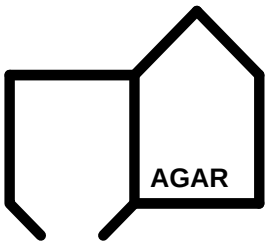

The Australian Group on Antimicrobial Resistance

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Enterococcus species Survey
2010 Antimicrobial Susceptibility and Vancomycin Resistant Enterococci (VRE) Characterisation Report

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Antimicrobial Susceptibility and VRE Characterisation Report of *Enterococcus* Isolates from the Australian Group on Antimicrobial Resistance (AGAR)

2010 Surveillance Report

Table of Contents

1 Executive Summary

Fifteen institutions around Australia conducted a period prevalence study of key resistances in isolates of *Enterococcus* species causing clinical disease amongst in- and outpatients in 2010. Each site collected up to 100 consecutive isolates and tested them 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 to similar surveys conducted in 1995, 1999, 2003, 2005, 2007 and 2009. In the 2010 survey, *E. faecalis* (1,201 strains) and *E. faecium* (170 strains) made up 98.9% of the 1,386 isolates tested. Ampicillin resistance was very common (85.3%) in *E. faecium* and absent for *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 three *E. faecalis* isolates. The *vanA* gene was detected in one *E. faecium* isolate but not in *E. faecalis.* All vancomycin-resistant *E. faecium* belonged to clonal complex 17. The most common sequence type (ST) was ST203 which was found in all regions with VRE. 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 8.2% in *E. faecalis* and 43.8% in *E. faecium*. Linezolid resistance was more common in *E. faecalis* (5.8%) than *E. faecium* (0.9%). 9.4% of *E. faecium* were resistant to quinupristin/dalfopristin (*E. faecalis* is intrinsically resistant).

It is important to have an understanding of the occurrence of VRE and high level aminoglycoside resistance in Australia to guide infection control practices, antibiotic prescribing policies and drug regulatory matters. Mapping of common MLST types provides useful epidemiological information on the types of *E. faecium* VRE in Australia compared to those reported from other countries.

2 Introduction

2.1 Objective of the Programme

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

1. Assessing susceptibility to ampicillin
2. Assessing susceptibility to glycopeptides
3. Assessing changes in resistance patterns over time using data collected in previous AGAR surveys
4. To determine the VRE clones circulating within Australia

AGAR commenced surveillance of antimicrobial resistance in *Enterococcus* species in 1995. Similar surveys were conducted in 1999, 2003, 2005, 2007 and 2009[1,2,3,4,5].

2.2 Importance of *Enterococcus* Species

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. In hospitals they 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. Enterococci are recognised as significant nosocomial pathogens causing urinary tract, blood stream, sterile site and wound infections.

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[6] and in the USA in the early 1990s[7]. The first VRE was reported in Australia in 1994[8] and a report on the emergence and epidemiology of VRE in Australia was described in 1998[9] when 69 isolates had been documented.

Multilocus sequence typing (MLST) of *E. faecium* has revealed that clonal complex (CC) 17 strains have become predominant in hospitals in many countries[10,11,12,13,14] and are characterised by ampicillin resistance and the presence of several genetic elements (*esp*, *hyl*) not present in colonising variants in humans and animals. 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 or incidence rates of 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[14,15,16,17,18]. The clinical impact of vancomycin resistance in enterococci has been reported to result in increases in mortality, length of stay and hospital costs[19,20,21]. Serious infections caused by vancomycin-resistant *E. faecium* are difficult to treat relying on recently introduced antimicrobials such as linezolid, quinupristin-dalfopristin and daptomycin which are not approved for all indications. Further complicating treatment of infections caused by VRE are reports of isolates resistant even to these newer agents[22,23].

The key to controlling VRE in the hospital system, particularly in low prevalence countries, appears to be controlling antimicrobial use and adhering to strict infection control guidelines[24]. Infection control measures can be used to eradicate the organism from a hospital in an outbreak setting and to help prevent it from becoming established[15]. Once prevalent in the hospital environment, cleaning of wards with bleach or hydrogen peroxide vapour has been shown to reduce VRE environmental contamination[25,26,27] providing a valuable tool for hospitals in reducing hospital-acquired infections.

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.

2.3 Antimicrobials Tested and Resistance

2.3.1 ß-lactams

Penicillin (IV benzylpenicillin) and ampicillin/amoxycillin (oral and IV) are the principal therapeutic agents used for the treatment of infections caused by enterococci.

Ampicillin: Testing of this agent is used to predict susceptibility to penicillin and amoxycillin. Resistance to penicillin/ampicillin most commonly results from alterations to penicillin binding proteins. Resistance is rarely mediated by a ß-lactamase.[28]

2.3.2 Glycopeptides

Vancomycin resistance is mediated by one of a number of gene clusters carried either on a transposon or on the chromosome. Organisms with a VanA phenotype are resistant to both vancomycin and teicoplanin whereas organisms with the VanB phenotype are resistant to vancomycin only. Both these phenotypes are located on transmissible genetic elements. Resistance is due to changes in the ligase gene that results in an alteration of the glycopeptide binding site. Several other genes in the cluster potentiate this alteration.

