Emergence of a Clinical Daptomycin-Resistant Staphylococcus aureus Isolate during Treatment of Methicillin-Resistant Staphylococcus aureus Bacteremia and Osteomyelitis

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The emergence of a clinically daptomycin-resistant Staphylococcus aureus isolate occurred during treatment of methicillin-resistant S. aureus bacteremia and probable vertebral osteomyelitis. The breakthrough isolate was indistinguishable from pretreatment daptomycin-susceptible isolates by pulsed-field gel electrophoresis. Daptomycin nonsusceptibility was confirmed by MIC and time-kill curve analyses.

The Food and Drug Administration (FDA) approved daptomycin in 2003 for the treatment of complicated skin and soft tissue infections (cSSTIs) caused by susceptible strains of Staphylococcus aureus, including methicillin-resistant S. aureus (MRSA) strains, and other gram-positive bacteria (1, 3). In a large worldwide survey, only 2 (0.1%) of 3,202 S. aureus isolates had decreased susceptibility to daptomycin (16). Mangili and colleagues recently reported a case of daptomycin-nonsusceptible MRSA infection (9), but they did not have pretreatment isolates to confirm that resistance to daptomycin emerged during treatment. We report on the emergence of a daptomycin-resistant S. aureus (DRSA) isolate, indistinguishable from pretreatment daptomycin-susceptible isolates by pulsed-field gel electrophoresis (PFGE), in a patient with breakthrough bacteremia during daptomycin treatment.

A 61-year-old man with acute myelogenous leukemia (AML) underwent nonmyeloablative allogeneic hematopoietic stem cell transplantation in April 2003. However, his AML relapsed and he received two donor lymphocyte infusions in September 2003. He subsequently developed gastrointestinal and cutaneous graft-versus-host disease (GVHD) that was treated with prednisone and tacrolimus.

On 16 October 2003, the patient was admitted to hospital with fever and chills. Initial blood cultures yielded MRSA. The isolate was susceptible to vancomycin, gentamicin, chloramphenicol, tetracycline, trimethoprim-sulfamethoxazole, and linezolid and was resistant to erythromycin and levofloxacin. A tunneled catheter was removed. Bacteremia persisted, despite 6 days of therapy with vancomycin and gentamicin. The patient declined further evaluations when persistent AML was found. He was discharged on 22 October and was given linezolid; his platelet count was 40,000/μL. He remained bacteremic through 1 November (isolate A8796). Linezolid was discontinued on 14 November, when his platelet count decreased to 7,000/μL. Blood cultures were sterile on this date.

On 8 December 2003 the patient presented to a clinic with a fever. Blood cultures grew MRSA (isolate A8797), and linezolid treatment was resumed. He was readmitted to hospital on 12 December. Vancomycin and gentamicin were started. A transthoracic echocardiogram demonstrated no vegetations. There was no evidence of upper-extremity thrombophlebitis. A gallium scan showed increased uptake at T11-T12. The findings on magnetic resonance imaging were consistent with osteomyelitis and diskits. The patient remained bacteremic through 15 December (isolate A8798) and was switched to 6 mg/kg of body weight/day of daptomycin in combination with gentamicin (7) at 1 mg/kg every 8 h. His blood cultures on 19 December were sterile. Gentamicin was discontinued on 21 December. The patient was discharged on 24 December to continue daptomycin at 6 mg/kg/day.

A surveillance blood culture on 5 January 2004 grew MRSA (isolate A8799). Blood samples for culture drawn on three subsequent days also grew MRSA. Disk diffusion testing demonstrated nonsusceptibility to daptomycin. His blood cultures cleared after 6 days of linezolid treatment, but he again developed severe thrombocytopenia a week later. His antimicrobial treatment was changed to vancomycin and rifampin for 8 weeks without evidence of breakthrough bacteremia. The regimen was then switched to doxycycline and rifampin. Surveillance blood cultures remained sterile. The patient died from refractory AML in May 2004.

Isolates A8796 to A8799 were further evaluated. The clonal relationships among them were assessed by PFGE with SmaI-macrorestriction genomic DNA (10, 17). Susceptibility to daptomycin (Cubist Pharmaceuticals, Lexington, MA) was studied by the broth macrodilution method. Starting inocula were prepared by using a direct colony suspension (final inoculum, ca. 106 CFU/ml) placed into cation-adjusted Mueller-Hinton broth (Becton Dickinson and Company, Franklin Lakes, NJ) supplemented with calcium to give a final calcium concentration of 50 to 55 mg/liter. S. aureus ATCC 29213 was used as a control. To confirm the reproducibility of the results, suscep-
tibility testing was performed on two separate occasions. To test the bactericidal activity of daptomycin, overnight cultures were diluted 1:10 in cation-adjusted Mueller-Hinton broth (supplemented to a final calcium concentration of 50 to 55 mg/liter) to obtain a starting inoculum of approximately log_{10} 7.50 CFU/ml. Baseline bacterial colony counts were obtained by sampling each culture, performing serial 1:10 dilutions in sterile saline, plating 25-μl aliquots of each dilution in duplicate onto sheep blood agar plates, and incubating the plates at 35°C for 24 h. In patients receiving 6 mg/kg/day of daptomycin, the mean serum steady-state minimum daptomycin concentration is approximately 8.9 μg/ml (12). Therefore, daptomycin at a final concentration of 8 μg/ml was added to each culture; and viable colony counts were assessed at 3, 6, and 24 h of incubation. Time-kill experiments were performed on two separate occasions, and the average log_{10} CFU/ml at each time point was determined for each isolate. The lower limit of detection for this method is log_{10} 2.30 CFU/ml.

