Risk Factors Associated with the Development of Infection with Linezolid- and Vancomycin-Resistant *Enterococcus faecium*

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This retrospective cohort study revealed that linezolid resistance in vancomycin-resistant *Enterococcus faecium* was dependent on prior linezolid exposure and duration of linezolid therapy. These strains of *E. faecium* were resistant to the entire class of oxazolidinones.

Vancomycin-resistant *Enterococcus* species were first reported in 1988, and the prevalence of resistance has increased from 0.3% of isolates during 1989–1993 to 47% [1]. Linezolid is a new oxazolidinone antibiotic that was approved by the US Food and Drug Administration in April 2000 for treatment of infections caused by vancomycin-resistant *Enterococcus faecium* (VREF), including cases with concurrent bacteremia [2]. Linezolid was effective in treating 67% of cases of VREF infection and was presumed to be less likely to induce resistance than are some older agents because of its synthetic derivation [2]. The development of resistance to linezolid during the manufacturer’s compassionate-use program was reported in 9 of 501 patients with enterococcal infections [3]. Patients infected with organisms that developed resistance to linezolid were treated for 4–6 weeks for *E. faecium* bacteremia and had abscesses, long-standing indwelling devices that could not be removed, or endocarditis [3]. In some cases, low doses of linezolid have also been incriminated as a risk factor for development of resistance [4]. The emergence of resistance to linezolid was recently detected in 5 patients at our network of 3 Chicago hospitals (University of Illinois at Chicago Medical Center, Westside Veterans Administration Hospital, and University of Chicago Hospital) [5]. The present study was undertaken to determine the risk factors associated with the development of resistance to linezolid in patients infected with VREF.

**Methods.** Approval from the institutional review boards was obtained before initiation of this case-control study. The study could only be performed at 2 of the 3 hospitals (University of Illinois at Chicago Medical Center and Westside Veterans Administration Hospital) at which linezolid-resistant VREF (LR-VREF) was initially detected, so we included data from only 4 of the 5 previously reported cases mentioned above [5]. Data were obtained retrospectively by a review of the charts for all patients treated with linezolid at our institutions from May 2000 through April 2001. Patients were excluded from the study if they were <18 years of age, if they had been treated with linezolid for ≈72 h, or if they had not received linezolid for a documented VREF infection. Case patients were those who developed infections with LR-VREF, and control patients were patients with VREF infections.

For each patient, we obtained data about demographic characteristics, antimicrobial use (including specific agents), receipt of cancer chemotherapy, date of last chemotherapy cycle, presence of any indwelling catheters, receipt of parenteral nutrition, receipt of assisted ventilation, receipt of hemodialysis, hospital location at the time of VREF isolation, site of infection, antimicrobial therapy received during hospitalization, and use of corticosteroids within 14 days of organism isolation. The total duration of hospitalization and the length of linezolid therapy were calculated.

Linezolid susceptibility testing of VREF isolates was not routinely performed at our institution in 2000. However, the documentation of LR-VREF resulted in the implementation of linezolid susceptibility testing (by use of Etest strips [AB Biodisk]) for all isolates of VREF recovered in 2001. Susceptibility to the investigational drug oxazolidinone, AZD 2563 (AstraZeneca Pharmaceuticals), was tested by use of an agar-dilution MIC method.

**Results.** Thirty-four patients were identified. Four patients were excluded from the study because linezolid had been prescribed for treatment of methicillin-resistant *Staphylococcus aureus* (2 patients), multidrug-resistant tuberculosis (1 patient), or an undocumented infection (1 patient). A summary of the specific characteristics of and clinical outcomes for each patient...
TABLE 1. Demographic characteristics of case patients and control patients in a study of linezolid- and vancomycin-resistant Enterococcus faecium.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Case patients</th>
<th>Control patients</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean years ± SD</td>
<td>49 ± 30</td>
<td>52 ± 15</td>
<td>.88</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td>.32</td>
</tr>
<tr>
<td>Male</td>
<td>4 (100)</td>
<td>19 (73)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>0 (0)</td>
<td>7 (27)</td>
<td></td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>2 (50)</td>
<td>5 (19)</td>
<td>.19</td>
</tr>
<tr>
<td>Black</td>
<td>1 (25)</td>
<td>16 (62)</td>
<td>.18</td>
</tr>
<tr>
<td>Hispanic</td>
<td>1 (25)</td>
<td>5 (19)</td>
<td>.44</td>
</tr>
<tr>
<td>Underlying condition or risk factor</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malignancy</td>
<td>3 (75)</td>
<td>8 (31)</td>
<td>.11</td>
</tr>
<tr>
<td>Receipt of transplant</td>
<td>3 (75)</td>
<td>9 (35)</td>
<td>.14</td>
</tr>
<tr>
<td>Abdominal surgery</td>
<td>1 (25)</td>
<td>4 (15)</td>
<td>.42</td>
</tr>
<tr>
<td>HIV infection</td>
<td>0 (0)</td>
<td>3 (12)</td>
<td>.64</td>
</tr>
<tr>
<td>Diabetes</td>
<td>0 (0)</td>
<td>7 (27)</td>
<td>.32</td>
</tr>
<tr>
<td>Congestive heart failure</td>
<td>1 (25)</td>
<td>3 (12)</td>
<td>.38</td>
</tr>
<tr>
<td>No. of comorbidities</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;2</td>
<td>3 (75)</td>
<td>11 (42)</td>
<td>.22</td>
</tr>
<tr>
<td>1 or 2</td>
<td>1 (25)</td>
<td>14 (54)</td>
<td>.24</td>
</tr>
<tr>
<td>0</td>
<td>0 (0)</td>
<td>1 (4)</td>
<td>.87</td>
</tr>
</tbody>
</table>

