Annual reports

Australian Group on Antimicrobial Resistance Enterococcus Surveillance Programme annual report, 2010

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Abstract

In 2010, 15 institutions around Australia conducted a period prevalence study of key resistances in isolates of *Enterococcus* species associated with a range of clinical disease amongst in- and outpatients. Each institution collected up to 100 consecutive isolates and tested these for susceptibility to commonly used antimicrobials using standardised methods. Vancomycin-resistant *Enterococcus faecium* and *Enterococcus faecalis* were characterised by pulsed-field gel electrophoresis. Multilocus sequence typing was performed on representative pulsotypes of *E. faecium*. Susceptibility results were compared with similar surveys conducted in 1995, 1999, 2003, 2005, 2007 and 2009. In the 2010 survey, *E. faecalis* (1,201 isolates) and *E. faecium* (170 isolates) made up 98.9% of the 1,386 isolates tested. Ampicillin resistance was very common (85.3%) in *E. faecium* and absent in *E. faecalis*. Non-susceptibility to vancomycin was 36.5% in *E. faecium* (similar to the 35.2% in 2009 but up from 15.4% in the 2007 survey) and 0.5% in *E. faecalis*. There were significant differences in the proportion of vancomycin-resistant *E. faecium* between the states ranging from 0% in Western Australia to 54.4% in South Australia. The vanB gene was detected in 62 *E. faecium* and 3 *E. faecalis* isolates. The vanA gene was detected in 1 *E. faecium* isolate. All vancomycin-resistant *E. faecium* belonged to clonal complex 17. The most common sequence type (ST) was ST203, which was found in all regions that had reports of vancomycin resistant enterococci. ST341 was detected only in New South Wales/Australian Capital Territory and ST414 only in South Australia and Victoria. High-level resistance to gentamicin was 34.1% in *E. faecalis* and 66.1% in *E. faecium*. A subset of isolates was tested against high-level streptomycin, linezolid and quinupristin/dalfopristin. High-level streptomycin resistance was found in 8.2% of *E. faecalis* isolates and 43.8% of *E. faecium* isolates. Linezolid non-susceptibility was more common in *E. faecalis* (5.8%) than *E. faecium* (0.9%). Overall 9.4% of *E. faecium* were resistant to quinupristin/dalfopristin (*E. faecalis* is intrinsically resistant). *Commun Dis Intell* 2013;37(3):E199–E209.

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

Introduction

Enterococci are part of the normal flora of the gastrointestinal tract. They can give rise to endogenous infections such as urinary tract infections outside of hospitals. Enterococci are recognised as significant nosocomial pathogens causing urinary tract, blood stream, sterile site and wound infections. In hospitals, enterococci can be transmitted through poor infection control practices and can give rise to a wide variety of infections usually in patients with co-morbidities. The two main species causing infections in humans are *Enterococcus faecalis* and *Enterococcus faecium* with only a very small number of other species being isolated from clinical specimens.

In the 1980s, enterococci were generally susceptible to amoxycillin and vancomycin. Since then *E. faecium* has become increasingly resistant to ampicillin/amoxycillin making vancomycin the treatment of choice for severe infections caused by this organism. The first vancomycin resistant enterococci (VRE) were described in the United Kingdom and Europe in 1988[1] and in the United States of America (USA) in the early 1990s.[2] The first VRE was reported in Australia in 1994[3] and a report on the emergence and epidemiology of VRE in Australia was described in 1998 when 69 isolates were documented.[4]

Multilocus sequence typing (MLST) of *E. faecium* has revealed that clonal complex (CC) 17 strains have become predominant in hospitals in many countries and are characterised by ampicillin resistance and the presence of several genetic elements (e.g. *esp* and *hyl*) not present in colonising variants in humans and animals.[5–9] There is some evidence that this additional genomic content assists in adaptation to the hospital environment and the ability to spread, therefore when CC17 strains acquired the *vanA* or *vanB* gene encoding vancomycin resistance, they were already primed for transmission in the hospital setting.

Prevalence and incidence rates for VRE in Australian hospitals are not routinely collected although there have been reports of individual hospital outbreaks of VRE infections and associated colonisation of other patients.[9–13] The clinical impact of vancomycin resistance in enterococci has been reported to include increases in mortality, length of stay and hospital costs.[14,15] Serious infections caused by vancomycin-resistant *E. faecium* are difficult to treat, and rely on recently introduced antimicrobials such as linezolid, quinupristin-dalfopristin, tigecycline and daptomycin which are not approved for all indications. Further complicating the treatment of infections caused by VRE are reports of isolates that are resistant even to these newer agents.[16,17]

It is important to have an understanding of the occurrence of enterococcal infection and antibiotic resistance in Australia to guide infection control practices, antibiotic prescribing policies and drug regulatory matters.

