Linezolid Resistance in Vancomycin-Resistant Enterococcus faecalis and Enterococcus faecium Isolates in a Brazilian Hospital

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Linezolid resistance in vancomycin-resistant enterococci (VRE) strains has been rarely reported, with Enterococcus faecium being the species most commonly associated with these few cases (1, 2, 3). Here, we report infections due to linezolid- and vancomycin-resistant Enterococcus (LRVRE) strains in patients who were treated with linezolid in a tertiary-care hospital in Brazil. To our knowledge, this is the first report of LRVRE strains in Brazil.

From August 2009 to December 2011, five E. faecalis strains and one E. faecium strain exhibiting high-level resistance to both vancomycin (MIC, >256 mg/liter) and linezolid (MIC, 8 to 64 mg/liter) were isolated from blood and urine cultures from different inpatients in a Brazilian hospital (Table 1).

All five subjects included were severely ill patients from intensive care units (ICUs). Patient 1 received linezolid for sepsis caused by VRE 10 days before an E. faecalis strain (18/755) was isolated from a blood culture. The regimen was changed to ampicillin, but the patient died 5 days later. Patient 2 data were not available. Patient 3 received linezolid for a period of 27 days. Two E. faecalis strains that were indistinguishable by pulsed-field gel electrophoresis (PFGE) were isolated from two blood cultures of this patient. The first strain (37/245), resistant to linezolid, was isolated at the 27th day of the total course of treatment with this drug, and the other (38/443), with intermediate resistance to linezolid, on day 53. Patient 4 received linezolid for 30 days. Eight days after the end of treatment, two strains from different species at different sites, one E. faecalis strain from a urine culture (40/1258) and a S. hominis strain from a blood culture, were obtained (4). Patient 6 received linezolid for a total of 32 days of treatment.

The E. faecium 42/448 strain was isolated 9 days after the end of treatment.

The strains were multidrug resistant, except the E. faecalis ST525 clone, which presented intermediate erythromycin and chloramphenicol MIC values, and the E. faecalis ST526 and E. faecium ST412 clones, which were susceptible to tetracycline. All E. faecalis strains were susceptible to ampicillin. Regarding glycopeptide resistance, the vanA gene was identified in all isolates. The G2576T mutation, which confers resistance to linezolid, was identified in the 23S rRNA gene in all linezolid-resistant strains, and the incomplete digestion of domain V with NheI suggested the presence of fragments with both G2576T mutant and wild-type sequences in these strains. The cfr gene was not identified in any isolate. Multilocus sequence typing (MLST) analysis revealed that the linezolid resistance was found to occur in two novel sequence types (STs) of E. faecalis (ST525 and ST526) and in ST412 of E. faecium. Strains 50/515 and 51/426 corresponding to the linezolid-susceptible E. faecalis and E. faecium control strains showed ST62 and ST838, respectively.

The emergency of LRVRE is a concerning issue. Our work contributes with data that enable observation of the course of

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**TABLE 1 Demographic data and antimicrobial susceptibility profiles of linezolid and vancomycin-resistant E. faecalis and E. faecium clinical strains**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Strain</th>
<th>ICU</th>
<th>Culture date</th>
<th>Total no. of treatment days</th>
<th>Clinical specimen</th>
<th>PFGE type</th>
<th>MLST result</th>
<th>Glycopeptide resistance gene</th>
<th>23S rRNA mutation</th>
<th>Resistance profile MIC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>E. faecalis 18/755</td>
<td>Yes</td>
<td>August 2009</td>
<td>10</td>
<td>Blood</td>
<td>A</td>
<td>ST525</td>
<td>vanA</td>
<td>G2576T</td>
<td>Lzd 8 &lt;256, 96 16 4 2 64 16 &gt;32 &gt;32</td>
</tr>
<tr>
<td>2</td>
<td>E. faecalis 28/279</td>
<td>Yes</td>
<td>April 2010</td>
<td>NA</td>
<td>Urine</td>
<td>A</td>
<td>ST525</td>
<td>vanA</td>
<td>G2576T</td>
<td>Lzd 16 &gt;256, 96 16 4 2 64 16 &gt;32 &gt;32</td>
</tr>
<tr>
<td>3</td>
<td>E. faecalis 37/245</td>
<td>No</td>
<td>November 2010</td>
<td>27</td>
<td>Blood</td>
<td>A</td>
<td>ST525</td>
<td>vanA</td>
<td>G2576T</td>
<td>Lzd 32 &gt;256, 96 16 4 2 64 16 &gt;32 &gt;32</td>
</tr>
<tr>
<td>4</td>
<td>E. faecalis 40/1258</td>
<td>Yes</td>
<td>January 2011</td>
<td>27</td>
<td>Blood</td>
<td>A</td>
<td>ST525</td>
<td>vanA</td>
<td>G2576T</td>
<td>Lzd 8 &gt;256, 96 16 4 2 64 16 &gt;32 &gt;32</td>
</tr>
<tr>
<td>5</td>
<td>E. faecalis 50/515</td>
<td>No</td>
<td>December 2011</td>
<td>30</td>
<td>Urine</td>
<td>C</td>
<td>ST62</td>
<td></td>
<td></td>
<td>Lzd 2 1 0.5 1 0 64 1 1</td>
</tr>
<tr>
<td>6</td>
<td>E. faecium 42/448</td>
<td>Yes</td>
<td>November 2011</td>
<td>32</td>
<td>Urine</td>
<td>D</td>
<td>ST412</td>
<td></td>
<td></td>
<td>Lzd 64 &gt;256, 96 &gt;32 512 &gt;256 &gt;256 1 &gt;256 &gt;32 &gt;32</td>
</tr>
<tr>
<td>7</td>
<td>E. faecium 51/426</td>
<td>No</td>
<td>December 2011</td>
<td>30</td>
<td>Urine</td>
<td>E</td>
<td>ST38</td>
<td></td>
<td></td>
<td>Lzd 2 &lt;0.25 0.5 8 1 8</td>
</tr>
</tbody>
</table>

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*a* ICU, intensive care unit; Lzd, linezolid; VAN, vancomycin; TEC, teicoplanin; PEN, penicillin; AMP, ampicillin; ERY, erythromycin; TET, tetracycline; CHL, chloramphenicol; CIP, ciprofloxacin; LEV, levofloxacin. NA, data not available. Gray shading represents resistance values; boldface type represents intermediate values.

*b* Strains 50/515 and 51/426 corresponding to the linezolid-susceptible E. faecalis and E. faecium control strains were recovered from clinical specimens obtained from other patients who were hospitalized at the same institution.
resistance to linezolid in VRE strains, and it strengthens the idea that combination therapies with ampicillin plus an aminoglycoside can still be good therapeutic options for serious enterococcal infections.

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We declare that we have no conflicts of interest.

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