Clinical Outbreak of Linezolid-Resistant Staphylococcus aureus in an Intensive Care Unit

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Context  Linezolid resistance is extremely uncommon in Staphylococcus aureus.

Objective  To report an outbreak with linezolid and methicillin-resistant S aureus (LRSA) in an intensive care department and the effective control measures taken.

Design, Setting, and Patients  Outbreak study of consecutive critically ill patients colonized and/or infected with LRSA at an intensive care department of a 1000-bed tertiary care university teaching hospital in Madrid, Spain. Patients were placed under strict contact isolation. Daily updates of outbreak data and recommendations for the use of linezolid were issued. Extensive environmental sampling and screening of the hands of health care workers were performed.

Main Outcome Measures  Linezolid use and clinical and epidemiological characteristics and outcomes using minimal inhibitory concentrations, pulsed-field gel electrophoresis, and polymerase chain reaction of LRSA isolates.

Results  Between April 13 and June 26, 2008, 12 patients with LRSA were identified. In 6 patients, LRSA caused ventilator-associated pneumonia and in 3 patients it caused bacteremia. Isolates were susceptible to trimethoprim-sulfamethoxazole, glycopeptides, tigecycline, and daptomycin. Genotyping identified 1 predominant clone and 3 other types. Cfr-mediated linezolid resistance was demonstrated in all isolates. Potential hospital staff carriers and environmental samples were negative except for one. Six patients died, 5 of them in the intensive care unit, with 1 death attributed to LRSA infection. Linezolid use decreased from 202 defined daily doses in April 2008 to 25 defined daily doses in July 2008. Between July 2008 and April 2010, no new cases have been identified in the weekly surveillance cultures or diagnostic samples.

Conclusions  The first clinical outbreak, to our knowledge, with LRSA mediated by the cfr gene developed at our center, was associated with nosocomial transmission and extensive usage of linezolid. Reduction of linezolid use and infection-control measures were associated with the termination of the outbreak.

JAMA. 2010;303(22):2260-2264 www.jama.com

For editorial comment see p 2293.
has been shown to confer linezolid-resistance to *S aureus* and *S epidermidis*. Nosocomial outbreaks with linezolid-resistant *E faecium*, *E faecalis*, and *S epidermidis* have been reported. Linezolid-resistant *S aureus* remains uncommon. We describe the first outbreak, to our knowledge, with linezolid- and methicillin-resistant *S aureus* (LRSA), and the infection-control measures that were applied.

**METHODS**

Hospital Clínic San Carlos is a 1000-bed tertiary care university teaching hospital with referral functions located in Madrid, Spain. The intensive care department consists of a neurotrauma unit (unit 1 has 14 beds), a mixed medical-surgical unit (unit 2 has 18 beds), and a cardiovascular unit (unit 3 has 14 beds). The mean length of stay in 2008 was 4.6 days, with a bed occupancy rate of 92.2%. There was a 2.4 patient-to-nurse ratio during the day shifts and 2.7 during the night shift.

Patients with LRSA in any of the 3 intensive care units (ICUs), detected between April 13 and June 26, 2008, were included in the study. The characteristics of the patients were recorded and analyzed with SPSS statistical software version 15.0 (SPSS Inc, Chicago, Illinois). All LRSA infections were reassessed for diagnostic criteria. Linezolid use in our department from November 2007 until May 2009 was reviewed. The local ethics review board reviewed and approved the study protocol and waived retrospective informed consent.

The preventive medicine service MRSA surveillance program screens the nose, pharynx, and axillae of all ICU patients with positive diagnostic cultures weekly. Local guidelines reinforce hand washing; use of gloves, masks, and disposable gowns; daily 4% chlorhexidine body wash; and 5 days of intranasal mupirocin ointment for management of proven nasal carriers. After decolonization treatment, patients are sampled weekly at the nose, throat, axillae, perineum, and groin and kept under strict barrier precautions until 3 consecutive negative weekly surveillance samples are obtained.

