STATUS OF HIGH LEVEL AMINOGLYCOSIDE RESISTANT ENTEROCOCCUS FAECIUM AND ENTEROCOCCUS FAECALIS IN A RURAL HOSPITAL OF CENTRAL INDIA

*DK Mendiratta, H Kaur, V Deotale, DC Thamke, R Narang, P Narang

Abstract

Considering the emergence of high level aminoglycoside resistance (HLAR) in enterococci this study was undertaken to determine their status in a rural setting. HLAR by disc diffusion and agar dilution, β-lactamase by nitrocefin disc and vancomycin resistance by agar dilution was determined in 150 enterococcal isolates, as per NCCLS guidelines. Only two species, Enterococcus faecalis (85.5%) and Enterococcus faecium (14.7%) were recovered, mostly from blood. Forty six percent showed HLAR. Multi drug resistance and concomitant resistance of HLAR strains to β-lactams were quite high. None showed β-lactamase activity or vancomycin resistance.

Key words: E. faecalis, E. faecium, high level aminoglycoside resistance.

Regarded as nosocomial pathogen of the 1990s, enterococci have become increasingly important not only because of their ability to cause serious infections but also because of their increasing resistance to many antimicrobial agents. Serious enterococcal infections are often refractory to treatment and the mortality is high.[1] Infections by enterococci have traditionally been treated with cell wall active agents (e.g., penicillin or ampicillin) in combination with an aminoglycoside (streptomycin / gentamicin), however emergence of high level resistance to aminoglycosides (HLAR), β lactam antibiotics and to vancomycin by some strains, together with association of HLAR with multi drug resistance has led to failure of synergistic effects of combination therapy.[1-3] Streptomycin was the aminoglycoside used clinically until 1970 when more than 50% of enterococci were found to be resistant to high level of this drug.[1] High level gentamicin resistance (HLGR) was first reported in E. faecalis 1979.[1]

The present study was undertaken considering the paucity of data on high level aminoglycoside resistance (HLAR) in enterococci, especially from a rural set-up and due to the fact that enterococci are the second leading cause of hospital acquired infection and the third leading cause of bacteraemia.[4]

Materials and Methods

Enterococci recovered from various specimens between June 2003 and January 2004 were identified and speciated by test scheme proposed by Facklam and Collins.[5] Our hospital primarily caters to the rural population of Central India.

Antibiotic susceptibility testing was done by Kirby Bauer disc diffusion method using discs and Mueller Hinton Agar (Himedia) as recommended by NCCLS.[6] High content gentamicin and streptomycin discs were prepared locally,[7] using pure powders obtained from Ranbaxy and Hindustan antibiotics respectively. Minimum inhibitory concentration (MIC) of streptomycin / gentamicin by agar dilution, β lactamase activity by nitrocefin disc and screening for vancomycin resistance by agar dilution was done as recommended by NCCLS.[6] Staphylococcus aureus ATCC 25923 and E. faecalis ATCC 29212 were used to check quality of routine and high content discs respectively.

Results

Of the 150 (1.16%) enterococcal isolates recovered from 12890 specimens, 128 (85.3%) were identified as E. faecalis and 22 (14.7%) as E. faecium which were isolated primarily from blood (77.3% and 24.2% respectively) and isolation of E. faecium was significantly higher (p<0.05) than E. faecalis. E. faecium was not isolated from urine as against the recovery of 13.3% of E. faecalis from urine.

A total of 69 (46%) isolates showed high level resistance to gentamicin and/or streptomycin by both, high content disc diffusion and agar dilution (MIC). HLAR among E. faecium isolates (95.5%) was significantly higher than E. faecalis (37.5%) (c1-c2 p < 0.05) and this was also evident when the two isolates were compared with respect to the two drugs considered individually (a1-b1 and a2-b2) or combined together (d1-d2) (Table 1).

Combined resistance to both the aminoglycosides was much higher in E. faecium (59.1%) as compared to E. faecalis (7.8%) (d1-d2, Table 1). There was no difference in resistance of E. faecalis to either gentamicin or streptomycin (14.8% each alone and 22.6% each overall - a1-a2 Table 1), however, in E. faecium it was higher.
Antibiotic resistance in enterococci is either intrinsic or acquired. Intrinsic traits expressed by enterococci include resistance to semisynthetic penicillinase resistant penicillins, cephalosporins, low level of aminoglycosides and low level of clindamycin, whereas acquired resistance includes resistance to chloramphenicol, erythromycin, high level of clindamycin, tetracycline, high level of aminoglycosides, penicillin, fluoroquinolones and vancomycin. HLR is due to release of various aminoglycoside modifying enzymes.

In the present study, 46% of the enterococci showed HLR and combined HLR and HLSR was significantly (p<0.05) higher in *E. faecium* (59.1%) than *E. faecalis* (7.8%), as also reported by Gordon et al.[13] High HLR in *E. faecalis* and HLSR in *E. faecium* observed has also been reported[8,10] as also vive a versa[13] and no such difference.[12]

We, in this rural set up, found the prevalence of HLSR *E. faecalis* and *E. faecium* and HLR *E. faecalis* to be lower while that of HLR *E. faecium* to be slightly higher than that reported from urban hospitals.[8,10,12,15] The reason for higher prevalence in urban hospitals could be because of the set up where chronic cases are prevalent and there is wider usage of broad spectrum antibiotics.