The objective of the 2010 surveillance program was to determine the proportion of clinical isolates of *Enterococcus* species demonstrating antimicrobial resistance with particular emphasis on:

1. assessing susceptibility to ampicillin;
2. assessing susceptibility to glycopeptides; and
3. assessing changes in resistance patterns over time using data collected in previous Australian AGAR surveys,
4. determining which VRE clones are circulating within Australia.

The Australian Group on Antimicrobial Resistance (AGAR) commenced surveillance of antimicrobial resistance in *Enterococcus* species in 1995. Similar surveys were conducted in 1999, 2003, 2005, 2007 and 2009 (www.agargroup.org).

Methods

Fifteen laboratories from all mainland Australian states and the Australian Capital Territory participated in the 2010 AGAR survey *Enterococcus*. To ensure institutional anonymity the New South Wales and the Australian Capital Territory data were combined.

From 1 January to 30 June 2010 each laboratory collected up to 100 consecutive clinically significant isolates of enterococci. Only 1 isolate per patient was tested unless subsequent isolates had a different antibiogram to the original isolate.

Species identification

All isolates were tested for pyrrolidonyl arylamidase with optional testing for growth in 6.5% sodium chloride, esculin hydrolysis in the presence of bile, Group D antigen and growth at 45°C. Isolates were identified to species level by either API® 20S (bioMérieux, Marcy l'Etoile, France), Vitek® 2 (bioMérieux, Marcy l'Etoile, France), Phoenix™ (BD, New Jersey, USA), polymerase chain reaction (PCR), or conventional biochemical tests. If biochemical testing was performed, the minimum tests necessary for identification were: motility, pigment production, methyl-α-D-glucopyranoside, fermentation of 1% raffinose, 1% arabinose, 1% xylose and utilisation of pyruvate.

Susceptibility methodology

Participating laboratories performed antimicrobial susceptibility tests according to each laboratory's routine standardised methodology (Clinical and Laboratory Standards Institute (CLSI) disc diffusion, Vitek® 2, Phoenix™, agar dilution or Etest® (bioMérieux, Marcy l'Etoile, France)). Ampicillin and vancomycin were tested by all laboratories. Vancomycin resistance was confirmed by PCR. Overall, 1,378 (99.4%) isolates were screened for high level gentamicin resistance, 932 (67.2%) were tested against linezolid, 503 (36.3%) were tested against quinupristin/dalfopristin and 146 (10.5%) were screened for high level streptomycin resistance. CLSI breakpoints were utilised for all antimicrobials.[18] Isolates with an intermediate and resistant category have been classified as non-susceptible.

Of the 178 invasive isolates, 116 (65.2%) were tested for ß-lactamase production using a chromogenic cephalosporin nitrocefin.

Epidemiological typing of vancomycin resistant enterococci

Pulsed-field gel electrophoresis (PFGE) of *SmaI*-digested DNA agarose plugs was performed as previously described on all VRE isolates.[19] MLST was performed as previously described on a representative of each PFGE pulsotype of vancomycin resistant *E. faecium*.[20]

Statistical analysis

The difference between proportions was tested using Chi-square test with alpha set at the 5% level and Fisher's exact test for 95% confidence limits (GraphPad® Prism Software).

Results

Both public (n=13) and private (n=2) laboratories participated in the survey. Participants included New South Wales (n=3), the Australian Capital Territory (n=1), Queensland (n=4), Victoria (n=1), South Australia (n=3), and Western Australia (n=3). In 2010 there were 1,386 isolates from 15 institutions (Table 1). *E. faecalis* was the most frequently isolated species (86.7%) followed by *E. faecium* (12.3%) (Table 2).

The majority of isolates (70.9%) were from the urinary tract (Table 3). They were predominately *E. faecalis* (91.3%). Invasive (blood, cerebrospinal fluid (CSF) and sterile body cavity) isolates comprised 12.8% of the total number of isolates collected. *E. faecium* was disproportionately represented in the invasive group (28.7%). Of the *E. faecalis* isolates, 9.9% were invasive compared with 30.0% of *E. faecium* isolates.

Susceptibility

Resistance to ampicillin was common in the *E. faecium* isolates (Table 4). Resistance in *E. faecium* was due to penicillin binding protein changes. No ß-lactamase positive *E. faecium* were detected amongst the subset (30/51, 59%) of invasive isolates tested. Resistance in invasive isolates was lower than for non-invasive isolates (72.9% and 90.7% respectively, $P=0.004$). Ampicillin resistance was not detected for *E. faecalis* and none of the 81 invasive isolates tested for ß-lactamase were positive.

