Antimicrobial resistance in *Enterococcus faecalis* at a tertiary care centre of northern India

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**Background & objectives**: Multiresistant enterococci are emerging as a leading nosocomial pathogen. Knowledge of the profile of antimicrobial resistance is essential to formulate treatment guidelines for infections caused by enterococci. This study reports the antimicrobial sensitivity of enterococci isolated during a one year period from clinical samples of patients admitted to a tertiary care hospital of Delhi.

**Methods**: A total of 444 isolates of *Enterococcus faecalis* were screened for antimicrobial susceptibility by the disk diffusion technique as recommended by the National Committee for Clinical Laboratory Standards (NCCLS). Screening for vancomycin resistance was done by the vancomycin screen agar method recommended by NCCLS, which was confirmed by determination of minimum inhibitory concentration (MIC) using microbroth dilution and E-test methods. Vancomycin resistance phenotypes were determined by polymerase chain reaction.

**Results**: A total of 115 (26%) isolates had high level aminoglycoside resistance, 293 (66%) were resistant to ampicillin, 391 (88%) to ciprofloxacin and 377 (85%) to erythromycin. Vancomycin resistance was found in five (1%) isolates, of which four had van A phenotype and one had van B phenotype.

**Interpretation & conclusion**: Emergence of vancomycin resistant enterococci is of concern due to the limited therapeutic options. Implementation of infection control measures can contain the spread of these resistant bacteria.

**Key words** Aminoglycoside resistance - *Enterococcus faecalis* - India - vancomycin resistant *Enterococcus*

Enterococci have become important nosocomial pathogens world-wide and are associated with a high mortality. The treatment of these infections poses a great challenge due to the inherent resistance of Enterococci to many antibiotics. A combination of penicillin and gentamicin had been the mainstay of treatment of enterococcal infections till now but with the emergence of high level aminoglycoside resistance (HLAR), vancomycin is the only alternative available. The widespread use of glycopeptides in hospitals has led to the emergence of vancomycin resistant *Enterococcus* (VRE) which is a major concern for health care professionals. VRE are frequently reported from hospitals in USA and Europe. However, VRE are not a cause for concern in many Asian countries due to a low prevalence. There is a paucity of information on vancomycin resistance in enterococci from our country.

In this study, we present the antimicrobial susceptibility of clinical isolates of *Enterococcus faecalis* recovered at a tertiary care hospital in New Delhi during January to December 2001 and report the emergence of vancomycin resistance in Enterococci.
Material & Methods

All isolates of *E. faecalis* recovered from clinical samples at the clinical bacteriology laboratory of the All India Institute of Medical Sciences, New Delhi during the study period were included. Antimicrobial susceptibility testing of the enterococci was performed by the disk diffusion method as recommended by the National Committee for Clinical Laboratory Standards (NCCLS)\(^1\). The antimicrobial disks (µg) used were ampicillin (10), ciprofloxacin (5), erythromycin (15), vancomycin (30) and teichoplanin (30). HLAR was determined using gentamicin (120 µg) disc. Control strains included *Enterococcus faecalis*, ATCC 51299 (ATCC E.f., vancomycin sensitive), *Staphylococcus aureus* ATCC 25923, WHO 3 (vancomycin resistant *E. faecalis*, Van A phenotype; vancomycin MIC 512 µg/ml), WHO 6 (glycopeptide intermediate *S. epidermidis*, vancomycin MIC 8 µg/ml, teichoplanin MIC 16 µg/ml), WHO 11 (vancomycin resistant *E. gallinarum*, vancomycin MIC 8 µg/ml, sensitive to teichoplanin), WHO 14 (vancomycin resistant *E. faecalis* Van B, vancomycin MIC 32 µg/ml, sensitive to teichoplanin) and WHO 20 (vancomycin resistant *E. faecalis*, Van B, vancomycin MIC 16 µg/ml)\(^2\). (WHO 3, 6, 11, 14 and 20 are characterized strains which were provided to us as a part of WHO/CDC External quality assurance and proficiency testing programme)\(^3\). Screening for low level vancomycin resistance was done by vancomycin screen agar method using 6 µg/ml vancomycin according to NCCLS recommendations\(^4\). Minimum inhibitory concentration (MIC) of vancomycin was determined by microbroth dilution method\(^5\) and E-test (AB Biodisk, Sweden)\(^6\).