Resistance to aminoglycosides in enterococci is often associated with multidrug resistance.[1] In our study, *E. faecalis* showed resistance to as many as nine and *E. faecium* to as many as eight drugs. Concomitant resistance of HLR and HLSR strains to the two β-lactam antibiotics (penicillin and ampicillin) was quite high in both the species and it was higher to penicillin than ampicillin in *E. faecalis* (Table 2). Concomitant HLR, high level penicillin resistance and resistance to vancomycin has been reported in 16% isolates by Aggarwal et al[10] from Nagpur. Resistance to penicillin and ampicillin, which is usually intrinsic, is primarily due to low affinity of the penicillin binding proteins[8] and it results in loss of synergistic effect between β-lactams and aminoglycosides leading to treatment failures.

HLGR has also been linked to β-lactam production, resistance to ciprofloxacin[14] and chloramphenicol.[1,14] In fact Schouten et al[14] reported that as the prevalence of HLR increases, β-lactamase production in Enterococci may also increase. Though none of our isolates were β-lactam producers, as also reported by Jessudason et al.[2] both *E. faecium* and *E. faecalis* showed concomitant resistance to ciprofloxacin and chloramphenicol (Table 2). Addition of resistance due to β-lactamase production to the intrinsic resistance already seen in enterococci is of major concern.

### Table 1: Distribution of the two high level aminoglycoside resistance enterococcal species with respect to resistance to aminoglycoside combination

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>E. faecalis (n=128)</th>
<th>E. faecium (n=22)</th>
<th>Total (n=150)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gentamicin alone</td>
<td>19 (14.8%)</td>
<td>05 (22.7%)</td>
<td>24 (16%)</td>
</tr>
<tr>
<td>Gentamicin and streptomycin</td>
<td>10 (14.8%)</td>
<td>13 (22.7)</td>
<td>23 (15.3)</td>
</tr>
<tr>
<td>Gentamicin and tetracycline</td>
<td>7.8%</td>
<td>59.1%</td>
<td>15.3%</td>
</tr>
<tr>
<td>Gentamicin and chloramphenicol</td>
<td>6 (20.6%)</td>
<td>4 (22.2%)</td>
<td>8 (27.5%)</td>
</tr>
<tr>
<td>Gentamicin and penicillin</td>
<td>24 (82.7%)</td>
<td>17 (94.4%)</td>
<td>24 (82.7%)</td>
</tr>
<tr>
<td>Gentamicin and ampicillin</td>
<td>26 (89.6%)</td>
<td>17 (94.4%)</td>
<td>26 (89.6%)</td>
</tr>
<tr>
<td>Total (n=150)</td>
<td>48 (37.5%)</td>
<td>21 (95.5%)</td>
<td>69 (46.0%)</td>
</tr>
</tbody>
</table>

* and $^{b}$ = HLGR, $^{a}$ and $^{b}$ = HLSR, $^{a1}$, $^{a2}$, $^{b1}$, $^{c1}$ and $^{d1}$ = P value < 0.05, (%) percentage from column total (n), [ ] percentage from row total [n]

### Table 2: Resistance of HLGR and HLSR *E. faecalis* and *E. faecium* to other antibiotics

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>HLGR No (%) (n=147)</th>
<th>HLSR No (%) (n=145)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin*</td>
<td>26 (89.6%)</td>
<td>26 (89.6%)</td>
</tr>
<tr>
<td>Ampicillin*</td>
<td>24 (82.7%)</td>
<td>24 (82.7%)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>22 (75.8%)</td>
<td>19 (65.5%)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>20 (68.9%)</td>
<td>20 (68.9%)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>19 (65.5%)</td>
<td>18 (62.0%)</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>6 (20.6%)</td>
<td>8 (27.5%)</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>13 (44.8%)</td>
<td>13 (44.8%)</td>
</tr>
</tbody>
</table>

*Overall resistance to β-lactam antibiotics: HLGR isolates to penicillin: 91.4%, to ampicillin: 87.2% and HLSR isolates to penicillin: 93.3%, to ampicillin: 88.8%.

to gentamicin (22.7% alone and 81.8% overall) than to streptomycin (13.6 alone 72.7% overall). None of these differences were however, statistically significant. None of the 150 enterococcal isolates showed β-lactamase activity or vancomycin resistance.

Both *E. faecalis* and *E. faecium* showed multi drug resistance, the former to as many as nine and latter to as many as eight drugs. It was observed that resistance of HLGR / HLSR isolates to various antibiotics (except to rifampicin and by HLSR to chloramphenicol) was more in *E. faecium* than *E. faecalis*, however this difference in the two species was statistically not significant (Table 2).

Concomitant resistance of HLGR and HLSR strains to β-lactam antibiotics was quite high in both the species. In case of HLGR, overall it was 91.4% with penicillin and 87.2% with ampicillin, while in case of HLSR it was 93.3% and 88.8% respectively (Table 2).

### Discussion

Recent years have witnessed increased interest in enterococci not only because of their ability to cause serious infections but also because of their increasing resistance to many antimicrobial agents.[1-3] In the present study only two species *E. faecalis*(85.3%) and *E. faecium* 22 (14.7%) were recovered in contrast to additionally more by others from India.[8,9] Our isolation rate was close to that from Nagpur[10] (*E. faecalis* 86% and *E. faecium* 14%) 80 km away and Coimbatore[11] (88% *E. faecalis*). However, report of higher isolation of *E. faecium* (80.7%) over *E. faecalis* (19.2%) has been there from Mumbai.[12]
Rifampicin is useful against multidrug resistant enterococci and often given in combination as alone has poor bactericidal activity.[15] Resistance to rifampicin was observed in both HLAR positive and negative isolates in our study, it being higher in latter, though not statistically significant.

The problem of VRE may not be very high in India[8,10,12] as also seen in our rural hospital at present but monitoring of VRE is the need of hour since it appears to be an emerging pathogen in India. This study revealed the prevalence of multidrug resistant HLAR strains of *E. faecalis* and *E. faecium* in this rural hospital.

References


Source of Support: Nil, Conflict of Interest: None declared.