Development of Oxacillin Resistance in a Patient with Recurrent *Staphylococcus aureus* Bacteremia

Eileen M. Burd, Mohammad Tauqueer Alam, Karla D. Passalacqua, Ameeta S. Kalokhe, Molly E. Eaton, Sarah W. Satola, Colleen S. Kraft, Timothy D. Read

Department of Pathology and Laboratory Medicine, Emory University School of Medicine, Atlanta, Georgia, USA; Department of Medicine, Division of Infectious Diseases, Emory University School of Medicine, Atlanta, Georgia, USA

Whole-genome sequencing was used to compare longitudinal isolates of *Staphylococcus aureus* that developed resistance to oxacillin (MIC up to 16 μg/ml). The meca gene was absent. A novel 5-bp TATCC frameshift insertion in a gene encoding an ABC transporter similar to that of the teichoic acid translocation ATP-binding protein TagH and a 3-bp GCT nonframeshift insertion in the pdhA pyruvate dehydrogenase gene were detected in the oxacillin-resistant isolates.

CASE REPORT

An 81-year-old man with an automated implantable cardioverter defibrillator (AICD) and pacemaker (PM) presented with 1 day of fever and chills without localizing symptoms. His past medical history was significant for diabetes mellitus, deep vein thrombosis, coronary atherosclerotic disease with remote bypass grafting, and ischemic cardiomyopathy (ICM) with a third-degree atrial flutter and an atrioventricular block requiring AICD/PM. His initial PM had been implanted 12 years previously in the left chest wall. The PM generator (and not the PM leads) had been extracted 4 years previously due to malfunction and replaced with an AICD/PM in his right chest wall.

Two months before the current admission, he received a 4-week course of intravenous nafcillin at an outside facility for methicillin-susceptible *Staphylococcus aureus* (MSSA) bacteremia resulting from cellulitis of his left toe (see Table 1 for details). A transthoracic echo (TTE) and tagged nuclear scan of the toe were negative for endocarditis and osteomyelitis, respectively. Within 4 days of treatment, he had defervesced and demonstrated clinical improvement, and blood cultures were negative for MSSA. Two days after stopping the treatment, however, the fever resumed and he was noted to have recurrent MSSA bacteremia identified by the BD Phoenix automated microbiology system (BD Biosciences, Franklin Lakes NJ). Transesophageal echocardiography (TEE) failed to demonstrate vegetations on the AICD/PM leads or valves. He was prescribed 2 weeks of oral linezolid treatment followed by 2 weeks of cephalixin. His blood cultures again cleared rapidly within 3 days of antibiotic initiation, and he clinically improved. The patient completed the linezolid course, but he self-discontinued the cephalixin (Keflex) after 5 days. He was readmitted to the outside hospital, having had fever over the preceding 24 h but no pain over his spine, joints, or cardiac device site. Physical exam findings were normal, with the exception of a temperature of 102.0°F, a pulse of 100 beats per minute, and an audible 3/6 systolic murmur at the left sternal border. Bilateral AICD/PM pocket sites were without erythema, tenderness, or swelling, and no peripheral stigmata of endocarditis were noted. Blood cultures again grew MSSA, with an oxacillin MIC of 2 μg/ml and cefazolin MIC of <2 μg/ml, and intravenous nafcillin was resumed. A TEE was repeated and demonstrated vegetations on the two right atrial wires. The patient was transferred to our institution for extraction of the cardiac device.

On transfer, repeat blood cultures were negative for bacterial growth. The patient underwent extraction of the AICD/PM and all device leads, with placement of a temporary dual-chamber AICD/PM in the right subclavian vein since the patient was PM dependent. On extraction, cultures from the AICD/PM generator pocket site and device leads were positive for methicillin-resistant *S. aureus* (MRSA), as detected by the MicroScan WalkAway plus (Siemens, Hoffman Estates, IL) automated susceptibility system using the Pos Combo 33 breakpoint panel. The treatment regimen was changed to intravenous vancomycin. After 2 weeks, the AICD/PM was reimplemented and the generator placed in a new, right-sided, subpectoral pocket, and he was discharged with 6 weeks of intravenous vancomycin treatment. At the 6-week follow-up, he denied fever and was clinically well, so the antibiotics were discontinued. At a 4-month reevaluation, he continued to feel well and had no recurrences of fever and no documented bacteremia.

