 multilocus sequence types (STs) were identified. Five STs had more than 10 isolates (Table 2). The 2 major STs, ST203 (159 isolates) and ST17 (47 isolates) were isolated across Australia. ST341 (38 isolates) and ST252 (11 isolates) were isolated only in the eastern regions of Australia, and ST555 (34 isolates) was isolated in the western and central regions. Using eBURST, 89.1% (304/341 isolates) of *E. faecium* isolates, including the 5 major STs, were grouped into clonal complex (CC) 17. *Van* genes were identified in the 5 major STs and in ST80 and ST414. Although only 8.5% (4/47) of ST17 isolates harboured *vanB*, *vanB* genes were identified in 50.6% (79/153) of ST203 isolates. Other STs harbouring the *vanB* genes included ST341 (33/38), ST555 (2/34), ST252

Table 1: Number and proportion of major *Enterococcus faecalis* pulsed-field gel electrophoresis pulsotypes, Australia, 2011, by state or territory

	ACT		NSW		NT		Qld		SA		Tas.		Vic.		WA		Aus.	
Type	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Efs1	10	30.3	57	33.3	2	28.6	55	32.2	18	25.4	6	40.0	31	33.7	12	19.4	191	30.7
Efs2	9	27.3	21	12.3	2	28.6	29	17.0	17	23.9	3	20.0	13	14.1	9	14.5	103	16.6
Efs3	0	0.0	6	3.5	0	0.0	1	0.6	2	2.8	1	6.7	2	2.2	1	1.6	13	2.1
Efs4	0	0.0	3	1.8	0	0.0	8	4.7	0	0.0	0	0.0	1	1.1	1	1.6	13	2.1
Efs5	0	0.0	11	6.4	0	0.0	1	0.6	0	0.0	0	0.0	0	0.0	0	0.0	12	1.9
Efs6	0	0.0	2	1.2	0	0.0	1	0.6	2	2.8	1	6.7	3	3.3	3	4.8	12	1.9
Efs7	1	3.0	3	1.8	0	0.0	5	2.9	2	2.8	0	0.0	1	1.1	0	0.0	12	1.9
Efs8	0	0.0	3	1.8	0	0.0	2	1.2	3	4.2	0	0.0	1	1.1	2	3.2	11	1.8
Efs9	0	0.0	4	2.3	0	0.0	3	1.8	2	2.8	0	0.0	0	0.0	1	1.6	10	1.6
Other	13	39.4	60	35.1	3	42.9	65	38.0	25	35.2	4	26.7	39	42.4	32	51.6	241	38.7
NT	0	0.0	1	0.6	0	0.0	1	0.6	0	0.0	0	0.0	1	1.1	1	1.6	4	0.6
Total	33	100.0	171	100.0	7	100.0	171	100.0	71	100.0	15	100.0	92	100.0	62	100.0	622	100.0

NT Not typed.

The category type 'other' includes the 117 pulsed-field gel electrophoresis pulsotypes that had less than 10 isolates per pulsotype.

Table 2: Number and proportion of major *Enterococcus faecium* multilocus sequence types, Australia, 2011, by state or territory

	ACT		NSW		NT		Qld		SA		Tas.		Vic.		WA		Aus.	
Type	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Efm ST203	9	40.9	37	39.8	2	33.3	34	60.7	14	25.9	2	50.0	36	57.1	25	58.1	159	46.6
Efm ST17	3	13.6	19	20.4	0	0.0	7	12.5	2	3.7	2	50.0	7	11.1	7	16.3	47	13.8
Efm ST341	7	31.8	26	28.0	0	0.0	3	5.4	0	0.0	0	0.0	2	3.2	0	0.0	38	11.1
Efm ST555	0	0.0	0	0.0	3	50.0	0	0.0	27	50.0	0	0.0	0	0.0	4	9.3	34	10.0
Efm ST252	0	0.0	2	2.1	0	0	3	5.4	0	0.0	0	0.0	6	9.5	0	0.0	11	3.2
Other	3	13.6	9	9.7	1	16.7	9	16.0	11	20.4	0	0.0	12	19.1	7	16.3	52	15.2
Total	22	100.0	93	100.0	6	100.0	56	100.0	54	100.0	4	100.0	63	100.0	43	100.0	341	100.0

The category type 'other' includes the 38 multilocus sequence types that had less than 10 isolates per sequence type.

(1/11), ST80 (2/6), ST414 (2/2), and a single non-CC17 isolate, ST863. Two CC17 STs harboured the *vanA* gene; ST341 and ST80.

Discussion

AESOP 2011 was the 1st ongoing sepsis program performed by AGAR, and was conducted primarily to determine the proportion of *E. faecalis* and *E. faecium* bacteraemia isolates demonstrating antimicrobial resistance, with particular emphasis on assessing susceptibility to penicillin and the glycopeptides and the distribution of different enterococcal clones. As this is the 1st year of an ongoing program, it is difficult to determine the public health significance of these results. We suggest these results should be used as a marker for future AESOPs.

Similar to the situation in the USA and in Europe,[16,17] enterococcal bacteraemia in Australia, and notably bacteraemia caused by multidrug-resistant *E. faecium*, has become a significant problem. In the AESOP 2011 study, approximately 1 in 3 cases of enterococcal bacteraemia was due to *E. faecium,* of which 90.4% (339 of 375 episodes) were ampicillin resistant and 36.9% (126 of 341 episodes) harboured either *vanA* or *vanB* genes. However, unlike Europe and the USA, where vancomycin resistance in *E. faecium* has predominately been due to the acquisition of the *vanA* operon, almost all of the AESOP 2011 *E. faecium* blood culture isolates harbouring *van* genes carried the *vanB* operon (98.4%). Recent studies however, have demonstrated a significant presence of *vanB E. faecium* in both North America and Europe.[18–20]

vanB vancomycin resistant enterococci (VRE) is now more prevalent than *vanA* VRE in several European centres including Sweden, Spain and Germany,[21–23] whilst recent Canadian national surveillance demonstrates *vanB* comprises 10% of their VRE.[24] It is thought this increased occurrence of *vanB*-positive *E. faecium* in the Northern Hemisphere may be due to the increased use of antibiotics selecting enterococci and VRE as well as to methodological reasons (e.g. reduced European Committee on Antimicrobial Susceptibility Testing (EUCAST) MIC breakpoints for glycopeptides; increased use and sensitive performance of chromogenic VRE agars; increased use of molecular diagnostic assays).[25] Twenty (16.1%) of the 124 *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, both enterococcal species were shown to be very polyclonal, confirming the enormous plasticity of the enterococcal genome. The majority of *E. faecium* isolates 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.

The study has a number of limitations. Although achieving national coverage, the participating laboratories service only a minority of the Australian hospitalised population. Further, MIC assays for vancomycin and teicoplanin were performed by a commercial gradient diffusion method and not the standard reference broth microdilution method.

Conclusions

The AESOP 2011 study has shown though predominately caused by *E. faecalis,* enterococcal bacteraemia in Australia is frequently caused by ampicillin-resistant *vanB E. faecium*. Molecular typing characterised over 50% of *E. faecalis* isolates as 2 PFGE pulsotypes and almost 90% of *E. faecium* isolates as CC17 clones of which approximately half were ST203. Further studies of the enterococcal genome will contribute to our understanding of the evolution of enterococci in the hospital environment and assist in preventing their nosocomial transmission.

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