Patients with LRSA were placed in isolation rooms under strict contact precautions. Concurrent cases were kept in a separate 4-bed ICU room under the care of dedicated nursing staff. Daily updates of outbreak data were provided and discussed on clinical rounds in each unit. Extensive cleaning and disinfection of the patient area (sodium hypochlorite, 1.000 ppm) and equipment (2% quaternary ammonium compound; Instrunet FA, Inibsa, Spain) were performed daily and after patient discharge.

Diagnostic and surveillance samples were processed according to standard methods. Minimal inhibitory concentrations (MICs) of linezolid in diagnostic samples were routinely determined by Etest (Abbiodisk, Solna, Sweden). Resistance was defined as greater than 4 mg/L. *S aureus* in surveillance samples is routinely tested by disk diffusion for oxacillin, mupirocin, fusidic acid, rifampicin, fosfomycin, and trimethoprim plus sulfamethoxazole. Linezolid resistance was studied by Etest during the outbreak. In all outbreak samples, susceptibility for linezolid, chloramphenicol, erythromycin, tigecycline, teicoplanin, clindamycin, and vancomycin was addi-
tially studied by agar dilution, while trimethoprim plus sulfamethoxazole and daptomycin were studied by Etest.

LRSA isolates were genotyped by pulsed-field gel electrophoresis using the methods of Murchan et al (with minor modifications). The agarose plugs were pretreated with proteinase K and lysozyme prior to digestion with Smal. The switch time was 5 to 40 seconds for 20 hours. The dendrogram was constructed using the dice correlation coefficient and the unweighted pair group method with a 3% band tolerance for the arithmetic mean. A cutoff for similarity of 80% and the criterion of a difference of 6 or less bands defined a cluster; a difference of 3 or less bands defined a probable outbreak strain.

Absence of the G2576T mutation was determined by sequencing the 5 copies of the domain V of 23S ribosomal RNA in 1 isolate per clone. Presence of the cfr gene was studied by polymerase chain reaction in all isolates. Ninety-one randomly selected surfaces were sampled (bedside equipment, furniture, clinical devices, and the nursing station). The hands of 47 health care workers of all categories from the ICU and external staff were sampled at declaration of the outbreak at week 5 (Figure). No indication for nasal swabs in LRSA was found in the literature. Environmental samples were processed according to standard methods.

Table 1. Characteristics of Patients With Linezolid- and Methicillin-Resistant Staphylococcus aureus (LRSA) (N = 12)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>No. (%) of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male sex</td>
<td>8 (66.7)</td>
</tr>
<tr>
<td>Age, mean (SD), y</td>
<td>58.5 (13)</td>
</tr>
<tr>
<td>APACHE II score, mean (SD)</td>
<td>17 (10)</td>
</tr>
<tr>
<td>Diagnostic group</td>
<td></td>
</tr>
<tr>
<td>Medical</td>
<td>8 (66.7)</td>
</tr>
<tr>
<td>Surgical</td>
<td>3 (25)</td>
</tr>
<tr>
<td>Trauma</td>
<td>1 (8.3)</td>
</tr>
<tr>
<td>Cancer</td>
<td>4 (33.3)</td>
</tr>
<tr>
<td>In ICU on day of index culture, mean (SD)</td>
<td>34.1 (19.1)</td>
</tr>
<tr>
<td>SOFA score, mean (SD)</td>
<td>5.2 (2.6)</td>
</tr>
<tr>
<td>Prior antibiotics</td>
<td></td>
</tr>
<tr>
<td>Broad-spectrum &gt;5 d</td>
<td>12 (100)</td>
</tr>
<tr>
<td>Fluoroquinolones</td>
<td>6 (50)</td>
</tr>
<tr>
<td>Linezolid</td>
<td>11 (91.7)</td>
</tr>
<tr>
<td>Length of use, median (IQR), d</td>
<td>7.5 (6-18.5)</td>
</tr>
<tr>
<td>Total dose, median (IQR), mg</td>
<td>9000 (7200-22 000)</td>
</tr>
<tr>
<td>Prior or concomitant LSSA</td>
<td>11 (91.7)</td>
</tr>
<tr>
<td>Time from LSSA to LRSA, median (IQR), d</td>
<td>10 (2-24)</td>
</tr>
<tr>
<td>Total length of stay, mean (SD), ICU</td>
<td>60.7 (37.8)</td>
</tr>
<tr>
<td>Mortality</td>
<td></td>
</tr>
<tr>
<td>ICU</td>
<td>5 (41.7)</td>
</tr>
<tr>
<td>Hospital</td>
<td>6 (50)</td>
</tr>
</tbody>
</table>

Abbreviations: APACHE, Acute Physiology and Chronic Health Evaluation; ICU, intensive care unit; IQR, interquartile range; LSSA, linezolid-susceptible methicillin-resistant S aureus; SOFA, sepsis-related organ failure assessment.

