Incidence of high-level gentamicin resistance in enterococci at Johannesburg Hospital

K. H. Keddy, K. P. Klugman, L. D. Liebowitz

Objective. To document the incidence of high-level gentamicin resistance (HLGR) in enterococcal isolates at Johannesburg Hospital.

Design. Survey of laboratory isolates.

Setting. Academic hospitals.

Bacterial strains. Consecutive samples of enterococcal isolates.

Main outcome measure. The incidence of HLGR in enterococcal isolates.

Results. The incidence of HLGR was 26.5% of *Enterococcus faecalis* isolates and 20% of *E. faecium* isolates grown during the study period.

Conclusions. High-level gentamicin resistance is common among enterococci isolated at Johannesburg Hospital, and this observation must be considered in defining strategies for the management of invasive enterococcal infections in the future.

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It is now well recognised that the penicillins, including ampicillin, are bacteriostatic against the enterococci and that for severe infections, particularly endocarditis and meningitis, bactericidal antimicrobial therapy is required.[1] The primary method of achieving bactericidal therapy depends on the synergistic activity of a cell-wall active agent, in combination with an aminoglycoside.[2] There has been a marked increase in the production of both β-lactamases and high-level gentamicin resistance (HLGR) in enterococci in the past 10 years.[1,2] It has now become mandatory to monitor enterococci for patterns of resistance.[3,4] Synergistic activity between penicillin or ampicillin and gentamicin is absent if there is HLGR, although synergy is maintained in the presence of low-level gentamicin resistance and penicillin susceptibility.[1-4]

The enterococci are among the three leading causes of nosocomial infections in the USA, and there are increasing numbers of reports from other countries regarding their burgeoning relevance as agents causing disease.[5,6] The clinical significance of the enterococcal isolates may be difficult to assess because they may be colonisers or contaminants in certain specimens, but as they can invade hospitalised patients with severe underlying conditions, the occurrence of HLGR in all isolates should be assessed to give an indication of the overall incidence of this problem.[1-4,6] The prevalence of HLGR in a tertiary care centre, Johannesburg Hospital, has therefore been documented.

Department of Medical Microbiology, South African Institute for Medical Research and University of the Witwatersrand, Johannesburg

K. H. Keddy, M.B. B.CH., B.SC. (MED.), D.T.M.&H.

K. P. Klugman, M.B. B.CH., M.MED., PH.D., F.F.PATH. (S.A.), M.R.C.PATH., D.T.M.&H.

L. D. Liebowitz, M.B. B.CH., B.SC. (PHARM.), PH.D., F.F.PATH. (S.A.), F.R.C.PATH., D.T.M.&H.

Material and methods

A total of 211 enterococcal isolates from all types of clinical specimens were collected between April and July 1994. These were identified as either *Enterococcus faecalis* or *E. faecium*, according to routine laboratory procedures.[7] Specimens were all obtained from laboratory isolates and site of infection could not always be determined, although specimen type was established. The organisms were inoculated into peptone water, adjusting the turbidity to a 0.5 McFarland standard, and then swabbed onto Mueller-Hinton agar with 5% laked blood (Oxoid, Basingstoke, UK). Antibiotic discs containing 120 µg gentamicin (Mast Diagnostics, Merseyside, UK) were applied to the plates, in addition to the antibiotics nomally used for testing susceptibility patterns. Replicate strains were included, as results showed that in three cases there was acquisition of resistance to gentamicin in consecutive isolates from the same patient.

Vancomycin susceptibility was monitored using the Kirby-Bauer method. Resistance to penicillin/ampicillin was documented by the Kirby-Bauer technique, but the mechanism of resistance was not established.

An enterococcal isolate was classed as having high-level gentamicin resistance if the zone diameter around the 120 µg disc was less than 10 mm.[8,9] This criterion was applied to both *E. faecalis* and *E. faecium*. Low-level resistance was not examined, since it would not alter the bactericidal effect of an aminoglycoside/penicillin combination, when compared with aminoglycoside-susceptible isolates.[1-4]

Student's *t*-test (two-tailed) was used for the statistical analysis, calculating the probability of acquiring the HLGR infection in relation to the length of the patient's in-hospital stay. The appropriate formulae for the large sample size (i.e. values pertaining to *E. faecalis*, $N = 196$) and for the smaller sample (values pertaining to *E. faecium*, $N = 15$) were used. Confidence intervals were calculated at 95%.

High-level wards were defined according to the incidence of the isolation of HLGR enterococci from each different ward, according to the results of this study, to facilitate patient management and infection control in these units.

Results

Enterococcal isolates were obtained from a range of specimens, from patients in all areas of the hospital. Fifteen of the 211 isolates (7.1%) were identified as *E. faecium*. Twelve patients had isolates of either species on consecutive specimens from each patient. In 3 of these patients, increasing resistance to gentamicin in different isolates of *E. faecalis* was observed — isolates that were

previously susceptible to the 120 µg disc (all had zone sizes greater than 20 mm initially), but showed no zones around the gentamicin discs 3 - 7 days later.