Trend data for *E. faecium* show that from 1995 to 1999, there was an increase in ampicillin resistance ($P=0.002$) with a plateau from 1999 to 2005 (Figure 1). Between 2005 and 2010, resistance has once again increased significantly ($P=0.005$). The gap between resistance in non-invasive versus invasive isolates narrowed over time, however in

Table 1: *Enterococcus* isolates in Australia, 2010, by region

Region	Number of institutions	Isolates	%
NSW/ACT	4	380	27.4
Qld	4	400	28.9
SA	3	207	14.9
Vic	1	100	7.2
WA	3	299	21.6
Total	15	1,386	100.0

Table 2: *Enterococcus* species isolated in Australia, 2010, by region

Region	*E. faecalis*	*E. faecium*	Other spp. or unspeciated	Total
NSW/ACT	334	41	5	380
Qld	381	18	1	400
SA	145	57	5	207
Vic	76	23	1	100
WA	265	31	3	299
Total	1,201 (86.7%)	170 (12.3%)	15 (1.1%)	1,386

Table 3: *Enterococcus* species isolated in Australia, 2010, by source

Source	*E. faecalis*	*E. faecium*	Other spp. or unspeciated	Total
Urine	897	82	3	982 (70.9%)
Wound	173	37	4	214 (15.4%)
Blood/CSF	77	34	5	116 (8.4%)
Sterile body cavity	42	17	3	62 (4.5%)
Other	12	0	0	12 (0.9%)
Total	1,201	170	15	1,386
Invasive*	119	51	8	178 (12.8%)
Non-invasive	1,082	119	7	1,208 (87.2%)

CSF Cerebrospinal fluid

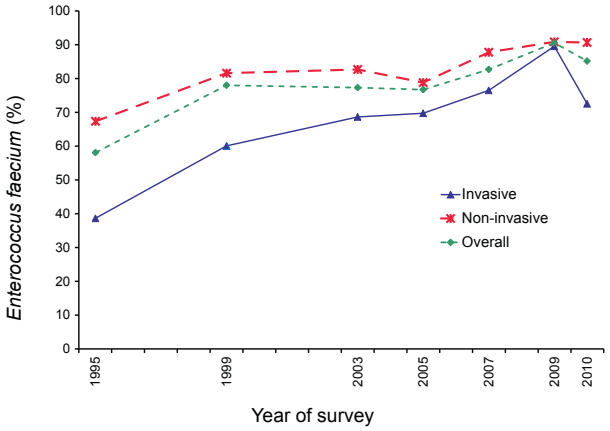
* Blood/cerebrospinal fluid/sterile body cavity

2010 there was a reversal of this trend with rates of resistance in invasive isolates falling significantly (P=0.04) compared to 2009 levels.

Vancomycin non-susceptibility was uncommon in *E. faecalis* (0.5%) (Table 5). Of the 6 non-susceptible *E. faecalis*, two harboured the *vanB* gene and four did not possess either *vanA* or *vanB*.

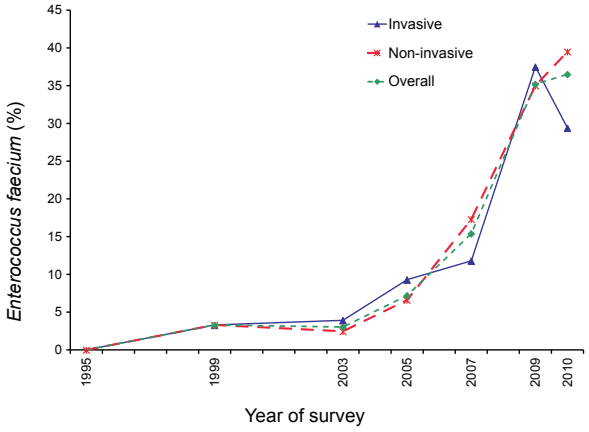
A total of 36.5% of *E. faecium* were vancomycin non-susceptible; a similar proportion to the 2009 survey (35.2%) but more than double that of the 2007 survey (15.4%, P<0.0001) (Figure 2). Vancomycin non-susceptible *E. faecium* were detected in all regions except Western Australia. Vancomycin non-susceptibility in the other regions ranged from 16.7% in Queensland to 54.4% in South Australia (Table 5). All of the vancomycin non-susceptible *E. faecium* were confirmed as VRE by PCR and were predominantly of the *vanB* genotype (61/62, 98.4%). In 2010, more than one third of urine, wound and blood *E. faecium* were vancomycin resistant. Trend data for *E. faecium* show there has been a marked increase in vancomycin resistance since 1995 (Figure 2). Vancomycin resistant *E. faecium* have occurred in all 5 regions over the 6 survey periods, with all regions except Western Australia showing increases in VRE over time (Figure 3).

