PREVALENCE OF ANTIMICROBIAL RESISTANCE IN *ENTEROCOCCUS* ISOLATES IN AUSTRALIA, 2005:

REPORT FROM THE AUSTRALIAN GROUP ON ANTIMICROBIAL RESISTANCE

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Abstract

Antibiotic resistance in *Enterococcus* species causing clinical disease was examined in a point-prevalence study in 2005. Twenty-two sites around Australia 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 and 2003. In the 2005 survey, *Enterococcus faecalis* (1,987 strains) and *E. faecium* (180 strains) made up 98.6% of the 2,197 isolates tested. Ampicillin resistance was common (77%) in *E. faecium*, but rare still in *E. faecalis* (0.2%). Resistance to vancomycin was 7.2% in *E. faecium* and 0.2% in *E. faecalis*; the *van*B gene was detected in all vancomycin-resistant isolates. High-level resistance to gentamicin was 35.8% in *E. faecalis* and 52.2% in *E. faecium*; the figures for high-level streptomycin resistance were 10.3% and 60.2% respectively. Compared to previous Australian Group on Antimicrobial Resistance surveys in 1995, 1999 and 2003, the proportions of vancomycin resistance and high-level gentamicin resistance in enterococci are increasing. It is important to have an understanding of the occurrence of vancomycin resistant enterococci and high level aminoglycoside resistance in Australia to guide infection control practices, antibiotic prescribing policies and drug regulatory decisions. *Commun Dis Intell* 2007;31:392–397.

Keywords: antibiotic resistance, enterococcus, vancomycin

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. In hospitals they can be transmitted through suboptimal 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. Enterococci, although resistant to many antibiotics, have been generally susceptible to amoxycillin and vancomycin. *E. 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 widespread in Europe and the United States of America (USA). The National Nosocomial Infections Surveillance System in the USA has demonstrated a rising resistance rate for enterococci causing infections in ICU patients with a 2003 rate of 28.5%.[1] The first vancomycin resistant enterococcal (VRE) isolate was reported in Australia in 1994[2] and a report on the emergence and epidemiology of VRE in Australia was described in 1998[3] 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.[4–8] The clinical impact of vancomycin resistance in enterococci has been reported to increase mortality, length of stay and hospital costs.[9–11] Intensive infection control measures can be used to eradicate the organism from a hospital population or to prevent it from becoming established.[4]

Enterococci cause 5%–18% of all cases of endocarditis, both on prosthetic and normal heart valves.[12–14] Combination therapy of a ß-lactam and an aminoglycoside (gentamicin or streptomycin)[15–17] has been the standard treatment for at least 50 years 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 greatly increases the likelihood of a cure. Synergy does not occur if the organism has high level gentamicin or streptomycin resistance (MIC > 500 mg/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 decisions.

Methods

Institutions

Participating laboratories were located in New South Wales (6), the Australian Capital Territory (1), Queensland (3), Victoria (4), South Australia (3), Western Australia (4) and Tasmania (1). To ensure institutional anonymity, data from New South Wales and the Australian Capital Territory and from Tasmania and Victoria have been combined.

Commencing on 1 January 2005, 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. Two thousand, one hundred and ninety-seven isolates were included in the survey. Results were compared with previous surveys conducted by the Australian Group on Antimicrobial Resistance (AGAR) in 1995, 1999 and 2003.

Laboratory methods

Participating laboratories were required to meet standards for species identification. All isolates were tested for pyrrolidonyl arylamidase 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, 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 pyruvate utilisation. Participating laboratories performed antimicrobial susceptibility tests according to each laboratory's routine standardised methodology[18–22] (CLSI, CDS or BSAC disc diffusion, Vitek, Vitek 2, agar dilution or CLSI broth microdilution). Antimicrobials that were tested by all laboratories included ampicillin and vancomycin. In addition, all isolates were screened for high level gentamicin and 1,201 (55%) isolates were screened for high level streptomycin resistance. Isolates were tested for ß-lactamase production using nitrocefin. All isolates that were resistant to vancomycin were referred to the appropriate state National VRE Network laboratory for molecular testing to confirm organism identification and resistance phenotype.