The series of S. aureus isolates recovered from the patient were indistinguishable from one another by PFGE (Fig. 1) (17). The MICs of daptomycin for the isolates are presented in Table 1. The daptomycin MIC for S. aureus A8799 was two- to eightfold higher than that for the pretreatment isolates, was above the susceptibility breakpoint (≥1 μg/ml), and, thus, by definition, was daptomycin nonsusceptible (4, 5).

The time-kill experiments with 8 μg/ml daptomycin demonstrated the attenuated killing of the daptomycin-nonsusceptible isolate (A8799) at 3, 6, and 24 h compared to that of the three daptomycin-susceptible isolates that were identical by PFGE (Fig. 2).

The emergence of resistance to daptomycin during treatment was rare at the time of approval of the drug for the treatment of cSSTIs (3), although bacteremic patients were excluded from the study (1). Selection for DRSA strains in vitro was difficult, with predicted resistance rates of <10^{-10} (14), and several studies have found only a few isolates with daptomycin MICs >1 μg/ml (6, 16).

We report on the emergence of a daptomycin-nonsusceptible S. aureus strain during daptomycin therapy that was indistinguishable by PFGE from pretreatment strains. Although a nonsusceptible S. aureus strain was encountered during a phase 2 study of daptomycin, the finding was attributed to underdosing of the drug (5). In the case reported by Mangili and colleagues (9), the patient received 4 mg/kg/day of daptomycin initially, but the dose was increased to 6 mg/kg/day 4 days later; a partial portal vein thrombophlebitis was identified as a probable septic reservoir.

![FIG. 1. Pulsed-field gel electrophoresis. The lanes contain the following from left to right, respectively: marker strain NCTC 8325 (17), S. aureus A8796, S. aureus A8797, S. aureus A8798, and S. aureus A8799.](image)

![FIG. 2. Time-kill curves for S. aureus A8796, A8797, A8798, and A8799 in the presence of 8 μg/ml daptomycin. The data at each time point represent the averages of two experiments.](image)

**TABLE 1. Daptomycin susceptibility testing of an MRSA strain isolated from a patient**

<table>
<thead>
<tr>
<th>Isolate</th>
<th>A8796</th>
<th>A8797</th>
<th>A8798</th>
<th>A8799</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIC (μg/ml)</td>
<td>0.5–1.0</td>
<td>0.25–0.5</td>
<td>0.5</td>
<td>2–4</td>
</tr>
</tbody>
</table>

*a Numbers assigned to the MRSA isolates recovered and in deposit in the Antimicrobial Resistance Laboratory, Division of Infectious Diseases, Beth Israel Deaconess Medical Center.

*b Date that the blood sample for culture that grew the isolate was drawn.

The MIC of daptomycin for each isolate was within 1 doubling dilution for each repetition. The ranges reflect the variation of results obtained in repeated assays. The MIC for the control strain, S. aureus ATCC 29213, was 0.5 μg/ml on both repetitions, which was within the acceptable quality control range for daptomycin (4, 5).
Throughout treatment our patient received 6 mg/kg/day of daptomycin, a dose being actively studied for the treatment of bacteremia and endocarditis (2, 15).

Information on the utility of daptomycin for treatment of osteomyelitis is limited. A rabbit model of MRSA osteomyelitis demonstrated the similar efficacies of daptomycin and vancomycin (8). Bone cultures remained positive in 59% of the animals after 28 days of daptomycin treatment, and the daptomycin levels in infected bone were 1.3% of those found in serum (8). The study modeled a divided daptomycin dosing regimen, which was pharmacodynamically suboptimal for this concentration-dependent bactericidal agent (2, 8, 15). Similar to our patient, Rezai and colleagues (K. Rezai et al., Abstr. 44th Intersci. Conf. Antimicrob. Agents Chemother., abstr. K-97a, 2004) have presented two cases of emergent S. aureus daptomycin resistance in patients with MRSA bacteremia and osteomyelitis after treatment with 6 mg/kg/day of daptomycin. In these cases, it may be postulated that the presence of non-debrided infected bone, subtherapeutic bone levels (8), or microenvironment interactions (13) may have contributed to the development of resistance. In addition, our patient was severely immunocompromised as a result of relapsed AML and GVHD treatment, which further impaired his ability to control the infection (11).

At the time of approval, FDA established only the daptomycin susceptibility MIC breakpoint at ≤1 μg/ml (3) due to the absence of data on daptomycin-resistant strains. The present report and the data reported by Mangili et al. (9) and Rezai et al. (44th ICAAC) lend further support to that susceptibility breakpoint value (3–5, 16). In addition, time-kill assays revealed that daptomycin had decreased bactericidal activity against S. aureus A8799 compared with that against the pretreatment strains.

The mechanisms of daptomycin resistance are being actively studied (15). Until these issues are investigated and resolved, clinicians should exercise caution when prescribing daptomycin to patients with bacteremia and osteomyelitis, especially if they have additional immunosuppressive conditions.

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REFERENCES