RESULTS

Between April 13 and June 26, 2008, 12 patients with LRSA were identified in the intensive care department (Table 1 and Table 2 and the Figure). At index culture, all patients required mechanical ventilation, central venous and indwelling urinary catheters, and total parenteral nutrition. Infection with LRSA was associated with moderate organ dysfunction. Two patients (22.2%) infected with LRSA and all 3 patients colonized with LRSA died. Patient 8 (Table 2) had severe acute respiratory distress syndrome and died 17 days after index culture. This patient experienced clinical and microbiological resolution after diagnosis of LRSA ventilator-associated pneumonia, but developed severe acute respiratory distress syndrome. Therefore, death was attributed to LRSA infection. Antibiotic therapy for LRSA infections was associated with a favorable clinical response in all cases. Total ICU consumption of linezolid decreased from 404 600 mg vials (202 defined daily doses) in April to 50 mg vials (25 defined daily doses) in July 2008. Per patient-day usage decreased from 0.31 vials in April 2008 to 0.04 vials in August 2008.

LRSA index cultures were obtained in patients on June 9 on a general surgical (third floor), June 12 on the orthopedic ward (fifth floor), and June 26 on the neurosurgical ward (sixth floor). Two patients had been discharged 14 and 66 days earlier after ICU stays of 4 and 20 days, respectively.

The linezolid MIC50 was 32 mg/L (range, 16-32 mg/L). All isolates were resistant to chloramphenicol (MIC50 of 128 mg/L), clindamycin (MIC50 >512 mg/L), and erythromycin (MIC50 of 512 mg/L) except clone C (erythromycin MIC of 0.5 mg/L), which had reduced susceptibility to glycopeptides (vancomycin MIC50 of 2 mg/L). All isolates were susceptible to tigecycline and daptomycin. Surveillance isolates displayed low-level resistance to mupirocin, except in patient 5 (Table 2), who received intranasal fusidic acid.

Pulsed-field gel electrophoresis showed 3 different banding patterns. A predominant clone A was identified in 11 patients, sequentially involving all 3 units of the department (Figure). The environmental sample showed clone B and patient 5 displayed clone C. No patient harbored more than 1 LRSA clone. Two of the 3 non-ICU patients (who had a prior ICU stay) had clone A while the third patient (without prior ICU stay) had a different clone D. Investigation of the mechanism of resistance ruled out the G2576T mutation and confirmed the presence of the cfr gene in all isolates. The cfr gene was also present in 2 of the 3 linezolid-resistant coagulase-negative staphylococci.

Fifteen of 91 surface samples (16.5%) were positive. Potential pathogens were found in 7 samples (7.7%); 5 samples (5.5%) showed high microbial counts and 1 sample (1.1%) grew LRSA obtained from an intravenous catheter connector. Because of the low level of environmental contamination during the outbreak, closure of the 3 units of the ICU was not deemed necessary. LRSA was not isolated from the hands of health care workers. The total duration of the outbreak was 17 weeks. Since June 27, 2008, no new cases of LRSA have been detected in the weekly surveillance cultures or diagnostic samples.

COMMENT

The outbreak involving 12 patients in the ICU is the first, to our knowledge, with LRSA to be reported, and the first
with \textit{cfr} gene–mediated linezolid resistance. LRSA is extremely uncommon in Spain\textsuperscript{19} and worldwide.\textsuperscript{20,21} Only 8 cases from the United States,\textsuperscript{9,15,28-32} 2 cases from Germany,\textsuperscript{33,34} and 1 case each from the United Kingdom,\textsuperscript{35} Brazil,\textsuperscript{36} and Colombia\textsuperscript{37} have been reported. The apparent risk factor is prior administration of linezolid.

