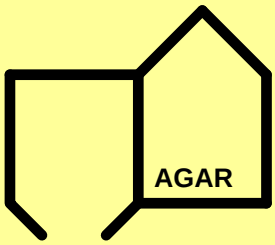

The Australian Group on Antimicrobial Resistance

http://www.antimicrobial-resistance.com

Enterococcus species Survey
2007 Antimicrobial Susceptibility Report

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

2007 Surveillance Report

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1 Executive Summary

Seventeen institutions around Australia conducted a period prevalence study of key resistances in isolates of *Enterococcus* species causing clinical disease amongst in- and outpatients in 2007. Each site collected up to 100 consecutive isolates and tested them for susceptibility to ampicillin, vancomycin, high-level gentamicin and/or high-level streptomycin using standardised methods. Results were compared to similar surveys conducted in 1995, 1999, 2003 and 2005. In the 2007 survey, *E. faecalis* (1520 strains) and *E. faecium* (156 strains) made up 98.9% of the 1695 isolates tested. Ampicillin resistance was very common (82.7%) in *E. faecium* and absent from *E. faecalis.* Resistance to vancomycin was 15.4 % in *E. faecium* (up from 7.2% in the 2005 survey) and 0.5% in *E. faecalis*; the *vanB* gene was detected in all the resistant *E. faecium* and in five of the eight *E. faecalis* isolates. High-level resistance to gentamicin was 30.8% in *E. faecalis* and 57.1% in *E. faecium*; the figures for high-level streptomycin were 13.9% and 58.9% respectively. For the first time, a subset of isolates was tested against linezolid and quinupristin/dalfopristin. Linezolid resistance was 4.8% in *E. faecalis* and absent in *E. faecium*. 9.8% 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.

2 Introduction

2.1 Objective of the Programme

The objective of the 2007 surveillance program was to determine the proportion of antimicrobial resistance in clinical isolates of *Enterococcus* spp 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

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

2.2 Importance of *Enterococcus* spp

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* (80–90%) and *Enterococcus faecium* (5-10%) 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. Although resistant to many antibiotics Enterococci have been generally susceptible to amoxycillin and vancomycin. *Enterococcus faecium* has become increasingly resistant to ampicillin/amoxycillin making vancomycin the treatment of choice for severe infections caused by this organism. Since 1988 resistance to vancomycin has emerged and increased worldwide and is now widespread in Europe and the USA. Recent reports from the USA indicate that rates of VRE in hospitalised adults are increasing at a rapid rate.[4] For device-associated infections the incidence is particularly high: the National Healthcare Safety Network (NHSN) reported 78.9% vancomycin resistance in *E. faecium* from central line-associated bloodstream infections[5]. The first vancomycin resistant enterococcal isolate (VRE) was reported in Australia in 1994[6] and a report on the emergence and epidemiology of VRE in Australia was described in 1998[7] when 69 isolates had been documented. 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.[8,9,10,11,12] The clinical impact of vancomycin resistance in enterococci has been reported to result in increases in mortality, length of stay and hospital costs.[13,14,15] Infection control measures can be used to eradicate the organism from a hospital or to prevent it from becoming established.[8]

Enterococci cause 5-18% of all cases of endocarditis, involving both prosthetic and normal heart valves.[16,17,18] Combination therapy of a ß-lactam and an aminoglycoside (gentamicin or streptomycin)[19,20,21] has been the standard treatment for endocarditis as use of ß-lactams alone are associated with high relapse rates (30-60%). Aminoglycosides are not routinely used to treat other enterococcal infections but in endocarditis the synergy between the two agents provides a cure. Synergy does not occur if the organism has high level gentamicin or streptomycin resistance (MIC> 500mg/L).

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.

2.3 Antimicrobials Tested and Resistance

2.3.1 ß-lactams

Penicillin (IV benzylpenicillin) and ampicillin/amoxycillin (oral and IV) are the principle 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.[22]

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 PCR.

2.3.3 Aminoglycosides

High level resistance to aminoglycosides (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-positives 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 species, including those resistant to other drug classes. *E. faecalis* is intrinsically resistant. It as used occasionally when resistance to other classes is a problem.

3 Methods

Seventeen institutions from all Australian states and the Australian Capital Territory (ACT) participated in the *Enterococcus* spp survey. Commencing on the 1st January 2007 each participating laboratory collected 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) and esculin hydrolysis in the presence of bile with optional testing for growth in 6.5% NaCl, Group D antigen and growth at 45°C. Isolates were identified to species level by one of the following methods: API 20S, rID32Strep, Vitek or Vitek 2, Microscan, 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[23,24,25,26,27] (CLSI, CDS or BSAC disc diffusion, Vitek, Vitek 2, agar dilution or Etest). Ampicillin and high-level gentamicin were tested by all laboratories. One thousand five hundred and ninety one isolates (94%) were tested against vancomycin. The remaining isolates were screened for vancomycin resistance with brain heart infusion agar supplemented with 6mg/L of vancomycin and if screen positive PCR for *vanA* and *vanB* genes was performed. In addition, 999 (59%) were screened for high level streptomycin resistance, 799 (47%) were tested against linezolid, 697 (41%) were tested against quinupristin/dalfopristin and 511 (30%) were tested against teicoplanin.