Figure 1: Percentage of *Enterococcus faecium* resistant to ampicillin, by survey year



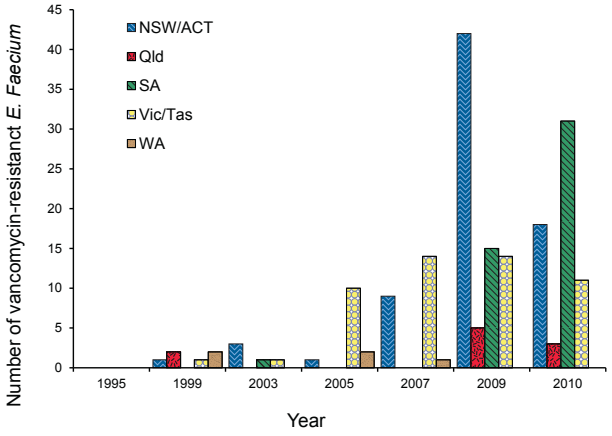
1995: invasive n=26, non-invasive n= 55, overall n=81.
1999: invasive n=30, non-invasive n= 152, overall n=182.
2003: invasive n=51, non-invasive n= 81, overall n=132.
2005: invasive n=43, non-invasive n= 137, overall n=180.
2007: invasive n=51, non-invasive n= 98, overall n=156.
2009: invasive n=48, non-invasive n= 165, overall n=213.
2010: invasive n=51, non-invasive n= 119, overall n=170

Figure 2: Percentage of *Enterococcus faecium* non-susceptible to vancomycin, by survey year



1995: invasive n=26, non-invasive n= 55, overall n=81.
1999: invasive n=30, non-invasive n= 152, overall n=182.
2003: invasive n=51, non-invasive n= 81, overall n=132.
2005: invasive n=43, non-invasive n= 137, overall n=180.
2007: invasive n=51, non-invasive n= 98, overall n=156.
2009: invasive n=48, non-invasive n= 165, overall n=213.
2010: invasive n=51, non-invasive n= 119, overall n=170

Figure 3: Regional location of vancomycin-resistant *Enterococcus faecium*, by survey year



* Tasmania did not contribute isolates in 2009 or 2010.

High level gentamicin (HLG) resistance was seen in *E. faecalis* (34.1%) and *E. faecium* (66.1%) (Table 6). Trend data (Figures 4 and 5) show significant increases for *E. faecium* from 1995 to 1999 (P<0.001) and again from 2003 to 2010 (P<0.0001). The increase from 2003 to 2010 was driven by resistance in non-invasive isolates as rates of resistance remained stable in invasive isolates during that time period despite year-to-year fluctuations (Figure 4) (P=0.09). HLG resistance in *E. faecalis* invasive and non-invasive isolates continued to increase until 2005 and then stabilised.

In this survey, high level streptomycin resistance (HLS) was tested only in New South Wales/Australian Capital Territory and South Australia. HLS resistance is more common for *E. faecium* than *E. faecalis* (Table 7), similar to HLG resistance. The trend from 1995 to 2010 for *E. faecium* was for relatively stable resistance despite year to year fluctuations (Figures 6 and 7). In *E. faecalis*,

Table 4: Number of ampicillin resistant *Enterococcus* species isolated in Australia, 2010, by region

	NSW/ACT			Qld			SA			Vic			WA			Aus		
	n	N	%	n	N	%	n	N	%	n	N	%	n	N	%	n	N	%
E. faecalis all	0	334	0.0	0	381	0.0	0	145	0.0	0	76	0.0	0	265	0.0	0	1,201	0.0
Invasive	0	46	0.0	0	14	0.0	0	22	0.0	0	8	0.0	0	18	0.0	0	108	0.0
E. faecium all	34	41	82.9	17	18	94.4	51	57	89.5	19	23	82.6	23	31	77.4	145	170	85.3
Invasive	12	18	66.7	1	2	50.0	17	20	85.0	3	3	100.0	4	8	50.0	37	51	72.5

Table 5: Number of vancomycin non-susceptible *Enterococcus* species isolated in Australia, 2010, by region