Results

Specimen source

The majority of isolates (73.6%) were from the urinary tract. These were predominantly *E. faecalis* (93.7%). Invasive (primarily blood, cerebrospinal fluid and sterile cavity) isolates comprised 10.3% of the total number collected (Table 1). *E. faecium* was disproportionately represented in the invasive group (18.9%). Of the *E. faecalis* isolates, 8.7% were invasive compared to 23.9% of *E. faecium*. 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* (63.9%) from women compared to men (36.1%).

Susceptibility results

Ampicillin

Resistance to ampicillin was predominantly in the *E. faecium* isolates where the proportion of resistance was similar across all the states except Queensland, where the rate was lower (Table 2). Resistance in all species was due to penicillin binding protein

Table 1. Source of isolates

Source	*E. faecalis*	*E. faecium*	Other spp.	Total	%
Urine	1,514	96	6	1,616	73.6
Wound	157	22	9	188	8.6
Blood/CSF	110	27	8	145	6.6
Sterile site	62	16	4	82	3.7
Other	144	19	3	166	7.6
Total	1,987	180	30	2,197	
Invasive	172	43	12	227	10.3
Non-invasive	1,815	137	18	1,970	89.7
Sex					
Female	1,041	115	9	1,165	53.0
Male	946	65	21	1,032	47.0

CSF Cerebrospinal fluid.

Table 2. Ampicillin resistance

	Qld		NSW/ACT		Vic/Tas		SA		WA		Aus	
	n	%	n	%	n	%	n	%	n	%	n	%
E. faecalis	0/286	0.0	1/619	0.2	0/449	0.0	0/280	0.0	2/353	0.6	3/1,987	0.2
invasive	0/22	0.0	0/76	0.0	0/35	0.0	0/8	0.0	0/31	0.0	0/172	0.0
E. faecium	7/12	58.3	57/72	79.2	36/47	76.6	10/13	76.9	28/36	77.8	138/180	76.7
invasive	2/4	50.0	18/20	80.0	8/12	66.7	0/0	0.0	4/7	57.1	30/43	69.8

changes. Two thousand and seventy-seven (94.5%) 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 2005 (Figure 1).

Vancomycin

Vancomycin resistance was uncommon in *E. faecalis* (0.2%). A total of 7.2% of *E. faecium* were vancomycin resistant with a greater proportion isolated from invasive infections. Resistant organisms were detected in New South Wales/Australian Capital Territory, Victoria/Tasmania and Western Australia. The 16 vancomycin resistant enterococci were all confirmed by PCR and were of the *van*B genotype. Thirteen (81.2%) were *E. faecium* (Table 3). Trend data for *E. faecium* show that after no vancomycin resistance was detected in 1995 there has been a marked increase, particularly for the invasive category (Figure 2) during the study periods. Vancomycin resistant *E. faecium* have occurred in all five regions over the four survey periods, with Victoria/Tasmania showing the greatest increases in VRE over time (Figure 3).

Figure 1. Ampicillin resistance: Enterococcus faecium

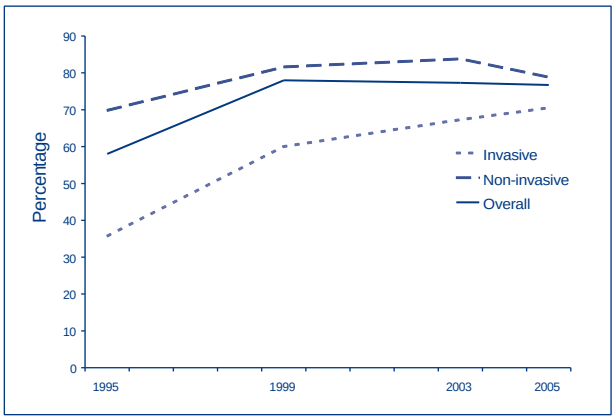


Table 3. Vancomycin resistant enterococci

Specimen source	*E. faecalis*	*E. faecium*	Genotype
Urine	3	5	*van*B
Wound		3	*van*B
Blood		1	*van*B
Sterile site		3	*van*B
Other		1	*van*B
Total	3	13	