The phenotype of vancomycin resistant strains was determined by PCR done according to standard protocols\(^7,8\). For this, DNA was isolated by the heat lysis method as described by Clark *et al*\(^9\). The extracted DNA (5 µl) was added to a PCR mixture (25 µl) containing 200 µM dNTPs; 2.5mM MgCl\(_2\), 1 x Taq buffer, 5 U reaction taq polymerase (Promega, USA) and each primer in 50 p moles concentration. The primers were selected from published sequences\(^10\) as follows : van A (product size 732 bp), A1, 5’-GGG AAA ACG ATT GC - 3’; A2, 5’-GTA CAA TGGGCGTGTA - 3’, van B (product size 635 bp), B1, 5’-ATG GTA GGA AGC CGA TAGTC - 3’; B2, 5’-GATTTCGTTCCTCGACC-3’; Van C (product size 822 bp), C1, 5’-GGTATCAAGGAACCTC-3’; C2, 5’-CTTCCGCCATCATAGCT-3’. Amplification of DNA was performed in an Ampliton thermolyne II thermocycler (Barnstead Thermolyne Corporation, Dubuque, USA) with predenaturation at 94°C for 2 min, followed by 30 cycles of 94°C for 1 min, 37°C for 1 min; and 72°C for 1 min. Final extension was done at 72°C for 10 min. Amplicons were analyzed by electrophoresis on 1 per cent agarose gels along with 100 bp ladder (Boehringer Mannheim, GmbH, W. Germany) and stained with ethidium bromide.

Results & Discussion

A total of 444 isolates of *E. faecalis* were recovered from various clinical samples during the study period, of which 217 (49%) were obtained from urine samples, 170 (38%) from blood, 45 (10%) from soft tissues and 12 (3%) from the lower respiratory tract. The maximum number of isolates [209 (47%)] were obtained from patients admitted to the intensive care units (ICUs), followed by surgical wards [155 (35%)], medical wards [36 (8%)] and oncology ward [44 (10%)].

Of the 444 isolates, 115 (26%) had high level aminoglycoside resistance, 293 (66%) were resistant to ampicillin, 391 (88%) to ciprofloxacin and 377 (85%) to erythromycin. Of the isolates showing HLAR, 54 were obtained from blood, 48 from urine, 12 from soft tissues and 1 from lower respiratory tract. In a recent multicenter study, the prevalence of HLAR was reported to be 37 per cent\(^2\). With the spread of strains showing HLAR, there is now rampant use of vancomycin in hospitals since it is the only available alternative for treatment. Simultaneously, vancomycin usage has also increased in hospitals following the emergence of methicillin resistant *S. aureus*. Excessive use of vancomycin has been found to be a risk factor for infection or colonization by *VRE*\(^16,17\).

Analysis by the WHONET software programme\(^12\) revealed a gradual decline in zone diameters of vancomycin sensitive strains (Fig.), thus showing a trend towards a decreasing susceptibility to vancomycin among *Enterococci*. Five (1%) isolates of *E. faecalis* were found to be resistant to vancomycin by the disk diffusion and agar screen methods (Table). Enterococci frequently cause bacteraemia in hospitalized patients especially those on prolonged intravenous lines or antibiotic therapy\(^18\). On PCR, four isolates had van A phenotype and one had van B phenotype. Van A is the most common phenotype world-wide and confers resistance to both vancomycin and teichoplanin\(^19\).
Fig. Vancomycin inhibition zone diameters of *E. faecalis* isolates analysed by WHONET programme (<14 mm - resistant, 14-16 mm - intermediate, >16 mm - sensitive).

