Emergence and epidemiology of vancomycin-resistant enterococci in Australia

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Abstract

Enterococci with acquired resistance to vancomycin and other glycopeptides (VRE) have emerged and spread rapidly through Europe and the United States since 1988. The first isolate of VRE in Australia occurred in 1994. Only one case was noted in 1995. Since March 1996 there has been a steady increase in the number of reports of VRE throughout the country. To August 1998 there have been 69 documented strains or clusters of strains detected in patients with documented infection, and about 3 times as many strains have been detected through screening procedures of contacts or in risk groups. 19% of strains whose source was known were blood isolates, while 34% came from urine and 47% came from other specimens. The strains have been found in 26 institutions in 10 widely separated cities or regions of the country (in 6/8 states or territories), without any obvious temporal associations in their appearance. All strains appear to have arisen locally except for one strain imported from the United Kingdom. Furthermore there was no direct evidence of interhospital transfer of strains. All clinical strains were examined by PCR to confirm species and to test for the presence of known vancomycin-resistance genes. Of the 69 strains, 42 were *vanB E. faecium*, 12 were *vanA E. faecium*, 9 were *vanB E. faecalis*, 3 were *vanA E. faecalis*. Three were negative for *vanA*, *vanB*, *vanC1*, *vanC2/C3* and *vanD*. PGFE profiles on 38 strains have revealed at least 8 types of *vanB E. faecium*, 6 of *vanA E. faecium*, 4 of *vanB E. faecalis* and 2 of *vanA E. faecalis*. Isolates containing *vanA* always had different profiles from those containing *vanB*. Clinical clustering was confirmed by PFGE, and supported by extended antibiogram. 14 of 15 *E. faecalis* were ampicillin susceptible compared to only 2 of 54 *E. faecium*. One *E. faecalis* strain was ß-lactamase positive. The epidemiology of VRE in Australia appears to be different from that of Europe or the United States, since *vanB E. faecium* predominates and strains have appeared in diverse locations independently and are highly polyclonal. *Commun Dis Intell* 1998;22:249-252.

Introduction

Vancomycin-resistant *Enterococcus faecium* and *E. faecalis* (VRE) were first described in Britain in 1988 and soon afterwards were reported from other European countries and the United States. In the United States they have become major nosocomial pathogens, rising in incidence from 0.3% in 1989 to 7.9% in 1993 as reported by the CDC,[1] and among patients in intensive-care units, now representing 14% of blood culture isolates of enterococci.[1] The rapid emergence of VRE in the United States has been attributed to the intensive clinical use of vancomycin in both parenteral and oral forms in that country,[2] on a background of high level usage of cephalosporins which promote enterococcal superinfection. In Europe, investigators have postulated an additional role for the use of the glycopeptide avoparcin as a growth promoter in intensive animal industries, resulting in colonisation with VanA *E. faecium* and subsequent transmission to humans via the food chain.[3] The first vancomycin-resistant *E. faecium* in Australia was isolated from a liver transplant recipient in Melbourne in 1994.[4] Since March 1996 multiple isolates of vancomycin-resistant *E. faecium* and vancomycin-resistant *E. faecalis* have occurred throughout Australia. Only a few of these strains have been reported in the literature.[5, 6, 7, 8] As a referral centre for antimicrobial resistance in Australia, we have collected isolates from virtually all known instances of VRE infection that have occurred since 1994. In order to characterise these strains further we have developed multiplex PCR assays for *vanA*, *vanB*, *vanC1* and *vanC2/3*,[9] and have used these to examine the genetic basis for vancomycin resistance in Australian isolates of VRE. Results have been compared to those obtained by conventional susceptibility testing against glycopeptides.

Methods

Bacterial Strains

Clinical isolates of *Enterococcus* spp. referred to the National Antimicrobial Resistance Surveillance Program were studied. For the purposes of analysis only strains isolated from clinical specimens were included (whether pathogenic or commensal at the site). Where there were clusters of epidemiologically related isolates with the same phenotype and genotype, this cluster was recorded as one isolate (index case). Although many additional isolates have been detected at several institutions around the country, these have not been included as there are insufficient data available to the Surveillance Program to determine whether these isolates are related or unrelated to the clinical isolates.

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Identification and antimicrobial susceptibility testing

Isolates were identified by a conventional test scheme.[10] A multiplex PCR assay based on specific detection of genes encoding D-alanine:D-alanine ligases (ddl)[11] was used to confirm identification of *E. faecalis* and *E. faecium*. MICs of vancomycin and teicoplanin were determined for each isolate by the E-test (AB Biodisk, Solna, Sweden) method on Mueller-Hinton agar. The interpretative criteria of the NCCLS[12] were used for determining susceptibility of the isolates.

