Low recovery rates of high-level aminoglycoside-resistant enterococci could be attributable to restricted usage of aminoglycosides in Indian settings

Intensive use of broad-spectrum antibiotics is responsible for the conversion of enterococci, the otherwise gut commensal bacteria, to opportunistic nosocomial pathogens (Huysse et al., 1998; Mohanty et al., 2005). There have been reports of the emergence of acquired drug-resistant enterococci in India but glycopeptide resistance is infrequently reported (Mohanty et al., 2005; Ghoshal et al., 2006). Nonetheless, there have been almost no reports on the faecal carriage of enterococci in India. Therefore, as a pilot analysis, we investigated the species distribution and antibiogram pattern of enterococci in 40 stool specimens of subjects with the symptoms of diarrhoea and a recent history of treatment with antimicrobial agents admitted to a government communicable-diseases hospital in Chennai, South India, in December 2006. Diarrhoeal specimens alone were investigated because the specimens were already being processed in the Bacteriology Laboratory and because the existence of drug-resistant enterococci in the hospital environment, often arising from patients with diarrhoea, is associated with nosocomial infection, particularly in peripheral limited-resource settings.

Stool specimens, collected in wide-mouthed containers, were inoculated on bile esculin agar (Himedia). Gram-positive cocci that produced black precipitates were tested for 6.5 % NaCl tolerance. Positive isolates that were identified as enterococci were subsequently inoculated on 5 % sheep blood agar. The isolates were further examined for microscopic morphology and catalase-negative reaction. Phenotypic and physiological characterization was carried out by standard tests, namely motility, pigment production, arginine dihydrolase test and carbohydrate fermentation tests (Valentina & Lalitha, 2001). Antibiotic susceptibility testing was carried out on Mueller–Hinton agar (Himedia) by the Kirby–Bauer disc diffusion method adhering to the Clinical and Laboratory Standards Institute (CLSI) guidelines using commercially available ampicillin (10 µg), vancomycin (30 µg) and linezolid (30 µg) antibiotic discs, with Enterococcus faecalis ATCC 29212 as the control. High content aminoglycoside discs, namely gentamicin (120 µg) and streptomycin (300 µg), were prepared in house. The isolates were confirmed as high-level aminoglycoside-resistant enterococci (HLARE) using the agar dilution susceptibility test (CLSI, 2005). Of the total 31 enterococci isolates from faecal cultures of 40 cases, 9 (29 %) were identified as E. faecalis and 22 (71 %) as Enterococcus faecium. However, all were sensitive to vancomycin and linezolid. Interestingly, 11 (50 %) of the E. faecium isolates and none of the E. faecalis isolates were resistant to ampicillin (Table 1).

Reports of the steady rise in the recovery rates of ampicillin-resistant enterococci (ARE) have been available in the recent past in India and more recently of glycopeptide resistance (Ghoshal et al., 2006). The prevalence of ARE carriers (27 %, 11/40) seems almost equivalent to that of European countries. High prevalence of ARE seems to be associated with a high relative proportion of E. faecium compared with E. faecalis (Fontana et al., 1998; Silverman et al., 1998; Torell et al., 1999; Torfoss et al., 1999). Intriguingly, high-level aminoglycoside resistance was observed in three E. faecalis isolates among which two were high-level streptomycin-resistant (HLSR) and one was high-level gentamicin-resistant (HLGR). None of the isolates were concurrently resistant to both the aminoglycoside antibiotics. Studies of infections caused by enterococci have reported a varying prevalence of HLARE from 7 to 44 % (Sifuentes-Osornio et al., 1996). We report a 2 % recovery rate of HLGR enterococci and a 4 % recovery rate of HLSR enterococci (faecal carriage) in Chennai. A greater sample size may be required to perform descriptive investigations on the existence and distribution of vancomycin-resistant enterococci (VRE) and other drug-resistant phenotypes. Although 100 % faecal carriage of enterococci might be expected, it is important to periodically monitor the emergence of aminoglycoside-resistant enterococci in hospital settings. Human carriage of ARE and HLARE could be an important source

### Table 1. Biotyping and antibiotic susceptibility pattern of enterococci in stool specimens

<table>
<thead>
<tr>
<th>Enterococcus</th>
<th>No. of isolates</th>
<th>Antibiotic resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ampicillin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S*</td>
</tr>
<tr>
<td>E. faecalis</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>E. faecium</td>
<td>22</td>
<td>11</td>
</tr>
<tr>
<td>Total</td>
<td>31</td>
<td>11</td>
</tr>
</tbody>
</table>

S, Streptomycin; G, gentamicin.
HLSR, HLGR.
of nosocomial infections among hospitalized patients. The low recovery rates of HLARE and the absence of VRE carriers could be attributed to restricted usage of antimicrobial agents in South India, but the high recovery rate of ARE could be indicative of selective antibiotic pressure resulting from the use of the cheaper antibiotics. A strategy for restricted antibiotic prescription combined with universal barrier precautions may seem more appropriate and feasible in limiting the spread of these bacteria. In conclusion, we observed a low recovery rate of HLARE with no detection of VRE in the diarrhoeal cases screened.

Acknowledgements
We are grateful to Dr Janarthanan and Dr James, Communicable Diseases Hospital, Chennai, for facilitating collection of stool specimens from the study participants involved.

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