The majority (1504, 89%) of isolates were tested for ß-lactamase production using nitrocefin.

3.3 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 for molecular testing to confirm organism identification and resistance phenotype. All isolates were stored at -70°C for further testing if required by AGAR.

4 Demographics

4.1 Regional Source of isolates

Both public (15) and private (2) laboratories participated in this study. Participants included New South Wales (4), ACT (1), Queensland (3), Victoria (3), South Australia (2), Western Australia (3) and Tasmania (1). There were 1695 isolates from 17 institutions (Table 1). *E. faecalis* was the most frequently isolated species (89.7%) followed by *E. faecium* (9.2%) (Table 2). To ensure institutional anonymity data from NSW and ACT and data from Tasmania and Victoria have been combined.

Table 1. Isolates by Region

Region	Participating Laboratories (n)	Isolates (n)	%
New South Wales/Australian Capital Territory (NSW/ACT)	5	499	29.4
Queensland (Qld)	3	300	17.7
South Australia (SA)	2	196	11.6
Victoria/Tasmania (Vic/Tas)	4	400	23.6
Western Australia (WA)	3	300	17.7
Total	**17**	**1695**	**100**

Table 2. Species by Region

Region	*E. faecalis*	*E. faecium*	Other Spp.	Total
NSW/ACT	428	62	9	499
Qld	287	11	2	300
SA	187	7	2	196
Vic/Tas	347	52	1	400
WA	271	24	5	300
Aus	1520 (89.7%)	156 (9.2%)	19 (1.1%)	1695

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 more common in women, in keeping with the greater incidence of urinary tract infections in that sex. Of note however is the greater proportion of *E. faecium* (59.4%) from women compared to men (40.6%) (Table 3). 794 (57.5%) patients were classified as hospital inpatients at time of collection and 624 (36.8%) were outpatients. Hospitalisation status was not available for 97.

Table 3. Age and Sex Distribution by Species

Age Range	*E. faecalis*	*E. faecium*	Other Spp.	Total (%)
<2	97	1	0	98 (5.8)
2-4	28	0	0	28 (1.7)
5-14	30	2	3	35 (2.1)
15-29	113	0	2	115 (6.8)
30-59	350	37	4	391 (23.1)
≥60	902	116	10	1028 (60.6)
Sex*				
Female	809	92	11	912 (54.6)
Male	688	63	8	759 (45.4)

*Gender not available for 24 patients

5 Specimen Source

The majority of isolates (71.9%) were from the urinary tract (Table 4). These were predominantly *E. faecalis* (93.7%). Invasive (blood, CSF and sterile cavity) isolates comprised 11.9% of the total number collected. *E. faecium* was disproportionately represented in the invasive group (25.4%). Of the *E. faecalis* isolates, 9.4% were invasive compared to 32.7% of *E. faecium.*

Table 4. Source of Isolates

Source	*E. faecalis*	*E. faecium*	Other Spp.	Total
Urine	1142	72	5	1219 (71.9%)
SSTI	174	25	6	205 (12.1%)
Blood/CSF	113	36	5	154 (9.1%)
Sterile Body Cavity	30	15	2	47 (2.8%)
Other	60	7	1	68 (4.0%)
Unknown	1	1		2 (0.1%)
Total	**1520**	**156**	**19**	**1695**
Invasive	143	51	7	201 (11.9%)
Non-invasive	1333	98	11	1442 (85.1%)
Unknown	44	7	1	52 (3.1%)

6 Susceptibility Testing Results: 2007 Study and Trend Data

6.1 Ampicillin

Resistance to ampicillin was common in the *E. faecium* isolates with no significant differences between the regions (Table 5). Resistance in *E. faecium* was due to penicillin binding protein

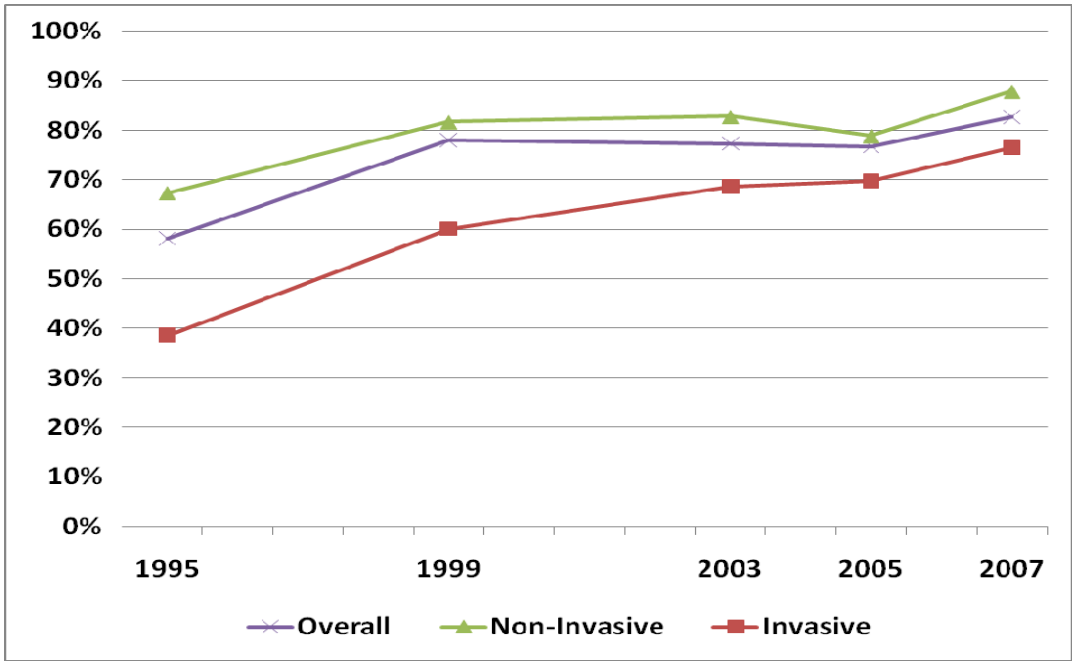
changes. No ampicillin resistance was found amongst the *E.* faecalis strains. 1507 (88.9%) of the isolates were tested for ß-lactamase; none were positive.