Figure 2. Vancomycin resistance: Enterococcus faecium

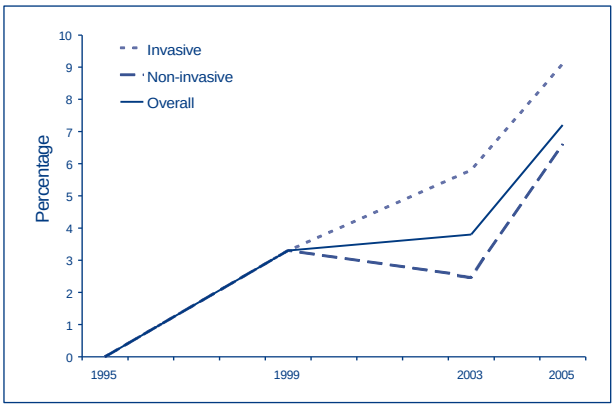
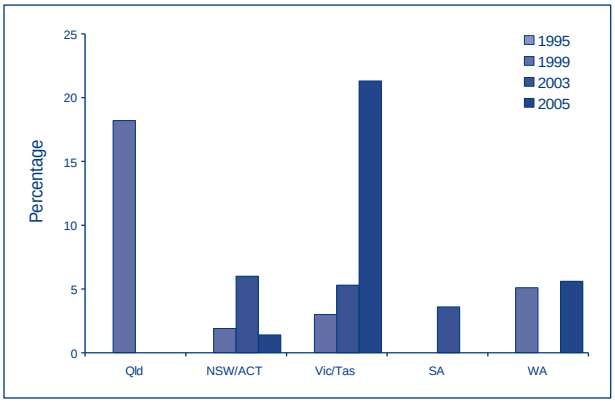


Figure 3. Regional location of vancomycin resistant Enterococcus faecium, 1995, 1999, 2003, 2005



Gentamicin

High level gentamicin resistance (HLG) was seen in both *E. faecalis* (35.8%) and *E. faecium* (52.2%) with comparable proportions in most regions (Table 4). Trend data for 1995 to 2005 (Figures 4 and 5) show an increase in HLG resistance over the last 10 years. However, in *E. faecium*, HLG has reached a plateau whilst in *E. faecalis* resistance is continuing to increase.

Figure 4. High level gentamicin resistance: Enterococcus faecium

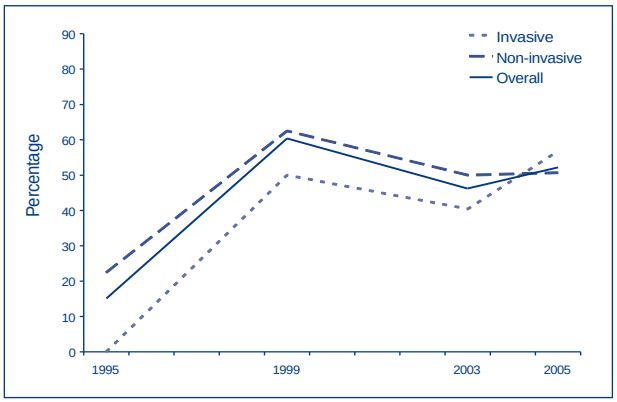
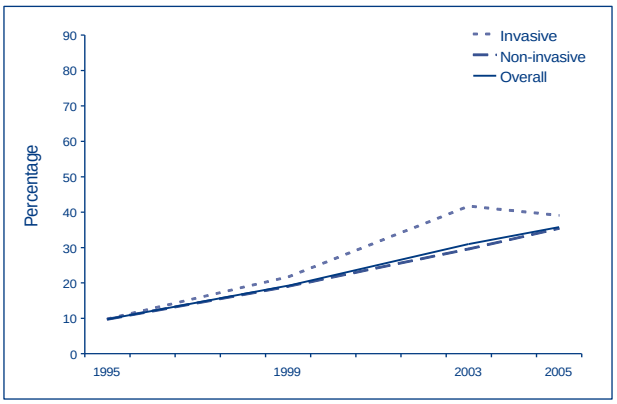


Figure 5. High level gentamicin resistance: Enterococcus faecalis



Streptomycin

High level streptomycin resistance (HLS) as with HLG resistance is more common for *E. faecium* than *E. faecalis* (Table 5). The trend since 1995 is for increasing resistance particularly for invasive isolates of *E. faecium* (Figure 6). The rate of increase in HLS is similar to that for HLG for *E. faecium*. In *E. faecalis*, the HLS is relatively stable with lower rates of expression than HLG (Figure 7).