In outpatients, durations of therapy of months\textsuperscript{34,36} and even years\textsuperscript{29,32} were described. Inpatients received approximately 50 defined daily doses of linezolid.\textsuperscript{9,28,30,31,33,35} Therefore, restriction of linezolid seemed a correct control measure at our institution. Importantly, however, \textit{cfr}-mediated resistance was documented after patients received therapy for a short duration during the outbreak. Resistance occurred after only 2 doses in the Colombian isolate\textsuperscript{37} and without prior exposure in the first strain in the United States.\textsuperscript{15} This suggests that, although the \textit{cfr} gene certainly confers a survival advantage, additional risk factors, like cross-resistance or transferal of the \textit{cfr}-harboring plasmid from an undetected microorganism, like a coagulase-negative \textit{Staphylococcus}, may have concurred.

The clinical spectrum of LRSA infections varies widely.\textsuperscript{9,15,28,29,33,35-37} Our outbreak occurred in a subgroup of high-risk critically ill patients with prolonged ICU stays and risk factors for antibiotic resistance. Although they developed potentially severe invasive infections, associated organ dysfunction\textsuperscript{27} was moderate, all infections improved, and we attributed only 1 death to LRSA infection.

Although LRSA was not detected on ICU staff, nasal swabs were not used. Negative surveillance cultures of 7 infected patients indicate that exogenous infection developed, implying breaks in hygiene. Conversely, LRSA colonization, detected in 3 of 6 ventilator-associated pneumonia episodes, suggests secondary endogenous ventilator-associated pneumonia development. The 5 patients carrying LRSA constitute the only identified reservoir for clone A. Therefore, infection-control strategies (without an environmental reservoir) may have facilitated the rapid control of the outbreak. In addition, our MRSA sur-

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Hospital Unit\textsuperscript{a}</th>
<th>No. of Days in ICU at Index Culture</th>
<th>Infection</th>
<th>Culture Site for LRSA</th>
<th>Antibiotic Therapy for LRSA Infection</th>
<th>Total Prior Dose of Linezolid, mg</th>
<th>ICU Outcome or Cause of Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>30</td>
<td>Primary bacteremia</td>
<td>Blood</td>
<td>Negative</td>
<td>Vancomycin</td>
<td>25 200</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>13</td>
<td>Primary bacteremia</td>
<td>Blood</td>
<td>Negative</td>
<td>Vancomycin</td>
<td>9600</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>11</td>
<td>Primary bacteremia</td>
<td>Blood</td>
<td>Negative</td>
<td>Vancomycin</td>
<td>7200</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>26</td>
<td>VAP</td>
<td>Bronchial aspirate + blood</td>
<td>Negative</td>
<td>Tigeccycline</td>
<td>8400</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>69</td>
<td>VAP</td>
<td>Bronchial aspirate</td>
<td>Positive\textsuperscript{c}</td>
<td>Tigeccycline</td>
<td>38 400</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>19</td>
<td>VAP</td>
<td>Bronchial aspirate</td>
<td>Positive\textsuperscript{d}</td>
<td>Tigeccycline</td>
<td>13 200</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td>48</td>
<td>Colonization</td>
<td>Negative</td>
<td>Positive\textsuperscript{e}</td>
<td>0</td>
<td>Died; had prior non-LRSA infection</td>
</tr>
<tr>
<td>8</td>
<td>2</td>
<td>24</td>
<td>Colonization\textsuperscript{f}</td>
<td>Blood</td>
<td>Positive\textsuperscript{g}</td>
<td>10 800</td>
<td>Died; VAP (\textit{Pseudomonas aeruginosa}) 17 d after index culture</td>
</tr>
<tr>
<td>9</td>
<td>2</td>
<td>42</td>
<td>VAP</td>
<td>Bronchial aspirate + catheter tip</td>
<td>Negative</td>
<td>Tigeccycline</td>
<td>7200</td>
</tr>
<tr>
<td>10</td>
<td>3</td>
<td>50</td>
<td>VAP</td>
<td>Bronchial aspirate</td>
<td>Negative</td>
<td>Vancomycin</td>
<td>28 800</td>
</tr>
<tr>
<td>11</td>
<td>2</td>
<td>18</td>
<td>Colonization</td>
<td>Bronchial aspirate + catheter tip</td>
<td>Negative</td>
<td>6000</td>
<td>Died; cardiogenic shock after heart surgery</td>
</tr>
<tr>
<td>12</td>
<td>3</td>
<td>59</td>
<td>VAP</td>
<td>Bronchial aspirate</td>
<td>Positive\textsuperscript{h}</td>
<td>Tigeccycline</td>
<td>7200</td>
</tr>
</tbody>
</table>