Resistance can be detected by the use of a screening plate or routine susceptibility testing. The result is confirmed by detection of the *vanA* or *vanB* genes by polymerase chain reaction (PCR).

2.3.3 Aminoglycosides

High level resistance to aminoglycosides (Minimum inhibitory concentration [MIC] >500–2000mg/L) is mediated by plasmid borne aminoglycoside modifying enzymes (most commonly a fused 6'-acetyltransferase-2'-phosphotransferase for gentamicin, tobramycin, amikacin and a 6-adenylyltransferase for streptomycin). Possession of these enzymes eliminates synergy between the aminoglycoside and the ß-lactam.

2.3.4 Oxazolidinones

The first of the new drug class of oxazolidinones (linezolid) was introduced into clinical practice in Australia in the middle of the first decade of this century. It has a novel mechanism of action, and there is no cross-resistance with other drug classes. With a strictly Gram-positive spectrum, it is a valuable reserve agent for the treatment of patients with (i) infections caused by Gram-positive bacteria resistant to, or (ii) who are intolerant of other drug classes.

2.3.5 Streptogramins

Quinupristin-dalfopristin is a combination antibiotic of members of the streptogramin B and A antibiotic classes. The agents act synergistically, and the combination is active even in the presence of resistance to the streptogramin B class, which is common and linked to macrolide and lincosamide resistance. The combination is active against many Gram-positive bacteria, including those resistant to other drug classes. *E. faecalis* is intrinsically resistant. It is used occasionally when resistance to other classes is a problem.

3 Methods

Fifteen institutions from all mainland Australian states and the Australian Capital Territory (ACT) participated in the survey. From the 1st January to the 30th June 2010, each participating laboratory collected up to 100 consecutive, significant, clinical isolates of enterococci. Only one isolate per patient was tested unless a different antibiogram was observed from routine susceptibility results. For each isolate the following information was obtained: date of collection, age, sex, specimen source, and inpatient or outpatient status.

3.1 Species Identification

All isolates were tested for pyrrolidonyl arylamidase (PYR) with optional testing for growth in 6.5% NaCl, esculin hydrolysis in the presence of bile, Group D antigen and growth at 45°C. Isolates were identified to species level by one of the following methods: API 20S, Vitek 2, Phoenix, PCR, or conventional biochemical tests. If biochemical testing was performed, the

minimum tests necessary for identification were: motility, pigment production, methyl-α-D-glucopyranoside (MGP), fermentation of 1% raffinose, 1% arabinose, 1% xylose and pyruvate utilisation.

3.2 Susceptibility Testing Methodology

Participating laboratories performed antimicrobial susceptibility tests according to each laboratory's routine standardised methodology[29,30,31] (CLSI disc diffusion, Vitek 2, Phoenix, agar dilution or Etest). Ampicillin and vancomycin were tested by all laboratories. In addition, 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.

116/178 (65.2%) of invasive isolates were tested for ß-lactamase production using nitrocefin.

3.3 VRE Characterisation

Pulsed-field gel electrophoresis (PFGE) of VRE was performed as previously described[32]. MLST on a representative of each pulsotype of *E. faecium* was performed as described at www.efaecium.mlst.net.

3.4 Quality Control

Additional quality control was not performed for this survey. As all participating laboratories are NATA accredited, routine QC testing of antimicrobial susceptibility test methods is an integral part of routine procedures. However, isolates that were resistant to vancomycin were referred to the appropriate state National VRE Network (NaVREN) laboratory or tested on-site to confirm resistance genotype by PCR.

4 Demographics

4.1 Regional Source of Isolates

Both public (13) and private (2) laboratories participated in this study. Participants included New South Wales [NSW] (3), Australian Capital Territory [ACT] (1), Queensland [Qld] (4), Victoria [Vic] (1), South Australia [SA] (3) and Western Australia [WA] (3). Data from NSW and ACT have been combined. 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).

Table 1. Isolates by Region

Region	Participating Laboratories (n)	Isolates (n)	%
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**

Table 2. Species 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
Aus	1,201 (86.7%)	170 (12.3%)	15 (1.1%)	1,386

4.2 Age and Sex Distribution

The age distribution of patients reflect the association of infection with other predisposing medical conditions more commonly seen in the elderly or very young. Isolation of enterococci was similar for males and females (females 51.2% 95%CI 48.6-53.9%). 829 (59.8%) patients were classified as hospital inpatients at time of collection and 541 (39.0%) were outpatients. Hospitalisation status was not available for 16 patients.