In total, 52 (26.5%) of *E. faecalis* specimens showed HLGR. Six of these were isolated either from blood cultures or from central venous catheters. Specimens that were susceptible to the high-level gentamicin discs had zone sizes ranging from 13 mm to 34 mm, with a mean of 24 mm (CI = 23.46 - 24.54), while resistant strains showed no zone around the disc.

Of the 15 specimens of *E. faecium*, 3 (20%) showed HLGR. Zone diameters of susceptible strains ranged from 20 mm to 28 mm, with a mean of 23.6 mm (CI = 22.16 - 25.04). No zone was observed around the resistant strains.

The sites of origin of the enterococcal isolates can be seen in Tables I and II. Notably, more than half of the isolates of *E. faecalis* obtained from blood cultures were resistant to high levels of gentamicin, and in isolates obtained from central venous catheters, the ratio of HLGR strains to non-HLGR strains of *E. faecalis* was 4:1. These patients were probably at the greatest risk of acquiring enterococcal endocarditis, because they had an established source of potential bacteraemia and were debilitated. No case of enterococcal endocarditis was diagnosed during the course of the study.

Table I. Sites of origin of *E. faecalis* isolates

	HLGR (*N* = 52)		Non-HLGR (*N* = 144)	
	No.	%	No.	%
Pus	27	51.9	44	30.6
Urine	15	28.9	63	43.6
Blood	4	7.7	7	4.9
CVP tip	4	7.7	1	0.7
Peritoneal fluid	1	1.9	4	2.8
Drain fluid	1	1.9	13	9
Sputum	0	0	3	2.1
Miscellaneous	0	0	9	6.3

CVP = central venous catheter.

Table II. Sites of origin of *E. faecium* isolates

	HLGR (*N* = 3)		Non-HLGR (*N* = 12)	
	No.	%	No.	%
Urine	2	67	2	17
Pus	1	33	4	33
CVP tip	0	0	3	25
Blood	0	0	2	17
Miscellaneous	0	0	1	8

CVP = central venous catheter.

The different wards from which the various strains were isolated are shown in Table III. High-risk wards are defined as those in which the percentage of HLGR strains recovered exceeded the percentage isolated from the hospital as a whole. It is hoped that by defining these wards clinicians will be alerted to problems in their wards regarding infection control and antimicrobial therapy.

Table III. Wards from which HLGR strains of enterococci were isolated (the higher percentages indicate the high-risk areas of the hospital)

	E. faecalis		*E. faecium*	
Ward	No.	%	No.	%
Surgery/trauma	17	36.9	0	0
Plastic surgery	8*	67	2	67
Intensive care	7	30	0	0
Medical wards	7	17.5	0	0
Urology	4	50	0	0
Paediatrics	4	26.7	1	50
Outpatients	3	20	0	0
Orthopaedics	2	13.3	0	0
Gynaecology	0	0	0	0

* Using Fisher's exact test, the probability of isolating HLGR *E. faecalis* from plastic surgery patients was significantly greater than the probability of isolating HLGR *E. faecalis* from patients in the hospital as a whole ($P = 0.006$).

The period of time from date of admission to the isolation of the resistant or susceptible *Enterococcus* species for each patient was not statistically different for either *E. faecalis* or *E. faecium*, although resistant strains tended to be isolated later in the hospital stay.

The mean duration of hospital stay for patients from whom HLGR *E. faecalis* was isolated was 20.60 days (CI = 14.30 - 26.90), while that for patients from whom non-HLGR strains were isolated was 13.80 days (CI = 9.95 - 17.65). The mean duration of hospital stay for patients from whom HLGR *E. faecium* was isolated was 21.70 days (CI = 0.00 - 51.10), while that for non-HLGR strains was 18.10 days (CI = 2.30 - 27.60).

No vancomycin-resistant isolates were observed in this study. Ampicillin/penicillin resistance was observed in 7 of the gentamicin-susceptible *E. faecalis* strains and 1 of the gentamicin-susceptible *E. faecium* strains. Four of the HLGR *E. faecalis* strains were resistant to ampicillin/penicillin, but none of the *E. faecium* strains was resistant. This can probably be ascribed to the low numbers of *E. faecium* isolated in this study.

Discussion

The most commonly encountered enterococcal isolates at Johannesburg Hospital are primarily *E. faecalis* and *E. faecium*, as is observed elsewhere.[5,6] Many of these organisms, when isolated from blood, may in fact be causing transient bacteraemia, but owing to their infective potential, a broad overview of resistance patterns is necessary to formulate an appropriate therapeutic strategy. Approximately 20 - 25% of both species of *Enterococcus* isolated from patients at Johannesburg Hospital demonstrate high-level gentamicin resistance, rendering available therapy bacteriostatic only. Furthermore, if the isolate proves to be resistant to ampicillin, vancomycin is the only suitable antibiotic currently available for treatment in this country.