	NSW/ACT			Qld			SA			Vic			WA			Aus		
	n	N	%	n	N	%	n	N	%	n	N	%	n	N	%	n	N	%
E. faecalis all	2	334	0.6	1	381	0.3	0	145	0.0	2	76	2.6	1	265	0.4	6	1,201	0.5
Invasive	2	41	4.9	0	12	0.0	0	40	0.0	0	5	0.0	1	21	4.8	3	119	2.5
E. faecium all	18	41	43.9	3	18	16.7	31	57	54.4	10	23	43.5	0	31	0.0	62	170	36.5
Invasive	6	18	33.3	0	2	0.0	8	20	40.0	1	3	33.3	0	8	0.0	15	51	29.4

Table 6: Number of high level gentamicin resistant *Enterococcus* species isolated in Australia, 2010, by region

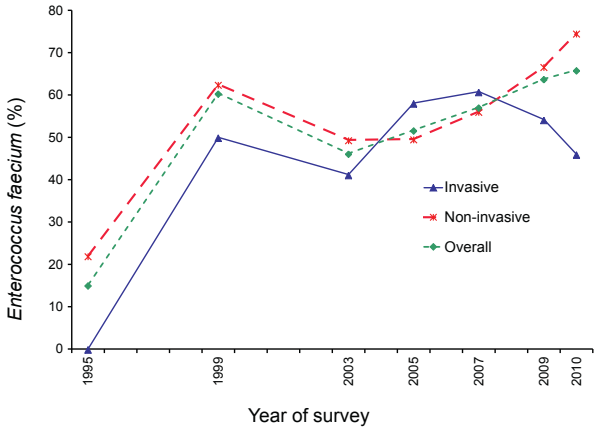
	NSW/ACT			Qld			SA			Vic			WA			Aus		
	n	N	%	n	N	%	n	N	%	n	N	%	n	N	%	n	N	%
E. faecalis all	141	334	42.2	150	381	39.4	38	142	26.8	18	76	23.7	62	265	23.4	409	1,198	34.1
Invasive	11	41	26.8	4	12	33.3	15	37	40.5	1	5	20.0	12	21	57.1	43	116	37.1
E. faecium all	30	41	73.2	16	18	88.9	30	52	57.7	16	23	69.6	17	31	54.8	109	165	66.1
Invasive	7	18	38.9	1	2	50.0	10	19	52.6	2	3	66.7	3	8	37.5	23	50	46.0

the HLS decreased significantly from 1995 to 2003 but has been relatively stable since then with lower rates of expression than HLG (Figures 5 and 7).

Linezolid non-susceptibility was present in 5.8% of *E. faecalis* (up from 4.0% in 2009) and in 0.9% of *E. faecium* (down from 2.1% in 2009) (Table 8). Forty-six of the 48 non-susceptible isolates had an minimum inhibitory concentration (MIC) in the intermediate resistant category; only two were classified as resistant (MIC ≥8 mg/L). The 2 resistant isolates were *E. faecalis* from Queensland.

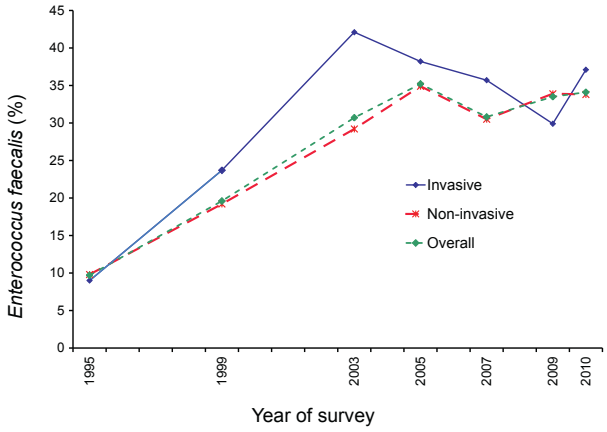
E. faecalis are intrinsically resistant to quinupristin/dalfopristin. Only 9.4% of the *E. faecium* were non-susceptible (down from 21.9% in 2009) with

Figure 4: Percentage of *Enterococcus faecium* resistant to high-level gentamicin, by survey year