Table 3. Age and Sex Distribution by Species

Age Range (years)	*E. faecalis*	*E. faecium*	Other spp. or unspeciated	Total (%)
<2	49	0	1	50 (3.6)
2-4	9	0	0	9 (0.6)
5-14	9	2	0	11 (0.8)
15-29	106	4	0	110 (7.9)
30-59	255	44	4	303 (21.9)
≥60	773	120	10	903 (65.2)
Female	605	97	8	710 (51.2)
Male	596	73	7	676 (48.8)

5 Specimen Source

The majority of isolates (70.9%) were from the urinary tract (Table 4). These were predominantly *E. faecalis* (91.3%). Invasive (blood, cerebrospinal fluid [CSF] and sterile body cavity) isolates comprised 12.8% of the total number collected. *E. faecium* was disproportionately represented in the invasive group (28.7%). Of the *E. faecalis* isolates, 9.9% were invasive compared to 30.0% of *E. faecium.*

Table 4. Source of Isolates

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%)

6 Susceptibility Testing Results: 2010 Study and Trend Data

6.1 Ampicillin

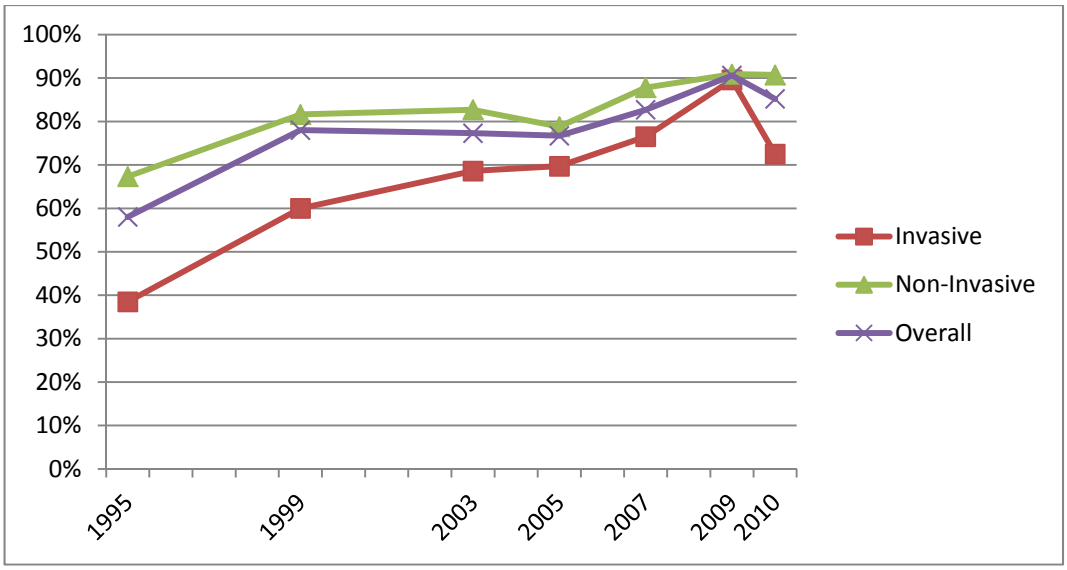
Resistance to ampicillin was common in the *E. faecium* (Table 5). Resistance in *E. faecium* was due to penicillin binding protein changes. No ß-lactamase positive *E. faecium* were detected amongst the subset (30/51, 59%) of invasive strains tested. Resistance in invasive strains was lower than for non-invasive strains (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 was positive.

Trend data for *E. faecium* show that from 1995 to 1999, there was an increase in ampicillin resistance (P=0.0017) with a plateau from 1999 to 2005. Since 2005, resistance has once again increased significantly (P=0.0053). The gap between resistance in non-invasive versus invasive strains narrowed over time, however in 2010 there was a reversal of this trend with rates of resistance in invasive strains falling significantly (P=0.0414) compared to 2009 levels (Figure 1).

Table 5. Ampicillin Resistance. Number Resistant/Total (%)

	NSW/ACT	QLD	SA	VIC	WA	AUS
E. faecalis	0/334	0/381	0/145	0/76	0/265	0/1,201
	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)
Invasive	0/46	0/14	0/22	0/8	0/18	0/108
	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)
E. faecium	34/41	17/18	51/57	19/23	24/31	145/170
	(82.9)	(94.4)	(89.5)	(82.6)	(77.4)	(85.3)
Invasive	12/18	1/2	17/20	3/3	4/8	37/51
	(66.7)	(50.0)	(85.0)	(100)	(50.0)	(72.5)

Figure 1. Ampicillin Resistance: *E. faecium*



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

6.2 Vancomycin

Resistant and intermediate resistant isolates have been combined and referred to as non-susceptible (NS). All isolates were tested for susceptibility to vancomycin and if NS tested for the presence of *van* genes. In addition 27 vancomycin susceptible *E. faecium* and 135 susceptible *E. faecalis* were tested for the presence of *van* genes as it is routine practice in one laboratory to perform PCR on all enterococci and in several laboratories to perform PCR on *E. faecium* from sterile sites regardless of vancomycin susceptibility.