Trend data for *E. faecium* show an initial increase in ampicillin resistance between 1995 and 1999 with a plateau from 1999 to 2007. Resistance in invasive strains continues to slowly rise but since 1999 the increase has not been statistically significant (Figure 1).

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

	NSW/ACT	QLD	SA	VIC/TAS	WA	AUS
E. faecalis	0/428	0/287	0/187	0/347	0/271	0/1520
	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)
invasive	0/62	0/21	0/12	0/37	0/11	0/143
	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)
E. faecium	46/62	9/11	6/7	46/52	22/24	129/156
	(74.2)	(81.8)	(85.7)	(88.5)	(91.7)	(82.7)
invasive	13/20	2/3	3/3	10/13	11/12	39/51
	(65.0)	(66.7)	(100)	(76.9)	(91.7)	(76.5)

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.

6.2 Vancomycin

Resistant and intermediate resistant isolates have been combined and referred to as non-susceptible (NS). Not all laboratories tested isolates against vancomycin: one laboratory screened for vancomycin resistance with brain heart infusion agar supplemented with 6mg/L of vancomycin and if screen positive, *vanA* and *vanB* PCR was performed. For the purpose of assigning resistance, an isolate from this laboratory with confirmed *vanA* or *vanB* genes has been called NS.

Resistance to vancomycin was uncommon in *E. faecalis* (0.5%) (Table 6). Of the eight NS *E. faecalis*, five were of the *vanB* genotype (Table 7). Two isolates that tested as intermediate resistance by disk diffusion (16mm) did not possess either the *vanA* or *vanB* genes. One isolate tested as intermediate resistance by Vitek (8mg/L) was susceptible by Etest (2mg/L): *van* gene PCR was not performed.

A total of 15.4% of *E. faecium* were vancomycin NS, more than double that of the 2005 survey (7.2%, p=0.0224) (Figure 2). Vancomycin NS *E. faecium* were detected in Vic/Tas, NSW/ACT and WA with Vic/Tas having the highest proportion (26.9%) (Table 6). The 24 vancomycin NS *E. faecium* were all confirmed as VRE by PCR and were of the *vanB* genotype. The average age of a patient with a confirmed VRE was 71 years (median 72 years) and the majority (72%) were female. 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). The proportion of NS *E. faecium* in invasive isolates continues to increase at a steady pace but a sharp increase is evident for non-invasive isolates since the last survey. This is due to an increase in VRE from urine samples (5.2% in 2005 to 15.3% in 2007, p=0.0346). Vancomycin resistant *E. faecium* have occurred in all 5 regions over the five survey periods, with NSW/ACT and Vic/Tas showing the greatest increases in VRE over time (Figure 3).

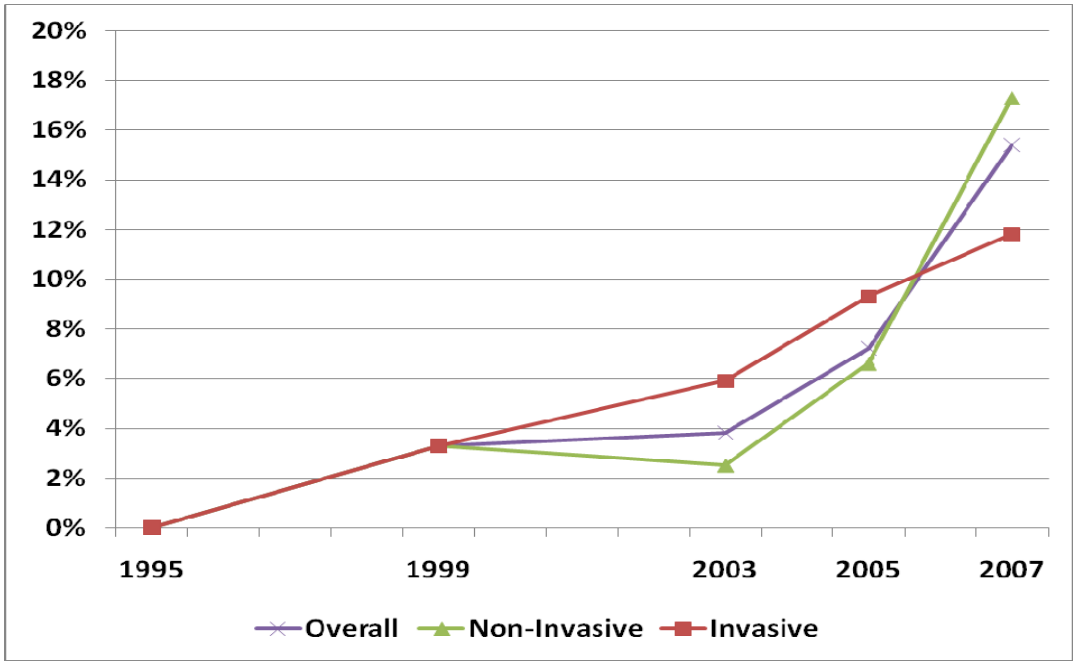
Table 6. Vancomycin non-susceptible. Number/Total (%)