Because the development of oxacillin resistance was noted by the Infectious Disease team caring for the patient, the two MSSA isolates (isolate 1 and isolate 2) from the most recent episodes of bacteremia were available and were obtained from the outside hospital. The MRSA isolate from the pacemaker pocket (isolate 3) was recovered in the clinical microbiology laboratory at our institution. Breakpoint testing for susceptibility to oxacillin and other antibiotics for each of the three isolates was repeated using the Pos Combo 33 Gram-positive identification/susceptibility panels and the MicroScan WalkAway plus instrument using both the Prompt inoculum preparation system and the turbidity standard tech-
Technique for preparation of inocula. MICs were also determined by testing with fresh subcultures on two separate days using Sensititre GPALL1F standard susceptibility MIC plates (Trek Diagnostic Systems, Inc., Cleveland, OH), with manual inoculation and reading of results. Reference broth microdilution was performed in the laboratory of David Lonsway at the Centers for Disease Control and Prevention (CDC) in Atlanta, GA, according to CLSI guidelines. The results of susceptibility testing for these isolates are shown in Table 2. No differences were seen in the MICs obtained by MicroScan whether the Prompt or the turbidity inoculation system was used. We identified a progressive increase in the MICs of oxacillin, ampicillin, penicillin, and cefoxitin for the three isolates. The oxacillin MIC for isolate 3 at 16/µg/ml was 32 times higher than that for pretreatment isolate 1 (0.5/µg/ml indicates susceptibility) and was above the susceptibility breakpoint (≥4/µg/ml indicates resistance). The oxacillin MIC for isolate 2 was variable and tested resistant by MicroScan and the broth microdilution Sensititre panel but susceptible by reference broth microdilution and by the BD Phoenix system at the outside hospital.

Isolate 1 tested resistant to penicillin, with an MIC of 1 to 2/µg/ml, and the MIC was increased approximately 2-fold for isolate 3, with an MIC of 4/µg/ml. Isolate 2 was also resistant to penicillin, but the MIC was variable and tested at 4/µg/ml using the Sensititre panel and at 1/µg/ml by reference broth microdilution. Similarly, all isolates were resistant to ampicillin, but the MIC of ampicillin increased from 1/µg/ml for isolate 1 to 2/µg/ml for isolate 3.

### Table 1: Culture results and treatment time course of our patient

<table>
<thead>
<tr>
<th>Day(s)</th>
<th>Culture</th>
<th>Treatment</th>
<th>Additional note(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, 3</td>
<td>Blood cultures grew MSSA</td>
<td>i.v. nafcillin for 4 wk</td>
<td>Presenting complaint was fever and cellulitis; the source of the bacteremia was left toe cellulitis</td>
</tr>
<tr>
<td>5</td>
<td>Blood cultures showed no growth</td>
<td></td>
<td>By TTE, the EF was 25%; there were no vegetations; a nuclear scan of left foot revealed no osteomyelitis</td>
</tr>
<tr>
<td>36</td>
<td>Blood cultures grew MSSA (isolate 1)</td>
<td>p.o. linezolid for 2 wk followed by p.o. cephalaxin for 5 days (prescribed for 2 wk but self-discontinued by patient)</td>
<td>Presenting complaint was fever; by TEE, the EF was 25%; there were no vegetations</td>
</tr>
<tr>
<td>39</td>
<td>Blood cultures exhibited no growth</td>
<td>i.v. vancomycin plus i.v. piperacillin-tazobactam for 2 days, followed by i.v. cefazolin for 5 days</td>
<td>Presenting complaint was fever; by TTE, the EF was 55%; there was right atrial vegetation on 2 pacer wires and possibly on the wire extending to the right ventricle</td>
</tr>
<tr>
<td>62, 63</td>
<td>Blood cultures grew MSSA (isolate 2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>70</td>
<td>Blood cultures exhibited no growth</td>
<td>i.v. vancomycin for 45 days</td>
<td>All AICD/PM leads and the generator were extracted; a temporary AICD/PM was implanted; on day 85, a permanent PM/AICD was reimplanted; at the 6-wk follow-up, the patient was clinically well and afebrile and exhibited no recurrence of bacteremia; at the 4-mo follow-up, the patient was clinically well and afebrile, and there was no recurrence of bacteremia</td>
</tr>
<tr>
<td>72</td>
<td>PM/AICD lead culture grew MRSA; the right generator pocket culture grew MRSA (isolate 3)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 2: Susceptibility testing results for β-lactam antibiotics