Resistance to vancomycin was uncommon in *E. faecalis* (0.5%) (Table 6). Of the six NS *E. faecalis*, two were the *vanB* genotype and four did not possess either *vanA* or *vanB* (Table 7). Of note, one vancomycin susceptible *E. faecalis* from a urine specimen possessed the *vanB* gene.

A total of 36.5% of *E. faecium* were vancomycin NS; 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 NS *E. faecium* were detected in all regions except Western Australia. Vancomycin NS in the other regions ranged from 16.7% in Queensland to 54.4% in South Australia (Table 6). All of the vancomycin NS *E. faecium* were confirmed as VRE by PCR and were predominantly (61/62, 98.4%) of the *vanB* genotype. In addition, one vancomycin susceptible *E. faecium* (vancomycin Etest MIC 1.5 mg/L) from a blood culture specimen possessed the *vanB* gene. In 2010 more than one third of urine, wound and blood *E. faecium* were VRE. The average age of a patient with a VRE was 68 years (median 72 years) and the genders were equally represented (56% female, 95%CI 43.3-67.2%). Trend data for *E. faecium* show that after no vancomycin resistance was detected in 1995 there has been a marked increase, particularly since 2003 (Figure 2). A big increase in NS *E. faecium* occurred from 2007 to 2009, and for non-invasive isolates the increase continued from 2009 to 2010 but not significantly (P=0.4540). Although the proportion of NS isolates in invasive isolates decreased from 37.5% in 2009 to 29.4% in 2010, this was not a significant drop (P=0.4042). Vancomycin resistant *E. faecium* have occurred in all five regions over the six survey periods, with all regions except WA showing increases in VRE over time (Figure 3).

Table 6. Vancomycin non-susceptible. Number/Total (%)

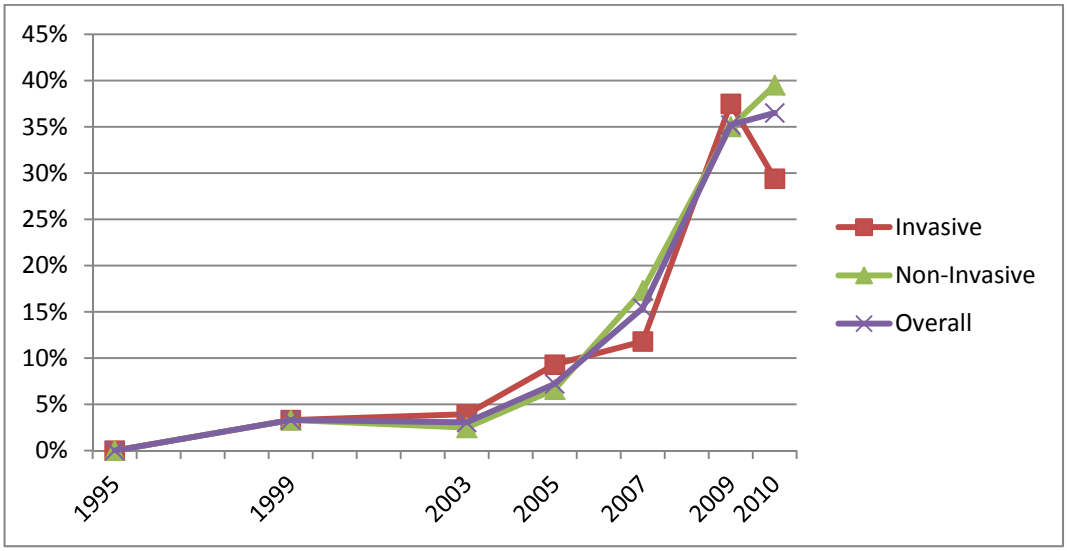
	NSW/ACT	QLD	SA	VIC	WA	AUS
E. faecalis	2/334	1/381	0/145	2/76	1/265	6/1,201
	(0.6)	(0.3)	(0.0)	(2.6)	(0.4)	(0.5)
Invasive	2/41	0/12	0/40	0/5	1/21	3/119
	(4.9)	(0.0)	(0.0)	(0.0)	(4.8)	(2.5)
E. faecium	18/41	3/18	31/57	10/23	0/31	62/170
	(43.9)	(16.7)	(54.4)	(43.5)	(0.0)	(36.5)
Invasive	6/18	0/2	8/20	1/3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

Table 7. Vancomycin Non-susceptible Enterococci: *van* gene PCR results

	E. faecalis			***E. faecium***		
Specimen	***vanA***	***vanB***	***vanA/B* not detected**	***vanA***	***vanB***	***vanA/B* not detected**
Urine		1	1		28	
Wound		1			19	
Blood			2	1	11	
Sterile body cavity			1		3	
Total		**2**	**4**	**1**	**61**	