	NSW/ACT	QLD	SA	VIC/TAS	WA	AUS
E. faecalis	2/428	0/287	0/187	5/347	1/271	8/1520
	(0.5)	(0.0)	(0.0)	(1.4)	(0.4)	(0.5)
invasive	1/62	0/21	0/12	0/37	0/11	1/143
	(1.6)	(0.0)	(0.0)	(0.0)	(0.0)	(0.7)
E. faecium	9/62	0/11	0/7	14/52	1/24	24/156
	(14.5)	(0.0)	(0.0)	(26.9)	(4.2)	(15.4)
invasive	3/20	0/3	0/3	3/13	0/12	6/51
	(15.0)	(0.0)	(0.0)	(23.1)	(0.0)	(11.8)

Table 7. Vancomycin Resistant Enterococci

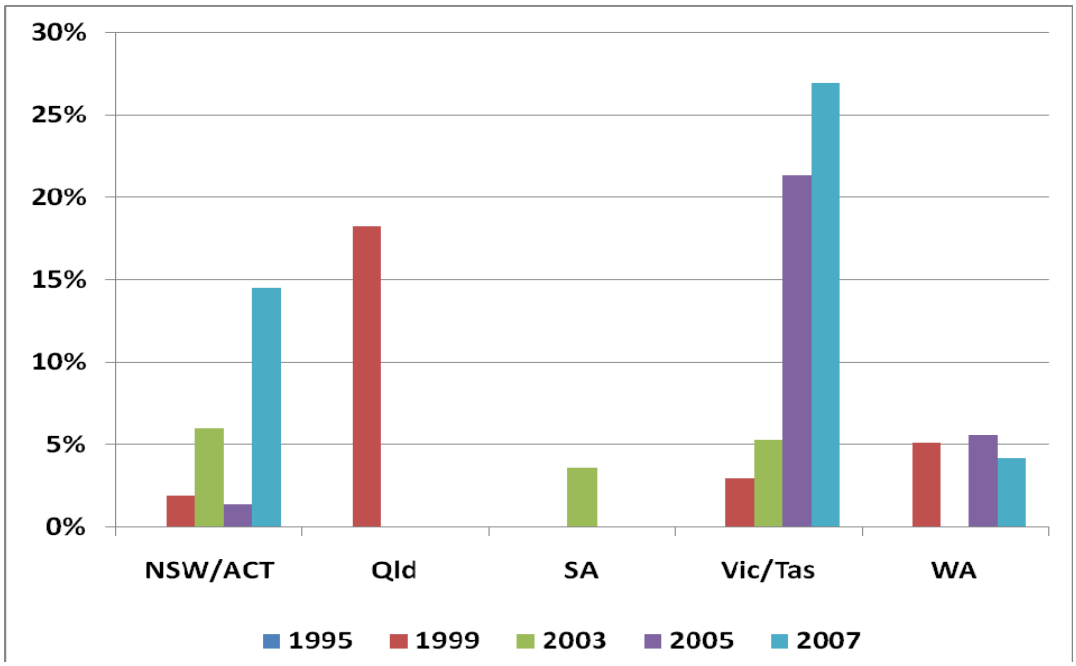
	E. faecalis	*E. faecium*	Genotype
Specimen source			
Urine	4	11	*vanB*
Wound	1	6	*vanB*
Blood/CSF		5	*vanB*
Sterile body cavity		1	*vanB*
Unknown		1	*vanB*
Total	**5**	**24**	

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.

Figure 3. Regional Location of Vancomycin Resistant *E. faecium* 1995, 1999, 2003, 2005 and 2007