Vancomycin Resistance Gene Typing by PCR

A multiplex PCR assay was used for the detection of *vanA*, *vanB*, *vanC1*, and v*anC2* or *vanC3* genes.[9] Genotype-negative VRE isolates, with vancomycin MICs 4 µg/ml, were also tested for the presence of *vanD* using primers described by Perichon et al.[13]

Pulsed-Field Gel Electrophoresis

Thirty eight strains were analysed by PFGE. Chromosomal DNA was digested with *Sma* 1 restriction endonuclease and patterns interpreted according to the criteria of Tenover.[14]

Results

Vancomycin-resistant genotypes

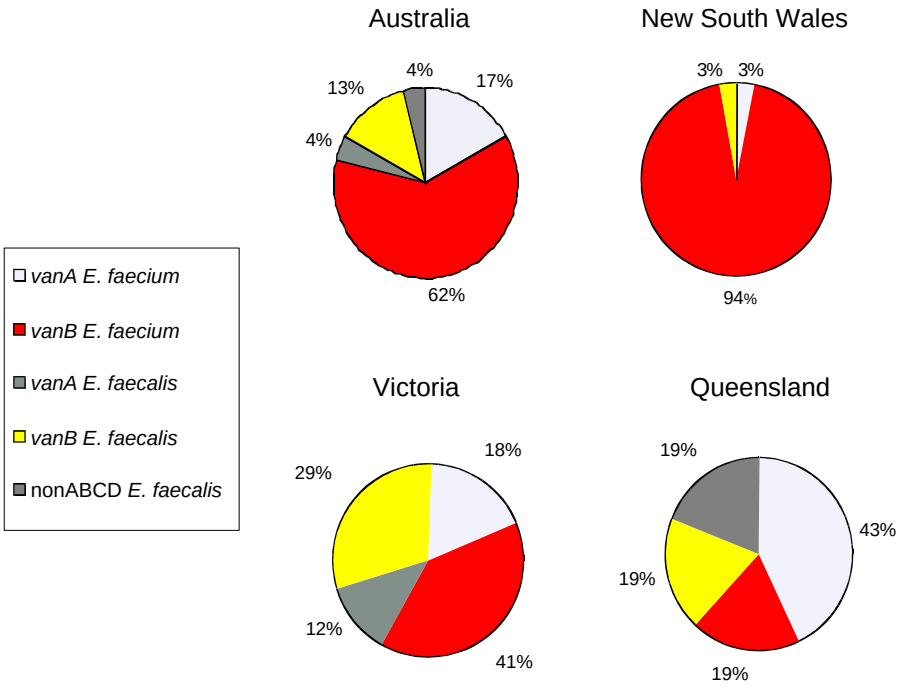
To the middle of September 1998, a total of 69 VRE isolates or clusters of isolates were found in 26 institutions in 10 cities throughout all mainland states of Australia (Figure 1). Results of PCR analysis of the van genotype are shown in Table 1. There were 42 (62%) *vanB E. faecium*, 12 (17%) *vanA E. faecium*, 9 (13%) *vanB E. faecalis*, three *vanA E. Faecalis* and three *van*-negative *E. faecalis* isolates. Two *E. faecium* and two *E. faecalis* isolates which were PCR-positive for *vanB* had intermediate resistance to vancomycin (MICs 8-16 µg/ml).

Figure 1. Evolution of clinical VRE isolates over time by institution.

Institution	Year and quarter																	Total for Institution
	1994		1995				1996				1997				1998			
- City*	q3	q4	q1	q2	q3	q4	q1	q2	q3	q4	q1	q2	q3	q4	q1	q2	q3	
A - Mel	1								1			3						5
B - Dar				1														1
C - New								1				2	1					4
D - Syd								1	1	4	3	2	1			1		13
E - Bri								1		1		1						3
F - Bri									1	1								2
G - Mel									1	1					2			4
H - Per									1									1
I - Bri									1	1	1	1				1	1	6
J - Syd									1							1		2
K - Mel										1								1
L - New										1		1	2	1	2			7
M - Mel												1						1
N - Syd												1						1
O - Ade													2					2
P - Mel														1				1
Q - New															1			1
R - Per															1			1
S - Mel															2		1	3
T - Vic																1		1
U – Bri																1	2	3
V – NSW																1		1
W – Bri																	2	2
X – Syd																	1	1
Y – NSW																	1	1
Z – Mel																	1	1
Total for quarters	1	0	0	1	0	0	0	3	7	10	4	12	6	2	8	6	9	69

Each institution designated by a letter. City abbreviations: Mel = Melbourne, Dar = Darwin, New = Newcastle, Syd = Sydney, Bri = Brisbane, Per = Perth, Vic = Victorian city not Melbourne, NSW = New South Wales city not Sydney or Newcastle

Figure 2. Prevalence of different VRE genotypes in Australia and different states.