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Test</th>
<th>Oxacillin MIC (µg/ml)</th>
<th>Interpretation</th>
<th>Ampicillin MIC (µg/ml)</th>
<th>Interpretation</th>
<th>Penicillin MIC (µg/ml)</th>
<th>Interpretation</th>
<th>Cefoxitin MIC (µg/ml)</th>
<th>Disk zone (mm)</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>MicroScan</td>
<td>Broth microdilution (Sensititre)</td>
<td>≤0.5</td>
<td>S</td>
<td></td>
<td>0.5</td>
<td>S</td>
<td>1</td>
<td>R</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Reference broth microdilution</td>
<td>0.5</td>
<td>S</td>
<td></td>
<td></td>
<td>2</td>
<td>R</td>
<td>4</td>
<td>25</td>
<td>S</td>
</tr>
<tr>
<td>2</td>
<td>MicroScan</td>
<td>Broth microdilution (Sensititre)</td>
<td>&gt;2</td>
<td>R</td>
<td></td>
<td>4</td>
<td>R</td>
<td>2</td>
<td>R</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Reference broth microdilution</td>
<td>0.25</td>
<td>S</td>
<td></td>
<td></td>
<td>1</td>
<td>4</td>
<td>28</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>MicroScan</td>
<td>Broth microdilution (Sensititre)</td>
<td>&gt;2</td>
<td>R</td>
<td></td>
<td>&gt;4</td>
<td>R</td>
<td>4</td>
<td>R</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Reference broth microdilution</td>
<td>16</td>
<td>R</td>
<td></td>
<td></td>
<td>&gt;2</td>
<td>R</td>
<td>8</td>
<td>19</td>
<td>R</td>
</tr>
</tbody>
</table>

R, resistant; S, susceptible.

In the MicroScan, MICs were the same regardless of inoculation method (Prompt inoculum versus turbidity technique). In the Sensititre assay, testing was done in replicate, with no difference in results.
Whole-genome sequencing was used to compare the three isolates as MLST sequence type 5 (ST5), with identical DNA sequences at all seven loci (2). Low-quality sequences (average Phred quality score, <20) were discarded using a stand-alone version of PRINSEQ v0.20.4 (3), and the remaining reads were processed at all seven loci (2). Low-quality sequences (average Phred quality score, <20) were discarded using a stand-alone version of PRINSEQ v0.20.4 (3), and the remaining reads were processed.

To determine if the three isolates represented the same strain or different strains, they were submitted to ARUP Laboratories (Salt Lake City, UT) for genomic analysis by restriction digestion using the repetitive regions (any repeated unit of a copy number equal to 3 or greater) of ST5, with identical DNA sequences at all seven loci (2). Low-quality sequences (average Phred quality score, <20) were discarded using a stand-alone version of PRINSEQ v0.20.4 (3), and the remaining reads were processed.

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include hyperproduction of blaZ-encoded β-lactamase (8), over-expression or modification of normal constitutive PBP genes (9–11), the presence of a methicillinase (12), or the expression of the divergent mecA homologue mecC (13).

The large census population numbers of bacteria in infections with S. aureus allow them to develop spontaneous mutations during the course of treatment (14). Conditions that support generation of a persistent population of bacteria, such as incubation in the presence of subinhibitory concentrations of an antibiotic at the site of infection, complex or chronic infections requiring a long duration of treatment, or repeated administration of antibiotics because of relapse or reinfection, are particularly conducive to the development of antibiotic-resistant mutants (15, 16).

Identification of the same ABC transporter gene mutation in the two breakthrough isolates supports its involvement in β-lactam MIC increases. Although the interactions are complex and not completely defined, there is evidence that the teichoic acid and peptidoglycan biosynthetic pathways in S. aureus are connected, and there are reports of an as-yet poorly defined functional interaction between teichoic acids and β-lactam antibiotics (17, 18).

That oxacillin resistance was detected in some clinical susceptibility assays undetected in others in the first breakthrough isolate may be related to the ability of the resistance to be induced after several rounds of replication in the presence of antibiotic. The additional mutation detected in the pdhA gene in the second breakthrough isolate was associated with stable oxacillin resistance. We do not have a ready explanation for how this mutation may function to enhance oxacillin resistance. The comparative genomics analysis between resistant S. aureus isolates and the susceptible parent strain described here provides leads for future functional genomics studies to uncover the specific mechanisms underlying the observed emergence of antibiotic resistance.

**Nucleotide sequence accession number.** The raw sequence reads from the project have been deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) database under accession number SRP042337.

**REFERENCES**