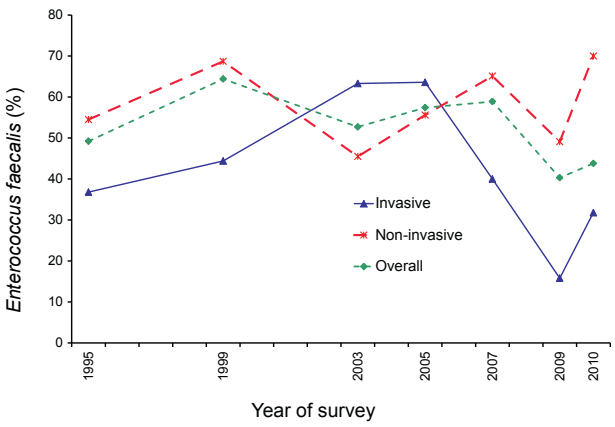
1995: invasive n=23, non-invasive n= 50, overall n=73.
1999: invasive n=30, non-invasive n= 152, overall n=182.
2003: invasive n=51, non-invasive n= 81, overall n=132.
2005: invasive n=43, non-invasive n= 137, overall n=180.
2007: invasive n=51, non-invasive n= 98, overall n=156.
2009: invasive n=48, non-invasive n= 165, overall n=213.
2010: invasive n=50, non-invasive n= 115, overall n=165.

Figure 5: Percentage of *Enterococcus faecalis* resistant to high-level gentamicin, by survey year



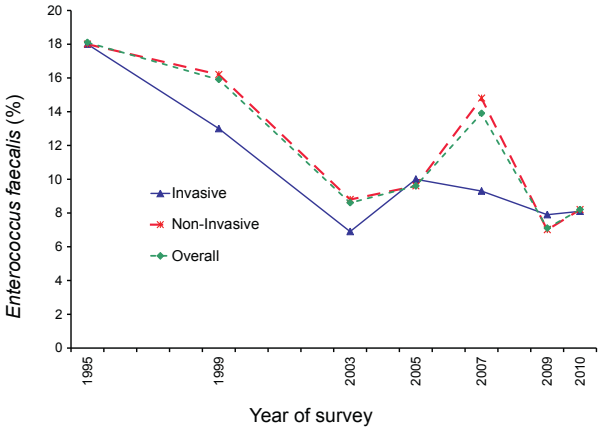
1995: invasive n=100, non-invasive n= 1109, overall n=1,211.
1999: invasive n=135, non-invasive n= 1,442, overall n=1577.
2003: invasive n=190, non-invasive n=1,432, overall n=1,622.
2005: invasive n=170, non-invasive n= 1,816, overall n=1,986.
2007: invasive n=143, non-invasive n= 1,333, overall n=1,520.
2009: invasive n=107, non-invasive n= 1005, overall n=1,112.
2010: invasive n=116, non-invasive n= 1082, overall n=1,198

Figure 6: Percentage of *Enterococcus faecium* resistant to high-level streptomycin, by survey year



1995: invasive n=19, non-invasive n= 44, overall n=63.
1999: invasive n=18, non-invasive n= 83, overall n=101.
2003: invasive n=30, non-invasive n= 44, overall n=74.
2005: invasive n=22, non-invasive n= 72, overall n=94.
2007: invasive n=25, non-invasive n= 43, overall n=73.
2009: invasive n=19, non-invasive n=53, overall n=72.
2010: invasive n=22, non-invasive n=10, overall n=32

Figure 7: Percentage of *Enterococcus faecalis* resistant to high-level streptomycin, by survey year



1995: invasive n=61, non-invasive n= 916, overall n=979.
1999: invasive n=92, non-invasive n= 916, overall n=1008.
2003: invasive n=102, non-invasive n=715, overall n=817.
2005: invasive n=80, non-invasive n= 1012, overall n=1092.
2007: invasive n=197, non-invasive n= 783, overall n=913.
2009: invasive n=38, non-invasive n= 229, overall n=267.
2010: invasive n=37, non-invasive n= 73, overall n=110.

four of the five non-susceptible isolates having an MIC in the resistant range (MIC >2 mg/L). All quinupristin/dalfopristin non-susceptible cases were identified in isolates originating in New South Wales/Australian Capital Territory, as was the case in 2007 and 2009 (Table 9).

Cross resistance to other agents was examined in vancomycin resistant isolates of enterococci (Table 10). Resistance to ampicillin and high levels of gentamicin was more common in vancomycin resistant *E. faecium*. Resistance to high levels of streptomycin, quinupristin/dalfopristin and linezolid was similar for VRE and non-VRE ($P>0.05$).