6.3 Aminoglycosides

6.3.1 Gentamicin

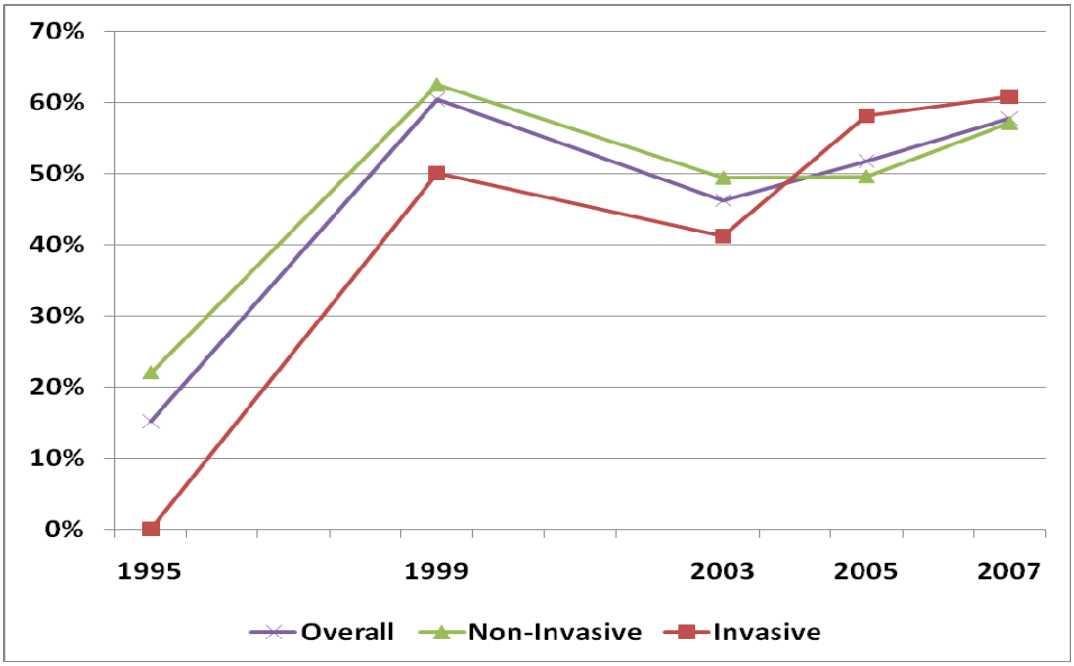
High level gentamicin (HLG) resistance was seen in both *E. faecalis* (30.8%) and *E. faecium* (57.1%) (Table 8). Resistance was higher in invasive isolates that in non-invasive isolates but this

did not reach significance. Trend data for 1995 to 2007 (Figures 4 and 5) show an increase in HLG resistance. However, in *E. faecium*, the initial increase in HLG has reached a plateau whilst in *E. faecalis* resistance continued to increase until 2005 and then decreased slightly in this survey.

Table 8. High Level Gentamicin Resistance

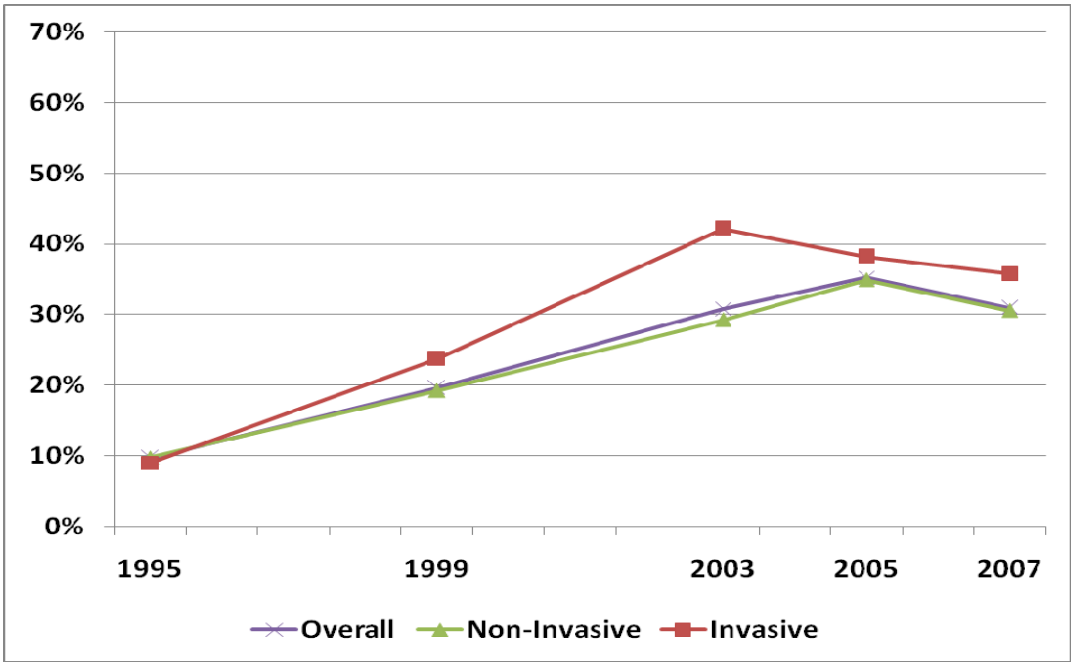
	NSW/ACT	QLD	SA	VIC/TAS	WA	AUS
E. faecalis	146/428	101/284	46/187	96/347	79/271	468/1520
	(34.1)	(35.6)	(24.6)	(27.7)	(29.2)	(30.8)
invasive	25/62	8/21	4/12	11/37	3/11	51/143
	(40.3)	(38.1)	(33.3)	(29.7)	(27.3)	(35.7)
E. faecium	37/62	7/11	5/7	29/52	11/24	89/156
	(59.7)	(63.6)	(71.4)	(55.8)	(45.8)	(57.1)
invasive	13/20	2/3	3/3	7/13	6/12	31/51
	(65.0)	(66.7)	(100)	(53.8)	(50.0)	(60.8)

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.