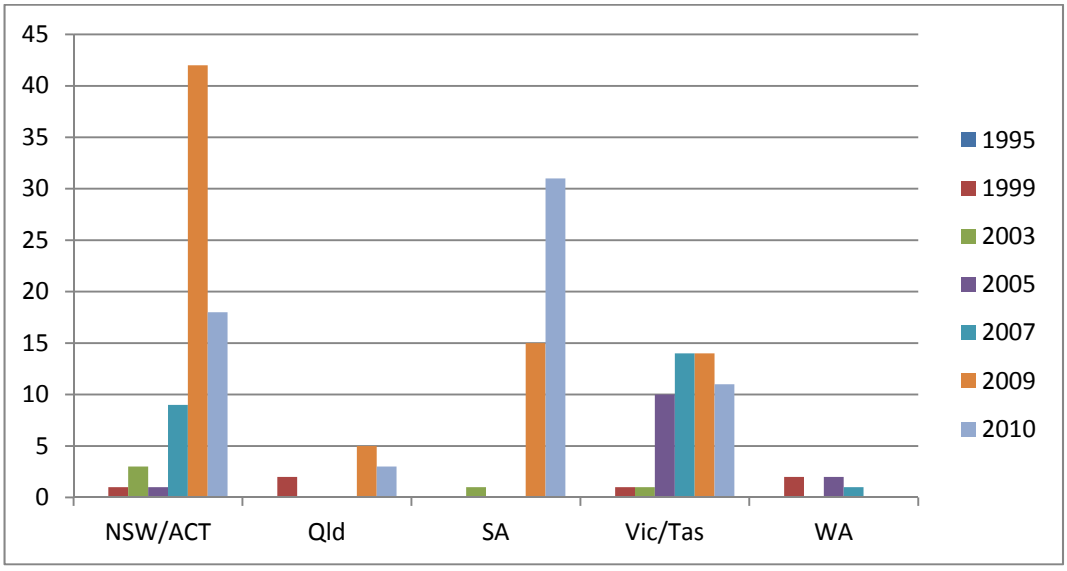
*Note: In addition, one *vanB E. faecalis* and one *vanB E. faecium* with vancomycin susceptible MICs were detected through routine PCR.

Figure 2. Vancomycin Resistance: *E. faecium*



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 *E. faecium* 1995, 1999, 2003, 2005, 2007, 2009 and 2010



Note: Tasmania did not contribute isolates in 2009 or 2010

6.3 Aminoglycosides

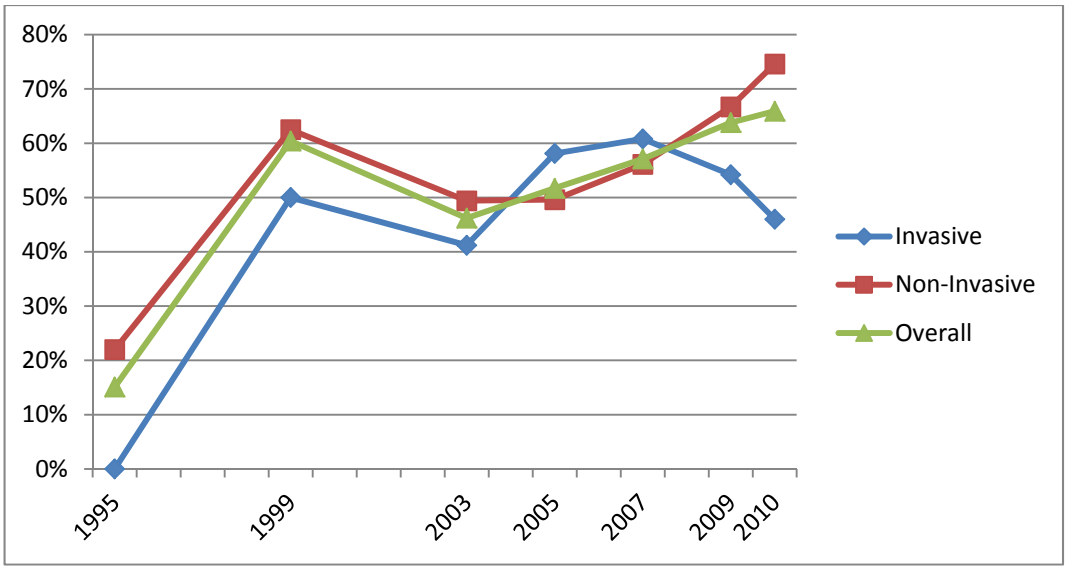
6.3.1 Gentamicin

High level gentamicin (HLG) resistance was seen in *E. faecalis* (34.1%) and *E. faecium* (66.1%) (Table 8). 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 strains as rates of resistance remained stable in invasive strains during that time period (P=0.09). HLG resistance in *E. faecalis* invasive and non-invasive isolates continued to increase until 2005 and then stabilised.