Abbreviations: ICU, intensive care unit; VAP, ventilator-associated pneumonia.
\textsuperscript{a}Unit 1 indicates neurotrauma (14 beds); unit 2, medical-surgical (18 beds); and unit 3, cardiovascular (14 beds).
\textsuperscript{b}Obtained for all patients.
\textsuperscript{c}From nasal, oropharyngeal, axillary, and perineal swabs.
\textsuperscript{d}From nasal, oropharyngeal, and groin swabs.
\textsuperscript{e}From oropharyngeal swabs.
\textsuperscript{f}Positive blood sample drawn through catheter. Simultaneous peripheral blood cultures and catheter tip were negative so possible line sepsis.
\textsuperscript{g}From nasal, oropharyngeal, axillary, groin, and perineal swabs.
\textsuperscript{h}Initial therapy with vancomycin; tigeccycline rescue treatment added later.
\textsuperscript{i}From nasal, oropharyngeal, groin, and perineal swabs.

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veillance program allows early detection so we consider undetected LRSA cases unlikely. In view of the current increase of community-acquired MRSA infections, our data have important implications for both hospitalized patients and outpatients. Combination therapy with rifampin and fusidic acid still needs to be evaluated in the clinical setting. 38

In conclusion, our data suggest that a combination of the emergence of linezolid resistance in S aureus with clonal spread and use of linezolid was responsible for the LRSA outbreak that occurred at our institution. The successful early control of the LRSA outbreak by infection-control measures and reduction of linezolid use is in line with these 2 assumptions.

Author Contributions: Dr Sánchez García and his coauthors had full access to all of the data in the study and Dr Sánchez García takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Sánchez García, Peláez, Tolón, Domínguez, Fieras, Picazo. Acquisition of data: De la Torre, Morales, Peláez, Tolón, Domínguez, Candel, Andrade, Arribi, García, Martinez Sagasti, Fatjó. Analysis and interpretation of data: Sánchez García, De la Torre, Morales, Peláez, Tolón, Domínguez, Candel, Andrade, Arribi, García, Martinez Sagasti, Fieras, Picazo. Drafting of the manuscript: Sánchez García, De la Torre, Morales, Peláez, Candel, Andrade, Arribi, García, Martinez Sagasti, Fieras. Critical revision of the manuscript for important intellectual content: Sánchez García, De la Torre, Morales, Peláez, Tolón, Domínguez, Candel, Andrade, Arribi, García, Martinez Sagasti, Fatjó. Statistical analysis: Sánchez García, Fieras. Administrative, technical, or material support: De la Torre, Morales, Peláez, Tolón, Domínguez, Candel, Andrade, Arribi, García, Martinez Sagasti, Fieras. Study supervision: Sánchez García, De la Torre, Domínguez, Candel, Andrade, Arribi, García, Martinez Sagasti, Fatjó.

Financial Disclosures: None reported.

Funding/Support: Dr Morales has a research contract of the Fundación para la Investigación Bio médica del Hospital Clínico San Carlos.

Role of the Sponsor: The sponsor had no role in the design and conduct of the study; data collection, management, analysis, and interpretation of the data; and preparation, review, or approval of the manuscript.

Additional Contributions: We thank Maria Paz Pacheco, PharmD, from the Pharmacy Department for providing the data on linezolid use. Dr Paz Pacheco received no financial compensation for her contribution. We also thank the nursing staff of the intensive care department for the swift implementation of infection-control measures that lead to the rapid control of the outbreak.

REFERENCES


OUTBREAK WITH LINEZOLID-RESISTANT S AUREUS

2264 JAMA, June 9, 2010—Vol 303, No 22 (Reprinted)

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