Figure 5. High Level Gentamicin Resistance: *E faecalis*



1995: invasive n=100, non-invasive n= 1109, overall n=1211. 1999: invasive n=135, non-invasive n= 1442, overall n=1577. 2003: invasive n=190, non-invasive n=14321, overall n=1622. 2005: invasive n=170, non-invasive n= 1816, overall n=1986. 2007: invasive n=143, non-invasive n= 1333, overall n=1520.

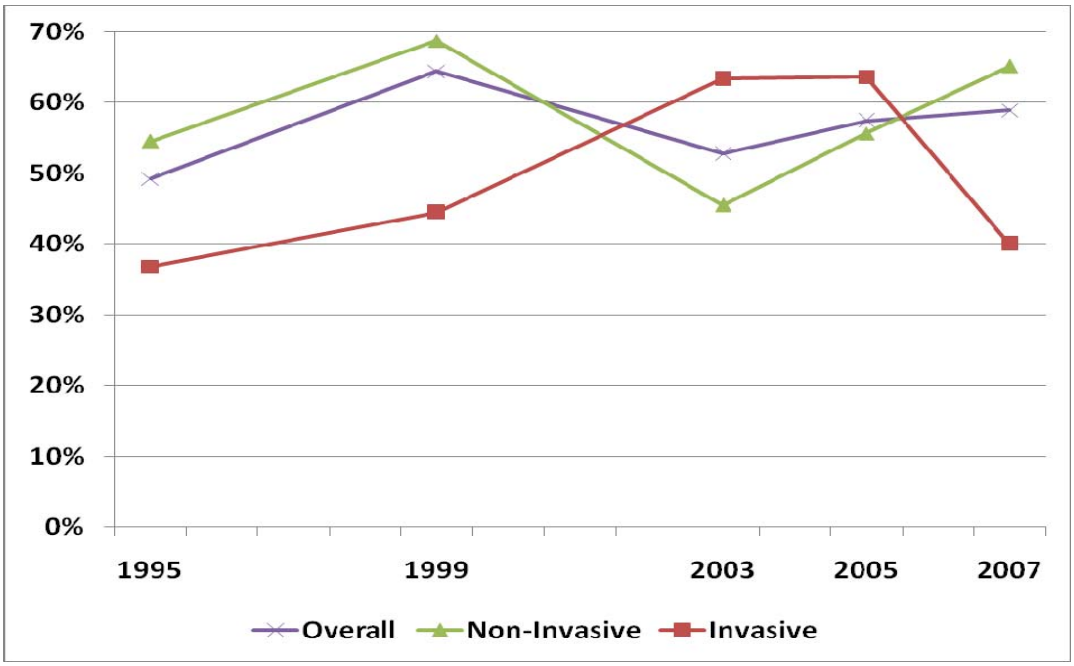
6.3.2 Streptomycin

High level streptomycin resistance (HLS) as with HLG resistance is more common for *E. faecium* than *E. faecalis* (Table 9). The trend from 1995 to 2005 for *E. faecium* was for increasing resistance particularly for invasive isolates (Figures 6 and 7) however in 2007 resistance in invasive isolates fell to 40.0% whereas resistance in non-invasive isolates rose compared with 2005 levels. In *E. faecalis*, the HLS is relatively stable with lower rates of expression than HLG.

Table 9. High Level Streptomycin Resistance

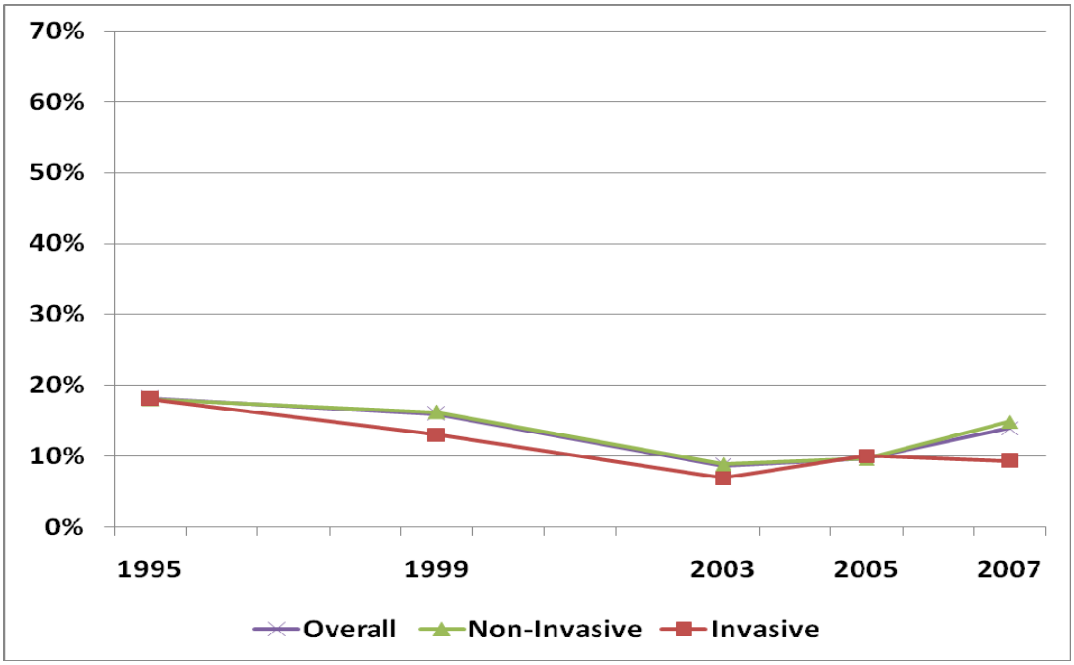
	NSW/ACT	QLD	SA	VIC/TAS	WA	AUS
E. faecalis	42/336	38/287	8/96	7/98	32/96	127/913
	(12.5)	(13.2)	(8.3)	(7.1)	(33.3)	(13.9)
invasive	6/55	1/21	0/8	1/12	1/1	9/97
	(10.9)	(4.8)	(0.0)	(8.3)	(100)	(9.3)
E. faecium	36/56	5/11	1/4	0/1	1/1	43/73
	(64.3)	(45.5)	(25.0)	(0.0)	(100)	(58.9)
invasive	7/18	2/3	1/3	0/1	1/1	10/25
	(38.9)	(66.7)	(33.3)	(0.0)	(100)	(40.0)

