HIGH LEVEL AMINOGLYCOSIDE RESISTANCE IN ENTEROCOCCAL BLOOD CULTURE ISOLATES

David Paterson[1], *Janet Bodman and Mee Len Thong, Royal Brisbane Hospital, Herston Road, Herston, Queensland 4006*

Abstract

Enterococci may display high level resistance to aminoglycosides, in which case synergy with cell-wall active antibiotics will be lost. All enterococcal blood culture isolates at Royal Brisbane Hospital have been screened by agar dilution for high level resistance to gentamicin and streptomycin since 1989. Of 110 isolates of *Enterococcus faecalis*, 16% displayed high level resistance to gentamicin and 10% showed high level resistance to streptomycin. Four isolates had high level resistance to both antibiotics. None of 23 *Enterococcus faecium* isolates displayed high level resistance to gentamicin and only one to streptomycin. Two *Enterococcus faecium* isolates were resistant to amoxycillin but none to vancomycin. There has been no apparent increase in high level aminoglycoside resistance from 1989 to 1996. High level gentamicin resistant isolates were relatively more common in liver transplant patients. Like vancomycin-resistant enterococci, isolates that are high level resistant to aminoglycosides can be spread by the hands of staff members. Preventing the nosocomial transmission of high level aminoglycoside-resistant enterococci follows the same general principles of preventing transmission of other resistant enterococci.
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Introduction

Enterococci intrinsically display resistance to low levels of aminoglycosides. However when an aminoglycoside is combined with a cell-wall active antibiotic (for example, amoxycillin or vancomycin), synergistic killing of the enterococcus results. In the last decade, high level resistance of enterococci to aminoglycosides has become an important clinical problem. If the organism exhibits high level resistance to an aminoglycoside, no synergy will be achieved when that aminoglycoside is combined with a cell-wall active antibiotic. This has most relevance in the treatment of serious infections such as endocarditis. Failure of cell-wall active agents used alone has been well described in this context.

Like vancomycin-resistant enterococci, high level aminoglycoside-resistant enterococci have been well recognised to be transmitted within hospitals[1]. Like many other antibiotic-resistant organisms, transmission is often via the hands of health care workers.

We reviewed the laboratory and clinical records of more than 100 patients with enterococcal bacteraemia from 1989 to 1996 to determine whether there was any rise in high level aminoglycoside resistance over that time and whether it has had any impact on the clinical outcome.

Methods

From January 1989 to July 1996, enterococcal blood culture isolates from the Royal Brisbane Hospital complex (Royal Brisbane Hospital, Royal Childrens Hospital and Royal Womens Hospital) were recorded on a database. A commercial system was used to detect growth (BACTEC NR 660 up to mid-1992, and then BacT / Alert). The organisms were identified to the genus level as *Enterococcus* using standard laboratory tests based on Gram stain, catalase reaction, bile tolerance, ability to hydrolyse aesculin, tolerance to 6.5% sodium chloride and pyruvate utilisation. If the organism did not utilise pyruvate, it was speciated using the API 20 STREP, yellow pigment production and motility test.

All enterococcal isolates were tested routinely by the agar dilution method using Steer's replicator. Enterococcal isolates were screened for high level resistance to aminoglycosides using agar plates containing gentamicin at 500 mg / L or streptomycin at 2000 mg / L.

A retrospective review of patients' charts was performed to collect data on underlying conditions, source of infection (nosocomial or community acquired), antibiotic usage and clinical outcome. Nosocomial acquisition of bacteraemia was defined as present if positive blood cultures were drawn more than 48 hours after hospital admission. Relapse was defined as blood culture positivity greater than 72 hours after the most recent positive blood culture was taken. Differences in outcome and other variables were assessed using the software package STATA.

Results

There were 136 episodes of enterococcal bacteraemia of which 110 were with *Enterococcus faecalis (E. faecalis)*, 23 with *Enterococcus faecium*, two with *E. durans* and one with *E. casseliflavus*. The resistance of these isolates to gentamicin and streptomycin is shown in detail in Table 1. Figures 1 and 2 illustrate the resistance patterns of the enterococcal blood culture isolates from 1989 to 1996. Over this period, 16% of *E. faecalis* isolates displayed high level resistance to

1. Current affiliation: JJ Sullivan, NJ Nicolaides and Partners, 134 Whitmore Street, Taringa, Queensland 4068.

Table 1. Blood culture isolates of enterococci at the Royal Brisbane Hospital, 1989 to 1996

Species of enterococcus	Number of blood culture isolates								
	1989	1990	1991	1992	1993	1994	1995	1996	Total
Enterococcus faecalis	13	17	10	13	13	19	15	10	110
High level resistance to gentamicin	1	2	3	1	1	4		1	13
High level resistance to streptomycin	1	2	1			2	1		7
High level resistance to both agents	1		1			1		1	4
Enterococcus faecium[1]	6	1	2	3	4	3	3	1	23
High level resistance to streptomycin	1								1
Amoxycillin resistant						2			2
Enterococcus durans					1			1	2
Enterococcus casseliflavus						1			1
TOTAL	19	18	12	16	18	23	18	12	136