**Table.** Characteristics of vancomycin resistant enterococci isolated in the present study

<table>
<thead>
<tr>
<th>Isolate no.</th>
<th>Source</th>
<th>Zone diameter (mm) (interpretation)</th>
<th>Vancomycin screen agar</th>
<th>MIC (µg/ml)</th>
<th>PCR phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Vancomycin</td>
<td>Teichoplanin</td>
<td>E-test</td>
<td>Broth dilution</td>
</tr>
<tr>
<td>1.</td>
<td>Blood</td>
<td>N (R)</td>
<td>N (R)</td>
<td>R</td>
<td>&gt;256</td>
</tr>
<tr>
<td>2.</td>
<td>Soft tissue</td>
<td>N (R)</td>
<td>N (R)</td>
<td>R</td>
<td>&gt;256</td>
</tr>
<tr>
<td>3.</td>
<td>Blood</td>
<td>N (R)</td>
<td>N (R)</td>
<td>R</td>
<td>&gt;256</td>
</tr>
<tr>
<td>4.</td>
<td>Blood</td>
<td>N (R)</td>
<td>N (R)</td>
<td>R</td>
<td>&gt;256</td>
</tr>
<tr>
<td>5.</td>
<td>Urine</td>
<td>N (R)</td>
<td>20 (S)</td>
<td>R</td>
<td>&gt;256</td>
</tr>
<tr>
<td>6.</td>
<td>ATCCE.f</td>
<td>-</td>
<td>22 (S)</td>
<td>S</td>
<td>4</td>
</tr>
<tr>
<td>7.</td>
<td>WHO 3</td>
<td>-</td>
<td>10 (R)</td>
<td>R</td>
<td>&gt;256</td>
</tr>
<tr>
<td>8.</td>
<td>WHO 11</td>
<td>-</td>
<td>N (R)</td>
<td>17 (R)</td>
<td>8</td>
</tr>
<tr>
<td>9.</td>
<td>WHO 14</td>
<td>-</td>
<td>N (R)</td>
<td>16 (S)</td>
<td>128</td>
</tr>
<tr>
<td>10.</td>
<td>WHO 20</td>
<td>-</td>
<td>12 (R)</td>
<td>20 (S)</td>
<td>128</td>
</tr>
<tr>
<td>11.</td>
<td>WHO 6</td>
<td>-</td>
<td>18 (S)</td>
<td>14 (S)</td>
<td>8</td>
</tr>
</tbody>
</table>

ATCCE.f, *E. faecalis* vancomycin sensitive ATCC 51299; WHO 3, VRE van A; WHO 11, Vancomycin resistant *E. gallinarum*; WHO 14 VRE van B; WHO 20, VRE van B; WHO 6 glycopeptide intermediate *S. epidermidis*; N, no zone; R, resistant; S, sensitive; MIC, minimum inhibitory concentration; - not done; E.f, *Enterococcus faecalis*; N, not applicable. All the clinical isolates were from patients admitted to the ICUs.
All five isolates of VRE were recovered from patients admitted to the ICUs. Two of these patients died following VRE bacteraemia. VRE was found to be an independent predictor of death in a study done on enterococcal bacteraemia\(^2\). Surveillance cultures of the wards from which the five isolates of VRE were recovered were sterile and cultures from the hands of health care personnel also did not grow enterococci. VRE can spread rapidly in hospitals due to cross contamination and person to person spread\(^4,20,21\). It has been found that being in the same ward as a VRE case carries the highest risk for acquisition of VRE\(^16\). Hence early detection and contact isolation are important in the management.

Infection by VRE was first reported in France and UK and since then it has been documented from many countries world-wide\(^1,19,22,23\).

Treatment of infections caused by VRE is a challenging task, especially because the resistance appears in strains, which are multiresistant\(^21\). The optimal therapy for such infections is not known and published reports are largely anecdotal\(^21\). Measures should be taken to prevent further development and transmission of these infections by strictly implementing infection control guidelines and antibiotic policies in hospitals\(^4,8,20\). Prudent use of vancomycin and a proper surveillance for VRE may permit early recognition and containment of spread of this emerging pathogen in our country.

References


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