Table 8. High Level Gentamicin Resistance

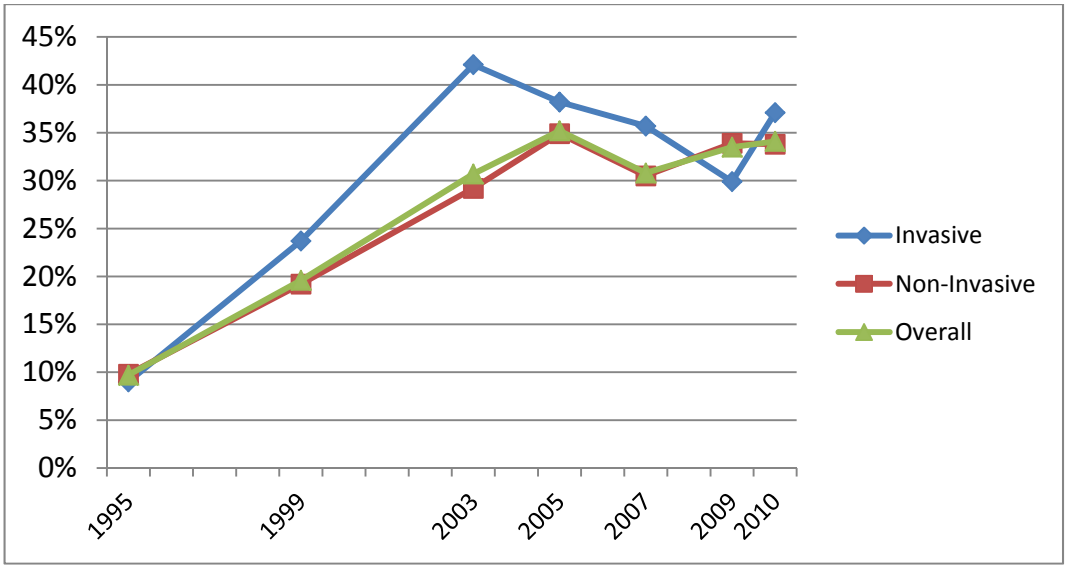
	NSW/ACT	QLD	SA	VIC	WA	AUS
E. faecalis	141/334 (42.2)	150/381 (39.4)	38/142 (26.8)	18/76 (23.7)	62/265 (23.4)	409/1,198 (34.1)
Invasive	11/41 (26.8)	4/12 (33.3)	15/37 (40.5)	1/5 (20.0)	12/21 (57.1)	43/116 (37.1)
E. faecium	30/41 (73.2)	16/18 (88.9)	30/52 (57.7)	16/23 (69.6)	17/31 (54.8)	109/165 (66.1)
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)

Figure 4. High level Gentamicin Resistance: *E. faecium*



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. High Level Gentamicin Resistance: *E faecalis*



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.

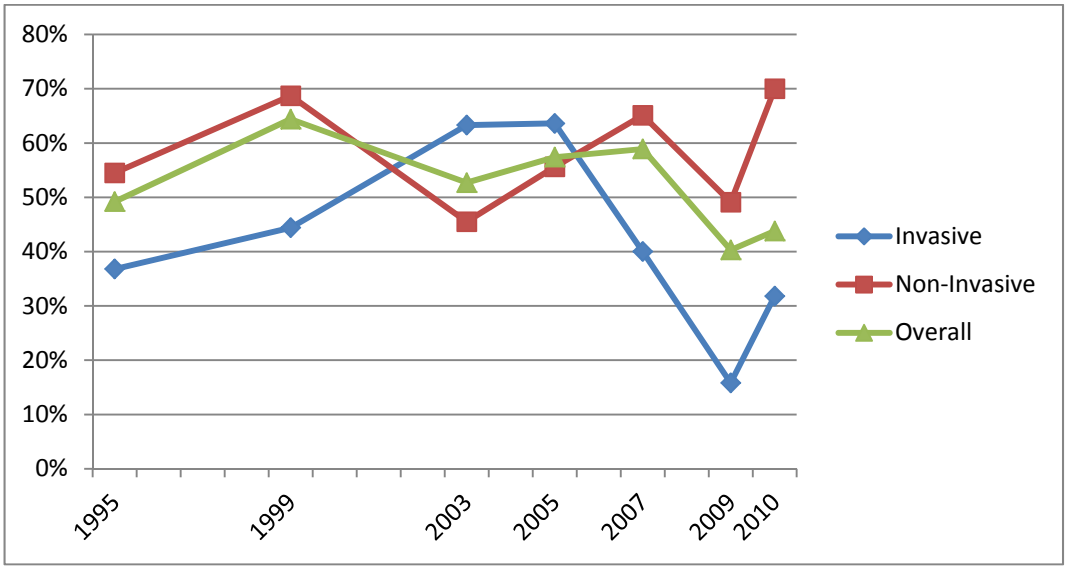
6.3.2 Streptomycin

In this survey, high level streptomycin resistance (HLS) was tested only in NSW/ACT and SA. HLS resistance as with HLG resistance is more common for *E. faecium* than *E. faecalis* (Table 9). 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*, the HLS decreased significantly from 1995 to 2003 but has been relatively stable since then with lower rates of expression than HLG.