One *vanA E. faecalis* isolate had a VanB phenotype (teicoplanin MIC of 4 µg/ml). One VanA *E. faecium* isolate which was vanB-positive had a teicoplanin MIC of 256 µg/ml. Three *E. faecalis* isolates were consistently negative for *vanA*, *vanB*, *vanC1*, *vanC2/3* and *vanD* in spite of exhibiting a VanB phenotype (vancomycin MICs 12-16 µg/ml). All three isolates were from the same institution. There was significant variation in both the species distribution and genotype of VRE isolates between States (Figure 2).

Table 1. Resistance Genotypes.

Species	*vanA*	*vanB*	nonABC	Total
E. faecium	12	42	-	54
E. faecalis	3	9	3	15
Total	15	51	3	69

Isolate sources

A high proportion of the 'index' case isolates were from blood (17%) (Table 2). The types of specimens from which VRE were isolated were otherwise typical of enterococci.

Other susceptibilities

Fourteen of 15 *E. faecalis* were ampicillin sensitive. The ampicillin resistant *vanB E. faecalis* was ß-lactamase positive. Similarly only 2 of 52 *E. faecium* were ampicillin sensitive. Both were *vanA* genotype.

Pulsed-Field Gel Electrophoresis

PFGE studies demonstrated at least 16 types of *E. faecium* (n=29) and at least 7 types of *E. faecalis* (n=9). The studies confirmed outbreak clusters. However, few institutions had strains in common, with only 4 PFGE types (*E. faecium* 3, *E. faecalis* 1) being detected in more than one institution. This finding was consistent with the fact that there is has been no known transfer of VRE between institutions. Moreover multiple unrelated were strains found even in a single institution.

Table 2. Specimen Source.

Source	*E. faecalis*	*E. faecium*	Total	
Urine	3	18	21	30%
Blood	3	9	12	17%
Other	4	25	29	42%
Stool, rectal, perianal	4	12	16	
Intra-abdominal		3	3	
Bile		4	4	
CAPD fluid		1	1	
Skin		5	5	
Unknown	5	2	7	10%
Total	15	54	69	

Discussion

The VRE isolated in Australia to date show considerable diversity in phenotype, genotype and geographic location. Cases have largely arisen sporadically and there has been no obvious geographic evolution, unlike in the USA where VRE strains have progressed from the North Eastern seaboard to the South East over several years. All four combinations of genotype and species have been found, with the commonest being *vanB E. faecium*. While the clinical profiles of VRE-affected patients appear to be similar to that recorded in the US and elsewhere,[2] the predominance of *vanB E. faecium* rather than *vanA E.*

faecium suggests different epidemiology from either Europe or the USA.

The origin of VRE in Australia remains unclear. One strain definitely appears to have been imported from the UK. Another strain occurred in a liver transplant recipient who was a New Zealand-born resident of Taiwan. This patient had entered Australia specifically for transplantation a few days prior to the procedure. *E. faecalis* of VanB phenotype was initially isolated from blood cultures after surgery. The patient was treated with teicoplanin, but several days later VRE was again isolated from blood cultures, with the isolate identified as *E. faecalis* of VanA phenotype. Genotyping showed both isolates to possess the *vanB* gene, and subsequent ribotyping confirmed the strains to be identical. Emergence of resistance to teicoplanin has been recorded previously, albeit rarely.[15]

Vancomycin usage in Australia is relatively high and has been increasing over the last decade (Eli Lilly Australia Pty Limited, personal communication). There is significant regional variation in its use due to the variation in prevalence of multi-resistant *Staphylococcus aureus*. Australia is also a high user of avoparcin as a growth promoter in the intensive animal industries. It is possible that the novel epidemiology of VRE in Australia may result from a combination of high usage vancomycin and avoparcin in humans and animals, respectively.

All three strains with the VanB phenotype, but lacking *vanA, vanB, vanC1, vanC2* or *vanC3*, or *vanD* came from a single institution and gave two distinct pulsed-field gel electrophoresis patterns. Our results are consistent with either the existence of a significant variant of a current *van* genotype or a novel one. The *van* loci of these strains are undergoing further analysis.

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