Follow-up of the 3 patients who developed HLGR enterococcal infections after previous isolation of a

susceptible strain showed that 1 of the 3 received gentamicin therapy, suggesting that, at least in the other 2, these organisms came from the contaminated hospital environment. All 3 patients were in surgical intensive care, but at different stages of their in-hospital stay, and this could not be classed as an outbreak. The first patient had been on gentamicin therapy for 24 days, since admission. Whether the first patient acquired a superinfection due to HLGR *E. faecalis*, or whether the initial isolate from this patient became resistant, could not be established because molecular analysis was not done.

The appropriate *in vitro* concentration of gentamicin to determine HLGR in the enterococci is still under debate. Most studies have utilised discs containing 120 µg gentamicin.[8-12] It has been suggested that 30 µg discs may detect HLGR adequately. More recently, it has been observed that laboratories utilising discs containing 30 µg gentamicin or less, tend to over-report HLGR in enterococci.[14,15] The best discrimination between susceptible enterococci and those with low-level resistance, using the Kirby-Bauer method, is achieved using 500 µg gentamicin discs.[16] Since differentiating between low-level gentamicin resistance and gentamicin susceptibility would not alter therapy in enterococcal infections, the 120 µg gentamicin disc was considered adequate for this study.

HLGR is frequently observed in conjunction with β-lactamase production, which may be either plasmid or chromosomally mediated.[17-20] Furthermore, the genes conferring HLGR in *E. faecalis* and *E. faecium* are highly homologous.[21] HLGR is mediated by a bifunctional enzyme, with 6′-acetylating activity and 2″-phosphorylating activity.[20-22] This high-level resistance can frequently be extrapolated to the other aminoglycosides.[1-3]

Streptomycin may be the exception to this trait.[23,24] Screening for synergy between streptomycin and a penicillin or for the lack of high-level streptomycin resistance in the presence of HLGR is therefore advocated.[2,3] A simple Kirby-Bauer or disc-diffusion method may be employed to this end, utilising either 300 µg or 1 000 µg streptomycin per disc.[8-12,25] Findings in this instance must be viewed with relative caution, because false susceptibility occurs more frequently with streptomycin-resistant isolates than with gentamicin-resistant isolates.[11] In this study, if the isolate was found to exhibit HLGR, an offer of testing for streptomycin susceptibility was made to the clinician concerned, but this was never accepted, possibly owing to the perceived toxicity associated with streptomycin usage and because streptomycin is rarely used for synergistic therapy with ampicillin at Johannesburg Hospital.

It is clear from the data in the study that the ward in which the patient is placed is a far greater risk factor than the length of time in hospital. Particularly high-risk wards for the acquisition of HLGR enterococci are plastic surgery, intensive care units and general surgery/trauma. An awareness on the part of the ward staff in these units may assist in controlling the spread of HLGR enterococci and of other nosocomially acquired pathogens and in facilitating the rapid introduction of appropriate treatment. Other studies observed that for certain high-risk patients, there is an increased incidence of colonisation or infection with resistant organisms.[4-26] Since these patients are normally extremely debilitated, with severe underlying conditions, optimal therapy must be obtained as soon as possible. Usage of the 120 µg gentamicin discs is an inexpensive and appropriate method, which can be applied in the clinical laboratory to give an adequate indication of therapeutic options. This method is easier to perform than both the 'checker-board' and time-kill synergy studies, and although it is not definitive, it can provide valuable preliminary information.[25]

Comparative studies from other centres in recent years have shown an incidence of HLGR ranging from 15% to 44%.[15,23,27,28] A Saudi Arabian study showed an overall incidence of 18%, which corresponded to high-level resistance to kanamycin (a marker for amikacin resistance), but not to streptomycin resistance, in most strains of enterococci examined.[23] The incidence of HLGR in enterococcal isolates in a retrospective study in the UK was found to be 44%.[15] This study also examined *Streptococcus agalactiae* for HLGR, but found none in the isolates studied. The authors included a comparison of various methods of detecting HLGR, including minimum inhibitory concentrations (MICs), 30 µg discs and 100 µg discs. They found good correlation between the MIC and the 100 µg disc in identifying HLGR, but the 30 µg disc was less discriminatory.[15] An important study from the USA clearly documented an increase in HLGR in enterococci over a period of 6 years, the initial incidence being 13.8% in 1986; the incidence more than doubled to 33.3% in 1991.[27]

The recommendation that all isolates of enterococci be screened for HLGR, where synergy between penicillin and an aminoglycoside will be required, such as in invasive enterococcal infections, is appropriate.[15] Monitoring of the incidence of HLGR by the clinical laboratory on an annual basis would assist in advising on optimal therapy for treatment of enterococcal infection. Compounding the problem of HLGR in the enterococci are recent reports of outbreaks of enterococcal infection resistant to both penicillin/ampicillin and vancomycin.[29,30] Knowledge of local patterns of resistance and continual updating of that knowledge is therefore no longer a matter of choice.

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