Table 9. High Level Streptomycin Resistance

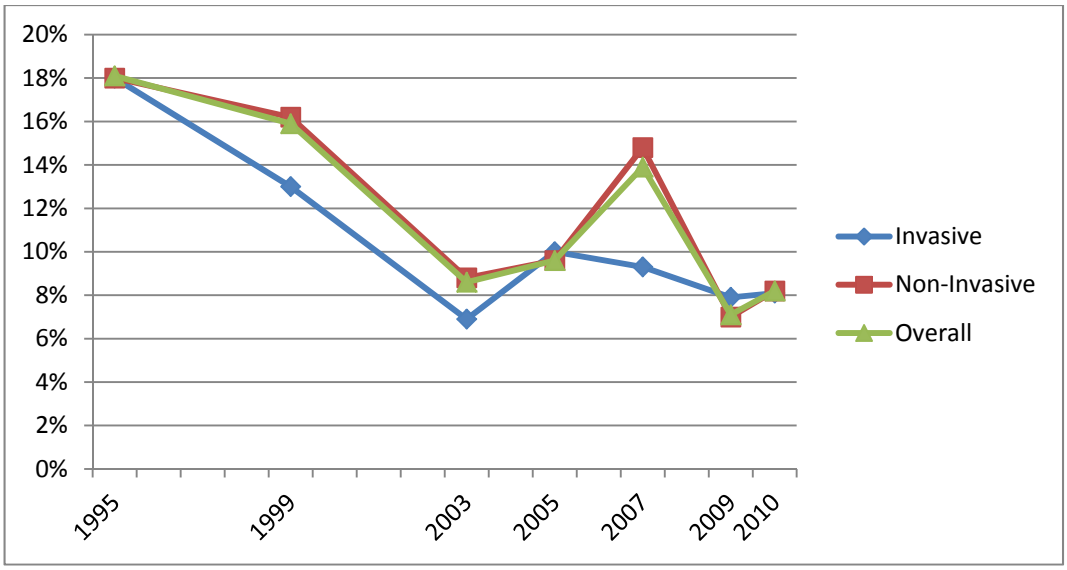
	NSW/ACT	QLD	SA	VIC	WA	AUS
E. faecalis	6/73 (8.2)	-	3/37 (8.1)	-	-	9/110 (8.2)
invasive	1/9 (11.1)	-	2/28 (7.1)	-	-	3/37 (8.1)
E. faecium	2/7 (28.6)	-	12/25 (48.0)	-	-	14/32 (43.8)
invasive	1/5 (20.0)	-	6/17 (35.3)	-	-	7/22 (31.8)

Figure 6. High Level Streptomycin Resistance: *E. faecium*



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. High Level Streptomycin Resistance: *E. faecalis*



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.

6.4 Linezolid

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

Table 10. Linezolid Non-susceptibility. Number Resistant/Total (%)

	NSW/ACT	QLD	SA	VIC	WA	AUS
E. faecalis	18/334	28/341	1/90	-	0/5	47/810
	(5.4)	(7.3)	(1.1)		(0.0)	(5.8)
invasive	2/41	0/12	1/32	-	0/5	3/90
	(4.9)	(0.0)	(3.1)		(0.0)	(3.3)
E. faecium	1/41	0/18	0/52	-	0/2	1/113
	(2.4)	(0.0)	(0.0)		(0.0)	(0.9)
invasive	1/18	0/2	0/18	-	0/1	1/39
	(5.6)	(0.0)	(0.0)		(0.0)	(2.6)

6.5 Quinupristin/dalfopristin

E. faecalis are intrinsically resistant to quinupristin/dalfopristin (Q/D). 9.4% of the *E. faecium* were NS (down from 21.9% in 2009) with four of the five NS isolates having an MIC in the resistant range (MIC >2 mg/L). As was the case in 2007 and 2009, all Q/D NS cases were identified in isolates originating in NSW/ACT.

Table 11. Quinupristin/dalfopristin Non-susceptibility. Number Resistant/Total (%)

	NSW/ACT	QLD	SA	VIC	WA	AUS
E. faecalis	246/258 (95.3)	154/177 (87.0)	2/3 (66.7)	-	5/5 (100)	407/443 (91.9)
invasive	33/34 (97.1)	6/6 (100)	0/1 (0.0)	-	5/5 (100)	44/46 (95.7)
E. faecium	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)

7 Cross Resistance

Cross resistance to other agents was examined in vancomycin resistant strains of enterococci (Table 12). Resistance to ampicillin and high levels of gentamicin was more common in vancomycin resistant *E. faecium*. Resistance to high levels of streptomycin, Q/D and linezolid was similar for VRE and non-VRE (P>0.05).