Figure 6. High Level Streptomycin: *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.

Figure 7. High Level Streptomycin: *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.

6.3.3 Relationship between HLG and HLS resistance by location

E. faecalis: High level gentamicin resistance for all regions except WA was the predominant feature for aminoglycosides (Figure 8).

E. faecium: The overall proportion of HLS in isolates of *E. faecium* was higher than that in *E. faecalis*. HLS was more common than HLG in isolates from NSW/ACT and WA whereas in the other states, HLG was the dominant aminoglycoside resistance (Figure 9).

Figure 8 *E. faecalis* Aminoglycoside (HLG, HLS) Resistance by Region

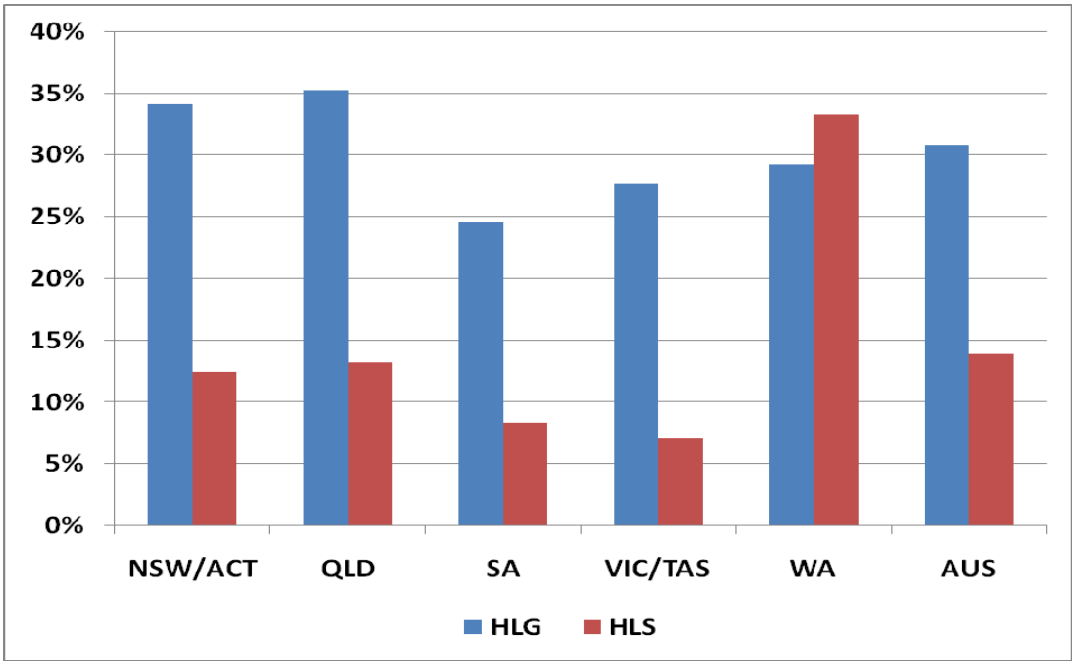
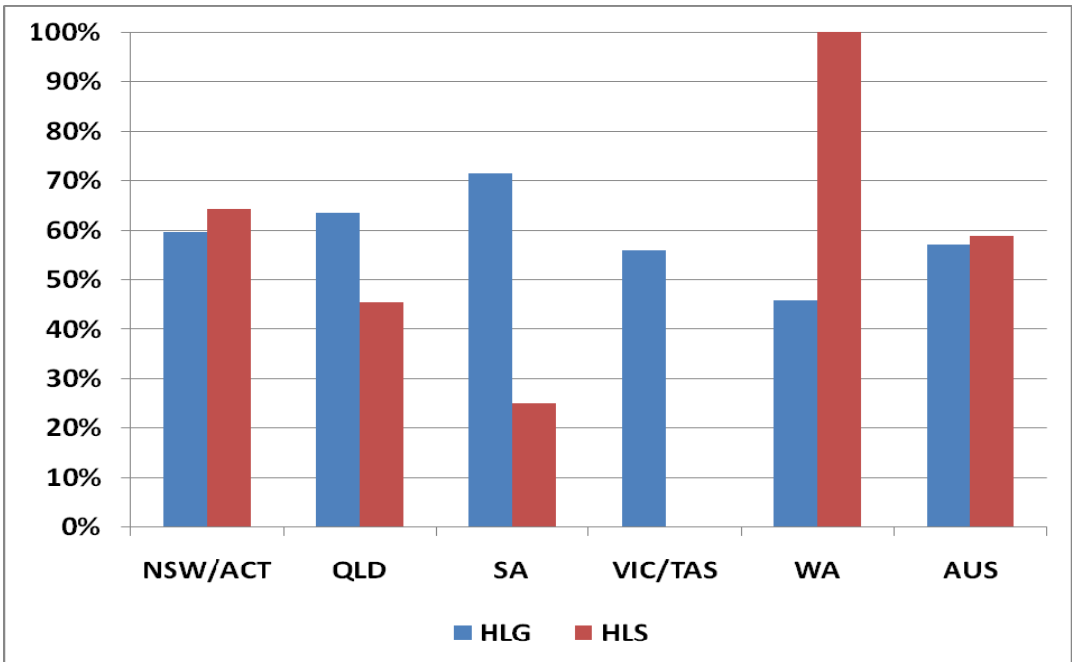


