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Eileen Burd, Emory University
Mohammad A. Alam, Emory University
Karla D. Passalacqua, Emory University
Ameeta Kalokhe, Emory University
Molly Eaton, Emory University
Sarah Satola, Emory University
Colleen S Kraft, Emory University
Timothy Read, Emory University

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

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

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

A 61-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. He then underwent a transcatheter aortic valve replacement (TAVR) and aortic valve replacement (AVR) to treat severe aortic valve disease with aortic regurgitation and severe mitral regurgitation. He also underwent coronary artery bypass grafting, and ischemic cardiomyopathy (ICM) with a third-degree atrial flutter and an atrioventricular block requiring AICD/PM. He was an inpatient with fever and positive blood cultures for MSSA, with no other localizing symptoms.

On admission, he denied any history of fever, chills, or any other symptoms of infection. He was afebrile and had a pulse of 100 beats per minute. He had no localizing symptoms of infection, and his physical examination was unremarkable. The patient was admitted to the hospital for further evaluation and treatment.

The patient was treated with intravenous nafcillin for 4 weeks, and then his antibiotics were changed to intravenous vancomycin. Despite this treatment, the patient developed recurrent fever and positive blood cultures for MSSA. A repeat TEE showed vegetations on the 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 reimplanted 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-
nique 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 mg/ml was 32 times higher than that for pretreatment isolate 1 (0.5 mg/ml indicates susceptibility) and was above the susceptibility breakpoint (≥4 mg/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.

<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 TEE, 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 AICD/PM 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>≤0.5</td>
<td>S</td>
<td>0.5</td>
<td>S</td>
<td>1</td>
<td>R</td>
<td>1</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Broth microdilution (Sensititre)</td>
<td>0.5</td>
<td>S</td>
<td>2</td>
<td>R</td>
<td>4</td>
<td>25</td>
<td>S</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>MicroScan</td>
<td>≥2</td>
<td>R</td>
<td>4</td>
<td>R</td>
<td>2</td>
<td>R</td>
<td>4</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Broth microdilution (Sensititre)</td>
<td>0.25</td>
<td>S</td>
<td>1</td>
<td>R</td>
<td>4</td>
<td>28</td>
<td>S</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>MicroScan</td>
<td>≥2</td>
<td>R</td>
<td>≥4</td>
<td>R</td>
<td>4</td>
<td>R</td>
<td>4</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Broth microdilution (Sensititre)</td>
<td>16</td>
<td>R</td>
<td>&gt;2</td>
<td>R</td>
<td>8</td>
<td>19</td>
<td>R</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| a | R, resistant; S, susceptible. |
| a | 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. |
for isolate 2 and to 4 μg/ml for isolate 3. The cefoxitin MIC was susceptible at 4 μg/ml for isolates 1 and 2 but resistant at 8 μg/ml for isolate 3. Cefoxitin results were confirmed by disk diffusion testing. All three isolates remained susceptible, with no increases in MICs, to all other antibiotics tested (chloramphenicol, daptomycin, gentamicin, linezolid, rifampin, trimethoprim sulfamethoxazole, quinupristin-dalfopristin, tetracycline, erythromycin, vancomycin, ciprofloxacin, levofloxacin, moxifloxacin, tigecycline, and clindamycin).

All three isolates tested negative in the Oxoid PBP2' latex agglutination test (Remel Inc., Lenexa, KS) and did not contain the mecA gene, as determined by a laboratory-developed PCR assay (1).

Oxacillin and cefoxitin induction studies were done at the CDC to determine whether prior exposure to these antibiotics could significantly increase the oxacillin or cefoxitin MICs. Suspensions (0.5 McFarland standard) of each isolate were prepared from the edge of the growth around the oxacillin and cefoxitin disk zones. Corresponding suspensions were inoculated onto Mueller-Hinton agar plates with a 1-μg oxacillin disk or a 30-μg cefoxitin disk and incubated at 35°C for 24 h. The resulting zone diameters were measured, and this process was repeated for four consecutive days. A significant change was defined as a change of 3 mm or greater than 5 bp) were excluded from calling SNPs. The nucleotide regions corresponding to the repetitive regions (any repeated unit of a copy number equal to or greater than 5 bp) which were missing from the sequenced strains, and the positions within the repetitive regions (any repeated unit of a copy number equal to or greater than 5 bp) were excluded from calling SNPs.

Comparative analysis of the whole-genome sequences revealed that isolates 2 and 3 differed from their parent strain (isolate 1) by a 5-bp TATCC frameshift insertion mutation in the putative gene encoding an ABC transporter similar to the teichoic acid translocation ATP-binding protein TagH (SA1688 locus in the N315 genome). This led to the production of a truncated protein that was only 87 amino acids long instead of the 505-amino-acid-long wild-type protein (Fig. 1). Isolate 3 had an additional nonframeshift insertion mutation of 3 bp, GCT, at codon 324 in the pyruvate dehydrogenase E1 component alpha subunit gene pdhA (SA0943-1 locus in the N315 genome). In addition to having the nonframeshift insertion mutation of 3 bp, GCT, at codon 324 in the pyruvate dehydrogenase E1 component alpha subunit gene pdhA (SA0943-1 locus in the N315 genome), it also contained the mecA gene. Whole-genome sequence results also confirmed that none of the isolates contained the mecA gene.

Resistance to oxacillin in S. aureus is typically caused by the acquisition of a mobile genetic element, the staphylococcal cassette chromosome mec (SCCmec), which encodes a penicillin-binding protein, PBP2a, that has low binding affinity for methicillin and other β-lactam antibiotics (6, 7). Oxacillin resistance in S. aureus isolates lacking a mecA gene is infrequently reported. Known mechanisms of non-mecA-mediated oxacillin resistance...
include hyperproduction of blaz-encoded β-lactamase (8), overexpression or modification of normal constitutive PBP genes (9–11), the presence of a 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 and 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