1. No *E. faecium* isolates displayed high level resistance to gentamicin or vancomycin resistance.

gentamicin and 10% to streptomycin. Four isolates were resistant to both antibiotics. Proportions of resistant isolates have fluctuated widely from year to year, but showed a slight decline since peaking in 1991. No *E. faecium* isolates displayed high level resistance to gentamicin and only one to streptomycin. Two isolates of *E. faecium* were amoxycillin resistant. Neither isolate was a beta-lactamase producer. No blood culture isolate was vancomycin resistant.

The medical records pertaining to 100 cases were available for review. Sixty-seven episodes occurred in 62 adult patients (three of whom were in the Royal Womens Hospital) and 33 episodes occurred in 30 paediatric patients (six of whom were in the Neonatal Intensive Care Unit). The ages of patients ranged from one day old to 93 years old. Four of the patients were Japanese children who came to Australia for liver transplantation. There have been no molecular epidemiologic studies performed to determine whether there was a common clone of resistant enterococci in patients from the liver transplant ward. Two adults (one American and one Indonesian) also came to the hospital for specialised medical care. The remaining patients were Australian residents who presumably had acquired their enterococcal species in Australia.

Twelve of the 100 patients whose charts could be reviewed had enterococcal isolates with high level resistance to gentamicin. Risk factors and outcomes associated with infection in patients with enterococcal bacteraemia with and without high level resistance to gentamicin is presented in Table 2. Patients with high level resistance to gentamicin were significantly more likely to be liver transplant recipients (p=0.04). Two patients with high level

Figure 1. Enterococcal bloodstream isolates with high level resistance to gentamicin, 1989 to 1996

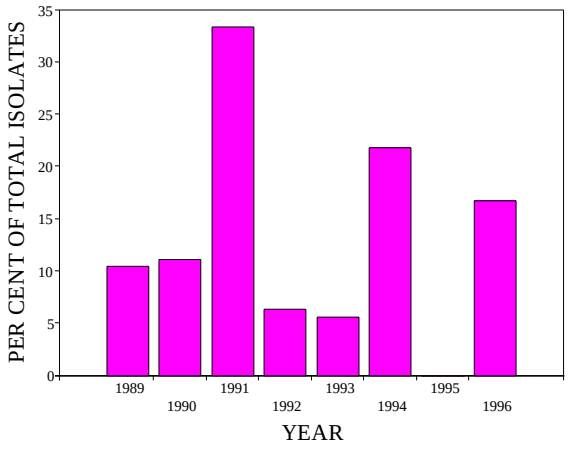

Figure 2. Enterococcal bloodstream isolates with high level resistance to streptomycin, 1989 to 1996

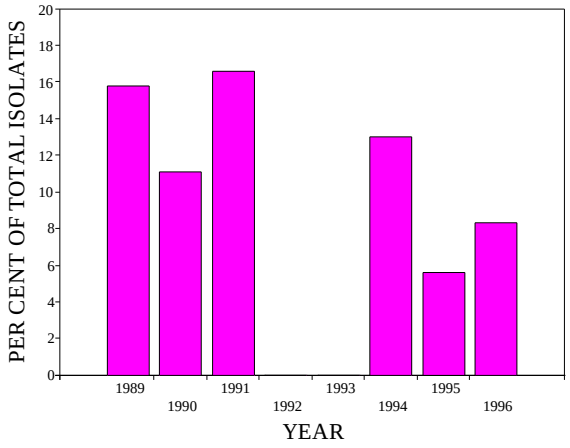

Table 2. Risk factors and outcomes associated with enterococcal bacteraemia with and without high level resistance to gentamicin

	Number with high level resistance to gentamicin (%) (n=12)	Number without high level resistance to gentamicin (%) (n=88)	Level of statistical significance
Risk factors			
Female	6 (50)	41 (47)	NS
Polymicrobial bacteraemia	5 (42)	30 (34)	NS
Nosocomial acquisition	11 (92)	63 (72)	NS
Organ transplant	3 (25)	4 (5)	p = 0.04
Primary bacteraemia	5 (42)	48 (55)	NS
Line-related sepsis	0 (0)	19 (22)	NS
Urosepsis	3 (25)	8 (9)	NS
Endocarditis	2 (17)	1 (1)	p = 0.04
Other site	2 (17)	12 (14)	NS
Outcome			
Relapse	1 (8)	8 (9)	NS
Death within one month	3 (25)	19 (22)	NS

NS: Not significant.

resistance to gentamicin had clinical diagnoses of endocarditis. One patient was treated with vancomycin alone and died of unrelated causes three weeks later. One patient was treated with penicillin and gentamicin, despite the in vitro susceptibility report, and survived.