**NOTE.** Data are no. (%) of patients, unless otherwise indicated.

With LR-VREF was published elsewhere [5]. There were no statistically significant differences between case and control patients with regard to demographic characteristics, underlying conditions or risk factors, and the number of comorbidities (table 1). The most common risk factors in both groups were receipt of a transplant and the presence of >2 comorbidities.

The incidence of admission to the intensive care unit was similar for case patients (100%) and control patients (77%). Use of parenteral nutrition, hemodialysis, and assisted ventilation was also similar for the 2 groups (table 2). The frequency of antimicrobial use before admission to the hospital was similar among case patients (2 of 4) and control patients (9 of 26). However, both of these 2 case patients received linezolid before admission, whereas only 1 control patient was treated with linezolid before admission (P = .04). The specific duration of linezolid use before admission to the hospital could not be ascertained. All patients who received antimicrobials before admission received ≥2 agents, and 50% of the patients received 3 agents. The incidence of steroid use 14 days before VREF isolation was significantly higher among case patients than it was among control patients (P = .04).

The use of cancer chemotherapy was similar for both groups. The number of exposures to antimicrobials was significantly higher among case patients (median, 9.5; range, 7–10) than it was among control patients (median, 5.5; range, 1–11; P = .046). In addition, the median duration of linezolid therapy was significantly higher for case patients (38 days) than it was for control patients (11 days; P = .04). The mean length of hospitalization was longer for case patients (63 days) than it was for control patients (46 days), but this difference was not statistically significant.

Steroid use 14 days before VREF isolation, exposure to multiple antimicrobials, use of linezolid before admission to the hospital, and longer duration of linezolid therapy were identified as risk factors associated with the development of linezolid resistance by univariate analysis. However, multivariable logistic regression demonstrated that exposure to linezolid therapy before hospital admission was independently associated with an increased risk of developing an LR-VREF infection (OR, 22.9; 95% CI, 1.39–379).

MICs of linezolid were 8–64 μg/mL in these isolates. MICs of AZD 2563 were elevated in parallel (range, 4–16 μg/mL) among all LR-VREF isolates (R. Jones, personal communication).

**DISCUSSION**

Linezolid was the first oxazolidinone antibiotic to be approved for use in patients in the United States and Europe. Other members of this class are in various stages of preclinical development at several companies. These agents are bacteriostatic against enterococci and staphylococci, and they exert their antibacterial effect by interfering with bacterial protein synthesis. They do this by binding to the 23S rRNA of the 50S subunit on the bacterial ribosome. In vitro experiments show that selection of resistance is relatively difficult to achieve, with resistant mutants occurring at a frequency of 10^-10 among staphylococci [6]. When LR-VREF have been studied by use of molecular methods, these in vitro mutants have been found to have a characteristic mutation in the central loop of domain V of the 23S ribosome, G2576U [7]. This is thought to be the site on the ribosome where linezolid exerts its effect [8]. This mutation has been previously described in 2 VREF isolates recovered from 2 separate patients in whom pathogens developed linezolid resistance during therapy [3]. In addition, G2576U has occurred in both enterococci and S. aureus selected for oxazolidinone resistance in the laboratory [7].

