Development of Daptomycin Resistance In Vivo in Methicillin-Resistant *Staphylococcus aureus*

M. K. Hayden, 1* K. Rezai, 1,2 R. A. Hayes, 1 K. Lolans, 2 J. P. Quinn, 1,2 and R. A. Weinstein 1,2

Rush University Medical Center, Chicago, Illinois, 1 and Stroger (Cook County) Hospital, Chicago, Illinois 2

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Daptomycin is a new lipopeptide antibiotic that is rapidly bactericidal against *Staphylococcus aureus*. We report daptomycin resistance and treatment failure in 2 patients with osteomyelitis due to methicillin-resistant *S. aureus*. Disk diffusion susceptibility testing failed to detect resistance. Daptomycin at high concentration retained bactericidal activity against resistant isolates.

Daptomycin is a cyclic lipopeptide antibiotic that is rapidly bactericidal in vitro against a broad spectrum of gram-positive bacteria. Its unique mechanism of action involves calcium-dependent binding to the bacterial plasma membrane and disruption of membrane function (10). Resistance is rare and the mechanism is not known. Daptomycin was approved by the United States Food and Drug Administration (FDA) in 2003 at the dose of 4 mg/kg of body weight/day for treatment of complicated skin and soft tissue infections caused by susceptible bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA) (2). We report daptomycin nonsusceptibility, hereafter referred to as resistance, and treatment failure in two patients with MRSA osteomyelitis. This investigation was reviewed and approved by the Rush University Medical Center and Stroger (Cook County) Hospital institutional review boards.

Patient 1 was an 86-year-old woman with bacteremic MRSA prosthetic knee septic arthritis. She underwent debridement, synovectomy, and revision arthroplasty. Vancomycin was started, but 7 days later bacteremia recurred. Evaluation for a source, including transthoracic echocardiogram, was unrevealing. Vancomycin was discontinued, and daptomycin was started at 6 mg/kg/day, the dose used in a recent clinical trial of bacteremia and infective endocarditis (3). On day 22 of daptomycin therapy, the patient’s creatinine clearance declined to 23 ml/min. The daptomycin dosing interval was increased to 48 h (2). Thirteen days later, her infection relapsed with MRSA bacteremia, epidural abscess, vertebral osteomyelitis, and diskitis at the level of the fifth lumbar and first sacral vertebrae. Blood and epidural tissue grew MRSA.

Patient 2 was a 61-year-old woman with MRSA bacteremia and sternal osteomyelitis complicating heart surgery. The infection resolved after 6 weeks of vancomycin therapy but relapsed 4 weeks later with high-grade MRSA bacteremia and osteomyelitis of the fourth and fifth lumbar vertebrae. Her symptoms resolved again after 6 weeks of daptomycin treatment (6 mg/kg/day), but 1 week later MRSA bacteremia recurred.

Twelve MRSA isolates from patient 1 and 15 MRSA isolates from patient 2 were available. Daptomycin disks (30 μg) and powder were obtained from Cubist Pharmaceuticals, Lexington, MA. Susceptibility testing, including time-kill experiments, was done using National Committee for Clinical Laboratory Standards guidelines (7, 8, 9). All testing was repeated at least three times.

Disk diffusion assays using Mueller-Hinton agar from two different manufacturers (Remel, Lenexa, KS, and BD Diagnostics, Cockeysville, MD) and the FDA-approved breakpoint of 16 mm (2, 9) indicated that all isolates were susceptible to daptomycin (Table 1). At the time of each patient’s clinical and laboratory evaluations, Cockeysville, MD) and the FDA-approved breakpoint...
microbiologic failure, available isolates were reevaluated by a microdilution method using Mueller-Hinton broth supplemented with 50 mg/liter Ca\(^{2+}\) and 10 mg/liter Mg\(^{2+}\). One of patient 2’s pretreatment isolates was resistant to daptomycin, as were all posttreatment isolates from both patients (Table 1). Resistance was stable after 30 serial subcultures on antibiotic-free tryptic soy agar containing 5% sheep blood (Remel). One of patient 2’s posttreatment isolates displayed heterogeneous intermediate vancomycin resistance; the vancomycin MIC, determined by a microdilution method (8), was 4 µg/ml, but the isolate grew on brain heart infusion agar containing 6 µg/ml vancomycin (Remel), and also grew at vancomycin concentrations as high as 16 µg/ml in population analysis profile assays (14).

Pulsed-field gel electrophoresis (6) revealed identical patterns for all of patient 2’s isolates. Likewise, all of patient 2’s
isolates were identical. However, the pulsed-field gel electrophoresis pattern of patient 1’s isolates differed from patient 2’s by more than six bands, indicating that the two patients were infected by unrelated MRSA strains (12).

We tested representative isolates in time-kill experiments (7). Daptomycin consistently showed bactericidal activity against susceptible and resistant isolates when tested at concentrations four times higher than the MIC, although regrowth at 24 h was common (Fig. 1). We determined the daptomycin MIC for 42 representative colonies picked from regrowth plates; the MIC of one of patient 2’s susceptible isolates was found to have increased from 0.5 μg/ml to 4 μg/ml.

Modified population analysis profile testing (10) of susceptible isolates demonstrated heterogeneous growth below the isolate MIC but did not identify a daptomycin-resistant subpopulation. Doubling times for susceptible and resistant isolates were comparable (13).

To our knowledge, this is the first report of de novo daptomycin resistance associated with treatment failure of S. aureus and only the second report of clinical failure associated with the development of daptomycin resistance during therapy (5). The bone infections in our patients may have disposed them to treatment failure. Clinical experience with daptomycin treatment of human osteomyelitis is anecdotal (11) and outcomes are mixed. In an experimental rabbit model, Mader and Adams demonstrated similar microbiologic eradications of MRSA from infected tibias of animals treated with vancomycin or daptomycin at comparable peak plasma levels but a poorer radiologic response to daptomycin. In that study, daptomycin concentrations in infected bone were lower than vancomycin concentrations and no daptomycin could be measured in healthy bone (4). Failure to debride patient 2’s infection may also have contributed to relapse.

Disk diffusion susceptibility testing did not detect daptomycin resistance in these two MRSA strains. This may be due to varied calcium concentrations in commercial Mueller-Hinton agar. Because of the unreliability of the disk diffusion method, it is no longer recommended, and the distribution of disks for investigational use has ceased.

We do not know the mechanism of daptomycin resistance in these isolates, nor do we know if the mechanisms of daptomycin resistance and the heterogeneous intermediate vancomycin resistance seen in patient 2’s MRSA strain are related. Another study found that daptomycin retained good activity against S. aureus with reduced vancomycin susceptibility (1).

Based on our experience, we urge caution in the use of daptomycin for treatment of osteomyelitis; e.g., use the maximum tolerated dose and monitor the patient closely for evidence of relapse. We also recommend testing the daptomycin susceptibilities of clinical isolates of S. aureus by an FDA-cleared MIC device before initiating therapy and in cases of microbiologic failure.

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REFERENCES