Paradoxically, patients with high level resistance to gentamicin were more likely to receive an aminoglycoside than patients without high level resistance (p=0.03). Eighty-three per cent of patients with high level resistance to gentamicin were treated with a cell-wall active antibiotic and an aminoglycoside. More than 50% of these patients received the combination therapy for more than one week. An adverse clinical outcome (death within one month or relapse of enterococcal bacteraemia) was not more common in patients with high level resistance to gentamicin, although the numbers of patients studied was small.

Discussion

High level aminoglycoside resistance in enterococci has been well established at Royal Brisbane Hospital since testing began in 1989. Since then, about 16% of *E. faecalis* isolates have displayed high level resistance to gentamicin and 10% have shown high level resistance to streptomycin.

The rates of high level resistance to gentamicin appear somewhat higher than the percentage of 7.3% (of 70 bacteraemic isolates) found in a recent multicentre Australia-wide survey[2]. However, the rates of high level resistance to streptomycin are lower than those found in other parts of Australia (17.7% for *E. faecalis* and 38.9% for *E. faecium* blood culture isolates). The percentage of *E. faecalis* isolates exhibiting high level resistance to gentamicin is certainly less than the 70% recently described in a study on enterococcal isolates in liver transplant recipients at the Mayo Clinic[3].

The opening of a new transplantation ward may have decreased crowding of patients and reduced environmental contamination with resistant enterococci, thereby in part explaining the decrease in high level gentamicin-resistant isolates in 1995 and 1996.

It is well known that inter-hospital and even inter-country transfer of resistant organisms can occur. Screening for rectal carriage of resistant enterococci and cephalosporin-resistant Enterobacteriaceae may be prudent in patients referred for transplantation or other specialised attention such as intensive care. Preventing the transmission of high level aminoglycoside-resistant enterococci follows the same general principles as preventing transmission of other resistant enterococci. Attention to hand washing by staff members is a key intervention. Single room isolation of patients with aminoglycoside-resistant enterococci has not been practised at our hospital.

It is surprising that more than 50% of patients with high level resistance to gentamicin were treated with this drug in combination with a cell-wall active agent despite knowledge of the in vitro susceptibility result. Theoretically the use of an aminoglycoside in this situation is more likely to result in adverse effects such as nephrotoxicity and ototoxicity, without any benefit being achieved for the patient. Drug toxicity was not determined in this study.

Uptake of aminoglycosides into enterococci depends on aerobic oxidative metabolism. The anaerobic metabolism of enterococci leads to their intrinsic resistance to low concentrations of these antibiotics. There are a number of mechanisms for acquiring high level resistance to the aminoglycosides[4]. High level resistance to gentamicin is mediated by aminoglycoside modifying enzymes (a fused 6'-acetyltransferase / 2''-phosphotransferase). These en-

zymes alter the aminoglycoside molecule so that it binds poorly to the ribosome (the site of action of aminoglycosides). The fused enzyme that produces high level gentamicin resistance produces resistance to synergy with all other clinically used aminoglycosides except streptomycin. Thus if a patient has high level resistance to gentamicin, streptomycin is the only option for synergy. Unfortunately enterococci with high level resistance to both gentamicin and streptomycin, and therefore all aminoglycosides, occur. Four such isolates were found in our series. Such a finding has dire consequences for a patient with enterococcal endocarditis.The genes coding for these aminoglycoside-modifying enzymes are found on plasmids, with the exception of a 6'-acetyltransferase of *E. faecium* which is chromosomally encoded. This enzyme is produced by all strains of *E. faecium*, and inactivates tobramycin, netilmicin and kanamycin[5]. These drugs should never be used for synergistic action against *E. faecium*.

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Update on bat lyssavirus

The second meeting of the Lyssavirus Expert Group was held on 3 December 1996. The group reviewed new information on the virus. This included the first identification of lyssavirus in an insectivorous bat. A yellow-bellied sheathtail bat, *Saccolaimus flaviventris*, was found on the ground and unable to fly, near Toowoomba, Queensland. Following euthanasia, the animal was found to have a non-suppurative encephalitis on histopathology and was lyssavirus positive by immunofluorescence.

Research priorities for the bat lyssavirus were discussed by the group. These included both wildlife and human aspects. This research will further inform public health action required for the control of the virus.

The group noted that while current advice to medical practitioners and public health authorities stands[1], there is the possibility of inapparent exposure to lyssavirus. This has been the experience with rabies in the United States of America[2,3]. The group recommended that neurologists and intensive care physicians be alerted to look for lyssavirus infection in cases of unexplained encephalopathy. The recommendations of the National Health and Medical Research Council for post-exposure vaccination of previously vaccinated persons for rabies should be applied to lyssavirus[4].

The group recommended that the National Health and Medical Research Council *Australian Immunisation Procedures Handbook* be updated to include advice on pre- and post-exposure prophylaxis for lyssavirus.

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