Emergence of linezolid resistance occurred infrequently among VREF isolates recovered during the compassionate-use program (1.8% of isolates). The true incidence of emergence of resistance at the network of 3 Chicago hospitals is not known, because, in 4 of the 5 cases described in our earlier report [5], we did not obtain linezolid-susceptible isolates before therapy to compare with our linezolid-resistant isolates. We are presently studying this issue in more detail by prospectively
Table 2. Characteristics of case patients and control patients at hospital admission in a study of linezolid- and vancomycin-resistant Enterococcus faecium.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Case patients (n = 4)</th>
<th>Control patients (n = 26)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Admission to the intensive care unit</td>
<td>4 (100)</td>
<td>20 (77)</td>
<td>.38</td>
</tr>
<tr>
<td>Receipt of parenteral nutrition</td>
<td>1 (25)</td>
<td>11 (42)</td>
<td>.36</td>
</tr>
<tr>
<td>Receipt of hemodialysis</td>
<td>1 (25)</td>
<td>6 (23)</td>
<td>.38</td>
</tr>
<tr>
<td>Receipt of assisted ventilation</td>
<td>2 (50)</td>
<td>12 (46)</td>
<td>.40</td>
</tr>
<tr>
<td>Antimicrobial use before admission</td>
<td>2 (50)</td>
<td>9 (35)</td>
<td>.34</td>
</tr>
<tr>
<td>Linezolid use before admission</td>
<td>2 (50)</td>
<td>1 (4)</td>
<td>.04a</td>
</tr>
<tr>
<td>Steroid use</td>
<td>3 (75)</td>
<td>5 (19)</td>
<td>.04b</td>
</tr>
<tr>
<td>Receipt of cancer chemotherapy</td>
<td>2 (50)</td>
<td>7 (27)</td>
<td>.28</td>
</tr>
<tr>
<td>Antibiotic exposure, no. of antibiotics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;10</td>
<td>2 (50)</td>
<td>3 (12)</td>
<td></td>
</tr>
<tr>
<td>7–9</td>
<td>2 (50)</td>
<td>5 (19)</td>
<td></td>
</tr>
<tr>
<td>&gt;7</td>
<td>4 (100)</td>
<td>8 (31)</td>
<td>.01a</td>
</tr>
<tr>
<td>4–6</td>
<td>0 (0)</td>
<td>9 (35)</td>
<td></td>
</tr>
<tr>
<td>1–3</td>
<td>0 (0)</td>
<td>9 (35)</td>
<td></td>
</tr>
<tr>
<td>Duration of linezolid therapy, median days (range)</td>
<td>38 (35–66)</td>
<td>11 (3–75)</td>
<td>.04b</td>
</tr>
<tr>
<td>Length of hospitalization, mean days ± SD</td>
<td>63 ± 30</td>
<td>46 ± 34</td>
<td>.38</td>
</tr>
</tbody>
</table>

NOTE. Data are no. (%) of patients, unless otherwise indicated.

* Statistically significant (i.e., P < .05).

observing all patients who receive this agent. In addition, in our stem cell transplantation unit, we have initiated a surveillance study in which rectal swabs are obtained weekly and cultured on selective media. Of 181 clinical isolates of VREF recovered in 2001 from patients who had never received this agent, none were resistant to linezolid. Therefore, we do not believe that de novo resistance to linezolid is common among VREF isolates.

This study expands the findings of our previous report by defining patient risk factors in more detail. At the University of Illinois at Chicago Hospital, linezolid is used primarily to treat serious VREF infections. VREF-infected patients are usually critically ill; 80% of treated patients received therapy in an intensive care unit, most had multiple comorbidities, and the mean length of hospital stay was 46 days, whereas the mean length of stay for all patients at our hospital during calendar year 2000 was 5.1 days.

The most important risk factors identified in our study were receipt of multiple antibiotics and a protracted course of linezolid therapy. A recent report about linezolid-resistant S. aureus was instructive [9] in that the affected patient also received a protracted course of linezolid (~30 days). Sequencing of rRNA from this patient’s isolate also confirmed the identical target site mutation.

Duration of therapy has also been shown to be the major risk factor for bone marrow suppression due to linezolid use [10]. We do not have comprehensive data on marrow toxicity for our patients, because that was not the primary focus of our study. In addition, the majority of our patients were treated in an intensive care unit and had multiple comorbidities, which makes assessment of this factor extremely difficult.

Patients who were previously exposed to linezolid and/or who received this agent for long periods of time may be at significant risk for emergence of linezolid resistance. In addition, these organisms may be transmitted from patient to patient in the hospital: nosocomial spread of LR-VREF in a transplant unit has recently been described [11]. To date, all of our tested linezolid-resistant isolates have remained susceptible to quinupristin/dalfopristin, so this agent may be a reasonable alternative therapy for patients infected with LR-VREF. However, emergence of in vitro resistance to quinupristin/dalfopristin among VREF has also been observed in several prospective studies; thus, we encourage clinicians to perform susceptibility testing for all isolates recovered from patients while they are receiving antimicrobial therapy for serious gram-positive infections [12, 13].

Acknowledgments

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References

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