Figure 9 *E. faecium* Aminoglycoside (HLG, HLS) Resistance by Region



6.4 Linezolid

Linezolid non-susceptibility was present in 4.8% of *E. faecalis* and absent in *E. faecium*. Thirty four of the 35 NS isolates had an MIC in the intermediate resistant category; only one was

classified as resistant (MIC ≥8mg/L). This agent was tested for the first time in the 2007 survey therefore trend data are not available.

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

	NSW/ACT	QLD	SA	VIC/TAS	WA	AUS
E. faecalis	10/158	10/287	0/96	9/98	6/96	35/735
	(6.3)	(3.5)	(0.0)	(9.2)	(6.2)	(4.8)
invasive	0/10	1/21	0/8	0/12	0/1	1/52
	(0.0)	(4.8)	(0.0)	(0.0)	(0.0)	(1.9)
E. faecium	0/38	0/11	0/4	0/1	0/1	0/55
	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)
invasive	0/9	0/3	0/3	0/1	NT	0/16
	(0.0)	(0.0)	(0.0)	(0.0)		(0.0)

NT: Not tested.

6.5 Quinupristin/dalfopristin

E. faecalis are intrinsically resistant to quinupristin/dalfopristin (Q/D). 9.8% of the *E. faecium* were NS with four of the five NS isolates having an MIC in the intermediate resistant range. This agent was tested for the first time in the 2007 survey therefore trend data are not available.

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

	NSW/ACT	QLD	SA	VIC/TAS	WA	AUS
E. faecalis	153/157	274/286	NT	92/98	93/96	612/637
	(97.5)	(95.8)		(93.9)	(96.9)	(96.1)
invasive	10/10	21/21	NT	12/12	1/1	44/44
	(100)	(100)		(100)	(100)	(100)
E. faecium	5/38	0/11	NT	0/1	0/1	5/51
	(13.2)	(0.0)		(0.0)	(0.0)	(9.8)
invasive	0/9	0/3	NT	0/1	NT	0/13
	(0.0)	(0.0)		(0.0)		(0.0)

NT: Not tested.

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 streptomycin was more common in resistant *E. faecium*. HLG resistance and Q/D non-susceptibility was similar for VRE and non-VRE. In *E. faecalis* HLG was more common in VRE.

Table 12. Cross Resistance in VRE

Species	Vancomycin Status	Ampicillin R (%)	Gentamicin R (%)	Streptomycin R (%)	Linezolid IR (%)	Q/D IR (%)
E. faecalis	**Not VRE**	0/1515 (0.0)	464/1515 (30.6)	127/913 (13.9)	35/735 (4.8)	612/637 (96.1)
	VRE	0/5 (0.0)	4/5 (80.0)	NT	NT	NT
E. faecium	**Not VRE**	107/132 (81.1)	75/132 (56.8)	35/63 (55.6)	0/46 (0.0)	4/42 (9.5)
	VRE	22/24 (91.7)	14/24 (58.3)	8/10 (80.0)	0/9 (0.0)	1/9 (11.1)

Q/D: Quinupristin/dalfopristin. NT: Not tested

8 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 2007 with the more recent inclusion of a number of private pathology laboratories. This may have influenced trend data.

9 Discussion

It is clear from this study and the examination of trends over the last 12 years that resistance is increasing significantly in *E. faecium*. Furthermore, this species is accounting for an increasing proportion of invasive disease. 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 (3 known instances in Australia in the last decade).[22] No ß-lactamase-producing strains of enterococci were detected in this survey. This survey has shown that ampicillin resistance is now the norm in *E. faecium* but is still uncommon 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 two years. It has been seen in all states and territories although rates in each region seem to 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[28], 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 the recently released daptomycin. Each of these agents presents its own challenges for treatment as well.

The increasing rate of high-level resistance to gentamicin is surprising. It is not clear what is driving this increase. For *E. faecium* it may well be the increase in resistant clones which are becoming established in some hospitals. Loss of susceptibility to high levels of aminoglycosides greatly compromises the ability to effectively treat enterococcal endocarditis.

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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