Table 7: Number of high level streptomycin resistant *Enterococcus* isolated in Australia, 2010, by region

	NSW/ACT			Qld	SA			Vic	WA	Aus		
	n	N	%		n	N	%			n	N	%
E. faecalis all	6	73	8.2	–	3	37	8.1	–	–	9	110	8.2
Invasive	1	9	11.1	–	2	28	7.1	–	–	3	37	8.1
E. faecium all	2	7	28.6	–	12	25	48.0	–	–	14	32	43.8
Invasive	1	5	20.0	–	6	17	35.3	–	–	7	22	31.8

Table 8: Number of linezolid non-susceptible *Enterococcus* isolated in Australia, 2010, by region

	NSW/ACT			Qld			SA			Vic	WA			Aus		
	n	N	%	n	N	%	n	N	%		n	N	%	n	N	%
E. faecalis all	18	334	5.4	28	341	7.3	1	90	1.1	–	0	5	0.0	47	810	5.8
Invasive	2	41	4.9	0	12	0.0	1	32	3.1	–	0	5	0.0	3	90	3.3
E. faecium all	1	41	2.4	0	18	0.0	0	52	0.0	–	0	2	0.0	1	113	0.9
Invasive	1	18	5.6	0	2	0.0	0	18	0.0	–	0	1	0.0	1	39	2.6

Table 9: Number of quinupristin/dalfopristin non-susceptible *Enterococcus* isolated in Australia, 2010, by region

	NSW/ACT			Qld			SA			Vic	WA			Aus		
	n	N	%	n	N	%	n	N	%		n	N	%	n	N	%
E. faecalis all	246	258	95.3	154	177	87.0	2	3	66.7	–	5	5	100.0	407	443	91.9
Invasive	33	34	97.1	6	6	100	0	1	0.0	–	5	5	100.0	44	46	95.7
E. faecium all	5	33	28.1	0	16	0.0	0	2	0.0	–	0	2	0.0	5	53	9.4
Invasive	4	15	26.7	0	2	0.0	–			–	0	1	0.0	4	18	22.2

Table 10: Cross resistant *Enterococcus* isolated in Australia, 2010

		Ampicillin			Gentamicin			Streptomycin			Linezolid			Quinupristin/ dalfopristin		
		n	N	%	n	N	%	n	N	%	n	N	%	n	N	%
E. faecalis	Not VRE	0	1,198	0.0	407	1,195	34.1	9	110	8.2	47	809	5.8	407	443	91.9
	VRE	0	3	0.0	2	3	66.7	–			0	1	0.0	–		
E. faecium	Not VRE	82	107	76.6	58	107	54.2	5	16	31.3	1	62	1.6	4	34	11.8
	VRE	63	63	100.0	51	58	87.9	9	16	56.3	0	51	0.0	1	19	5.3

VRE Vancomycin resistant enterococci

Vancomycin resistant enterococci characterisation

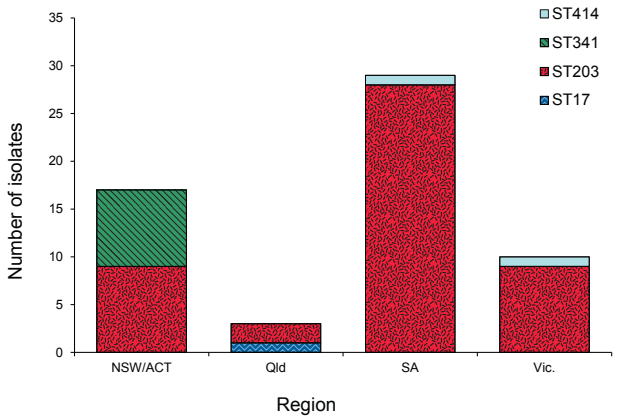
Three (100%) *vanB E. faecalis*, 1 (100%) *vanA E. faecium* and 59/62 (95%) *vanB E. faecium* isolates were available for molecular typing.

Two of the *vanB E. faecalis* were classified as pulsotype A and one was classified as pulsotype B (Table 11). The *vanA E. faecium* was a pulsotype C and sequence type (ST) 117. Six pulsotypes and 4 STs were identified in the *vanB E. faecium*. ST203 was the most common ST (comprising 81% of *vanB E. faecium*) and was found in all regions that reported VRE. ST341 was found only in New South Wales/Australian Capital Territory, ST414 only in South Australia and Victoria and ST17 only in Queensland (Figure 8). The *E. faecium* isolates belonged to CC 17.

Discussion

It is clear from this study and the examination of trends over the last 15 years that antimicrobial resistance has increased significantly in *E. faecium*.

Figure 8: Distribution of vanB *Enterococcus faecium* multi-locus sequence types, by region



Treatment options for this species are becoming ever more limited as resistance to ampicillin and other penicillins is now very frequent, and glycopeptide resistance is increasing. In some instances only expensive and/or potentially toxic treatment options such as linezolid, quinupristin-dalfopristin, tigecycline or daptomycin are available.