Figure 6. High level streptomycin: Enterococcus faecium

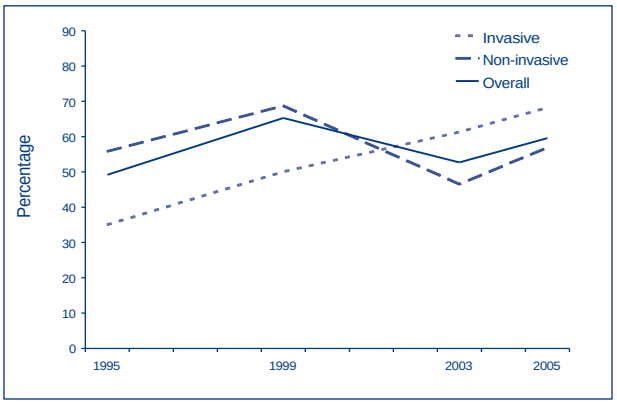


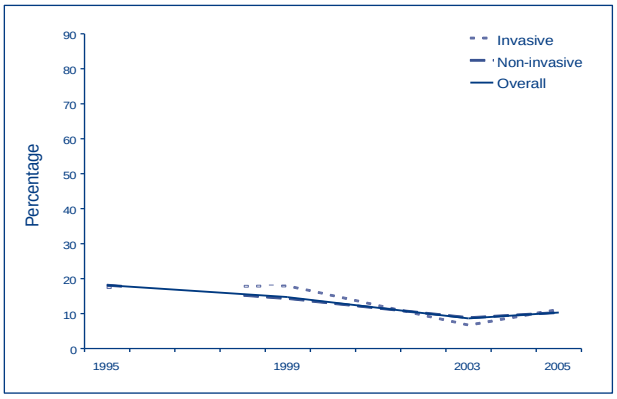
Table 4. High level gentamicin resistance

	Qld		NSW/ACT		Vic/Tas		SA		WA		Aus	
	n	%	n	%	n	%	n	%	n	%	n	%
E. faecalis	101/286	35.3	243/619	39.4	145/448	32.4	58/280	20.7	163/353	46.2	710/1,986	35.8
invasive	7/22	31.8	34/76	44.7	10/35	28.6	2/8	25.0	15/31	48.4	68/172	39.5
E. faecium	7/12	58.3	48/72	66.2	12/47	25.5	9/13	69.2	18/36	50.0	94/180	52.2
invasive	2/4	50.0	16/20	80.0	2/12	16.7	0/0	0.0	5/7	71.4	25/43	58.1

Table 5. High level streptomycin resistance

	Qld		NSW/ACT		Vic/Tas		SA		WA		Aus	
	n	%	n	%	n	%	n	%	n	%	n	%
E. faecalis	40/286	14.0	32/348	9.2	11/90	12.2	22/280	7.9	8/88	9.1	113/1,092	10.3
invasive	2/22	9.1	5/36	13.9	1/9	11.1	0/8	0.0	1/5	20.0	9/80	11.2
E. faecium	6/12	50.0	25/50	50.0	7/8	87.5	9/13	69.2	9/11	81.8	56/94	60.2
invasive	3/4	75.0	8/13	61.5	2/2	100	0/0	0.0	2/3	66.7	15/22	68.2

Figure 7. High level streptomycin: Enterococcus faecalis



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. 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 2005 with the more recent inclusion of a number of private pathology laboratories. This may have influenced trend data.

Discussion

It is clear from this study and the examination of trends over the last 10 years that resistance problems are 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 (7% across Australia, range 0%–21% in 2005).

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).[23] 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 slowly increasing in Australia. 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 *van*B complex,[24] in contrast with the situation in Europe and the USA where *van*A dominates. Vancomycin-resistant strains causing serious infection are very challenging to treat. The choices are linezolid, quinupristin-dalfopristin and the recently released tigecycline. Each of these agents presents its own challenges for treatment as well.

The increasing rates of high-level resistance to aminoglycosides (except for streptomycin resistance in *E. faecalis*) is surprising. It is not clear what is driving this increase. For *E. faecium* it may well be the increase in resistant clones that 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.

A full detailed report of this study may be found on the Australian group on Antimicrobial Resistance website: http://www.antimicrobial-resistance.com under 'AMR Surveillance'.

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