Table 12. Cross Resistance in VRE

Species	Vancomycin Status	Ampicillin R (%)	Gentamicin R (%)	Streptomycin R (%)	Linezolid IR (%)	Q/D IR (%)
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)	51/58 (87.9)	9/16 (56.3)	0/51 (0.0)	1/19 (5.3)

Q/D: Quinupristin/dalfopristin.

8 VRE Characterisation

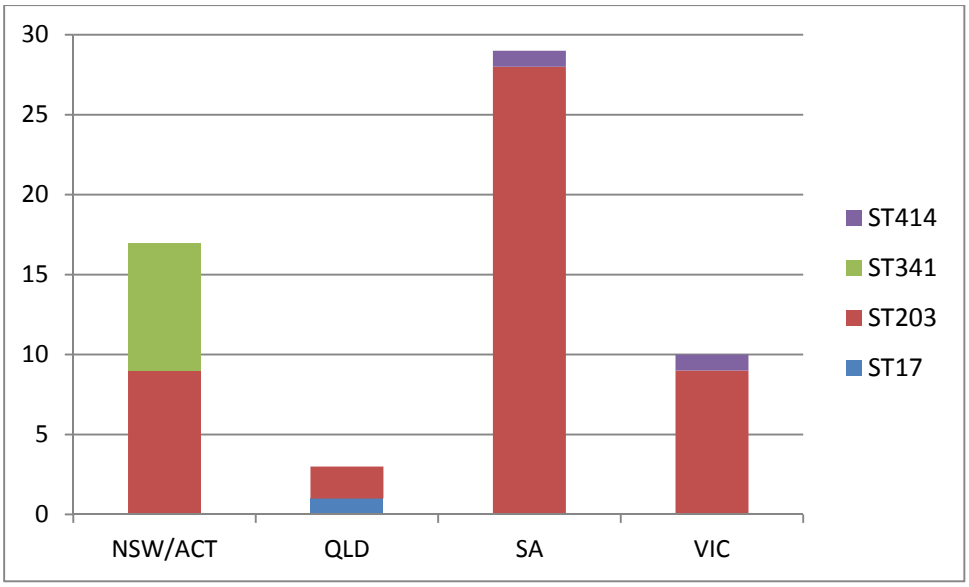
Three (100%) *vanB E. faecalis*, one (100%) *vanA E. faecium* and 59/62 (95%) *vanB E. faecium* were available for molecular typing. PFGE was performed on all isolates. MLST was performed on the *E. faecium*.

Two of the *vanB E. faecalis* were classified as pulsotype A and one was classified as pulsotype B (Table 13). The *vanA E. faecium* was a pulsotype C and ST117. Six pulsotypes and four sequence types (ST) were identified in the *vanB E. faecium*. ST203 was the most common ST (81% of *vanB E. faecium*) and was found in all regions with VRE. ST341 was found only in NSW/ACT, ST414 only in SA and Vic and ST17 only in Qld (Figure 8). The *E. faecium* strains belonged to clonal complex (CC) 17.

Table 13. Molecular Characterisation of VRE

Van gene	Species	PFGE	MLST	NSW/ACT	Qld	SA	Vic
vanB	*E. faecalis*	A	N/A				2
vanB	*E. faecalis*	B	N/A			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

Figure 8: MLST of *vanB E. faecium* by region



9 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 UTI.

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

As only a maximum of 100 isolates were collected per institution 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.

10 Discussion

It is clear from this study and the examination of trends over the last 15 years that resistance is increasing significantly in *E. faecium*. Treatment options for this species are becoming ever more limited as resistance to ampicillin and other penicillins is now very high, 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.

In *E. faecium*, ampicillin resistance is the result of changes in penicillin-binding proteins. This is also true for most strains of *E. faecalis*, although ß-lactamase production has been seen rarely (three known instances in Australia in the last two decades)[28]. This survey has shown that ampicillin resistance is now the norm in *E. faecium* but is rare in *E. faecalis*. Ampicillin resistance in enterococci presents considerable challenges when infections are serious, as the strains will not be susceptible to any ß-lactam, 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 five 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 our 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[9,33], in contrast with the situation in Europe and the USA where *vanA* dominates. Vancomycin-resistant strains 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 as well.

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

Molecular characterisation of the VRE strains 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[14,34,35]. Containing additional genetic content thought to assist in survival and spread in the hospital environment, CC17 pose 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[12,15,34,36]. In addition VRE belonging to CC17 are causing severe infections, in particular bacteraemia, in increasing numbers[14,34].

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 strains 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 reserve antibiotics.

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