Ampicillin resistance in *E. faecuim* is the result of changes in penicillin-binding proteins. This is also true for most isolates of *E. faecalis*, although ß-lactamase production has been seen rarely (3 known instances in Australia in the last 2 decades).[21] This survey has shown that ampicillin resistance is now usual in *E. faecium* but is rare in *E. faecalis*. Ampicillin resistance in enterococci presents considerable challenges when infections are serious, as the isolates will not be susceptible to any ß-lactam antibiotic, and the drug of choice becomes vancomycin, which is only slowly bactericidal. Further, for endocarditis the combination of vancomycin with an aminoglycoside creates significant toxicity problems.

Unfortunately vancomycin resistance in enterococci is increasing in Australia particularly over the past 5 years. It has been seen in all states and territories although rates in each region vary considerably. It is widely recognised that rates of colonisation far exceed the rates of infection with VRE, and thus the amount of VRE seen in this survey does not truly reflect the size of the VRE reservoir. The survey results are also consistent with the previous Australian experience that the dominant type of resistance is encoded by the *vanB* complex[4,22] in contrast with the situation in Europe and the USA where *vanA* dominates. Vancomycin-resistant isolates causing serious infection are very challenging to treat. The choices are linezolid, quinupristin-dalfopristin, tigecycline and daptomycin. Each of these agents presents its own challenges for treatment.

Table 11: Molecular characterisation of vancomycin-resistant enterococci isolated in Australia, 2010

van Gene	Species	PFGE	MLST	NSW/ACT	Qld	SA	Vic
vanB	*E. faecalis*	A	n.d.				2
vanB	*E. faecalis*	B	n.d.			1	
vanA	*E. faecium*	C	ST117	1			
vanB	*E. faecium*	D	ST203	5	1	28	9
vanB	*E. faecium*	E	ST203	2	1		
vanB	*E. faecium*	F	ST203	2			
vanB	*E. faecium*	G	ST341	8			
vanB	*E. faecium*	H	ST414			1	1
vanB	*E. faecium*	I	ST17		1		
Total				18	3	30	12

High-level resistance to gentamicin has increased in recent years after apparently reaching a plateau in the early 2000s. This greatly compromises the ability to treat enterococcal endocarditis effectively.

Molecular characterisation of the VRE isolates in this study has revealed that *E. faecium* belonging to CC17 are now established in Australia. CC17, including ST203 and ST414 both found in this study are considered to be hospital-associated clones and have been responsible for outbreaks in several countries including Australia.[9,23,24] Containing additional genetic content thought to assist in survival and spread in the hospital environment, CC17 poses a challenge for hospital infection control as standard measures may not be enough to control spread in the long term. Extensive screening of patients, confinement of colonised or infected patients, antimicrobial restrictions and additional cleaning protocols are often required to reduce VRE in the hospital environment.[7,10,24,25] In addition, VRE belonging to CC17 are causing severe infections, in particular bacteraemia, in increasing numbers.[9,23]

The data provided by this survey will be useful in informing microbiologists, infectious diseases physicians and infection control practitioners about the increasing importance of VRE in Australia. It will help to guide prescribers treating presumptive enterococcal infections in empirical choices; e.g. ampicillin/amoxycillin still being active against the vast majority of isolates of *E. faecalis* when treating infections caused by this organism. Finally, the data will assist regulators and the pharmaceutical industry on the growing importance of VRE in Australia, and guide decision makers about controls that might be required on the prescribing of reserve antibiotics.

Limitations of the study

The enterococci in this study were tested against a limited range of antimicrobials. In part, this was driven by the presence of intrinsic resistances in this genus. Enterococci are intrinsically resistant to cephalosporins, macrolides, lincosamides and conventional therapeutic levels of aminoglycosides when used alone. Other agents which are usually active against enterococci in urinary tract infection, including fluoroquinolones and nitrofurantoin, were not examined, largely because few clinical treatment problems have been encountered up to now with enterococcal urinary tract infection.

It is likely that the number of wound isolates in this study under-represents the true proportion, as it is common for microbiology laboratories not to proceed with identification or susceptibility testing of enterococci when they are found in mixed cultures from wound infections.

Only a maximum of 100 isolates were collected per institution, therefore only a portion of actual clinical isolates are represented.

There have been changes in participating laboratories in the AGAR Enterococcus surveys over time from 1995 through to 2010 with the more recent inclusion of a number of private pathology laboratories. This may have influenced trend data.

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