Vancomycin-Intermediate *Staphylococcus aureus* with Phenotypic Susceptibility to Methicillin in a Patient with Recurrent Bacteremia

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Vancomycin-intermediate *Staphylococcus aureus* (VISA) are an emerging problem. We observed a statistically significant inverse relationship in the MICs of vancomycin and oxacillin in *S. aureus* isolates from a patient undergoing hemodialysis who received 26 weeks of treatment with vancomycin during November 1999 through April 2000. All isolates were meca positive and were indistinguishable by pulsed-field gel electrophoresis. The evolving susceptibility patterns of this strain highlight the challenges of detecting and treating VISA infections.

Vancomycin-intermediate *Staphylococcus aureus* (VISA) isolates and glycopeptides have been reported in several countries [1, 2]. To date, all VISA organisms have also been methicillin (oxacillin)—resistant. Because vancomycin is the primary antimicrobial agent for treating infections caused by oxacillin-resistant *S. aureus*, VISA strains are clinically important. Although the mechanism responsible for decreased susceptibility to vancomycin is associated with changes in the thickness and composition of the *S. aureus* cell wall [3–5], the genetic mechanisms by which these changes occur are not fully understood. According to NCCLS interpretive criteria, organisms with vancomycin MICs of ≤4, 8–16, or ≥32 μg/mL are considered to be susceptible, intermediately resistant, or resistant, respectively.

We report a case of VISA infection in a patient undergoing hemodialysis in whom VISA isolates were oxacillin susceptible, despite the continued presence of the meca gene. Multiple additional *S. aureus* isolates were obtained from this patient during a 6-month period; overall, there was a statistically significant inverse relationship between the MICs of vancomycin and oxacillin.

**Methods.** Identification of *S. aureus* was confirmed with a tube coagulase test (Difco Laboratories) [6]. MICs of vancomycin and oxacillin were determined by both broth microdilution and Etest (AB Biodisk) gradient diffusion [7]. In addition, vancomycin screening plates with 6 μg/mL vancomycin (Becton Dickinson) and oxacillin screening plates with 6 μg/mL oxacillin and 4% NaCl (Becton Dickinson) were inoculated with all isolates. The susceptibility breakpoints established by the NCCLS [7] were used. To detect heteroresistant colonies of *S. aureus* by Etest, organisms were plated with an inoculum of 2.0 McFarland turbidity level (instead of the 0.5 McFarland turbidity level used for standard susceptibility testing) and were incubated on the plate for 48 h (instead of 24 h) [8].

Molecular subtyping of isolates was performed by PFGE with 2 endonuclease restriction enzymes, *Sma* and *Apa*. PFGE restriction-fragment patterns were compared using Bionumerics software (Applied Maths) with a 1% molecular weight sensitivity. PCR testing was performed to confirm the presence of the meca gene [9].

**Results.** The patient was a 59-year-old man undergoing hemodialysis who had diabetes, hypertension, atrial fibrillation, peripheral vascular disease, and hepatitis C. In May 1998, the patient had bacteremia caused by methicillin-resistant *S. aureus* (MRSA) that was associated with an infected foot ulcer. In April 1999, the patient had MRSA bacteremia associated with an infected hemodialysis arteriovenous fistula and was treated with vancomycin for 4 weeks. In August 1999, he had MRSA bacteremia resulting from infection in a hemodialysis arteriovenous synthetic graft and was treated with vancomycin for 4 weeks; the graft was replaced. In November 1999, the patient had a below-the-knee amputation and began a 6-week course of vancomycin to treat MRSA bacteremia associated with an infected, nonhealing leg ulcer. Sterilization of the blood was documented by negative blood cultures. In late January 2000, the patient had MRSA bacteremia associated with septic arthritis in a metacarpal-phalangeal joint. In addition to surgical debridement, he was treated with a 6-week course of vancomycin combined with a 2-week course of gentamicin.

In March 2000, the patient presented with fever and back...
Figure 1. PFGE of Staphylococcus aureus isolates obtained in February (lane 2), April (2 isolates; lanes 3 and 4), and June (lane 5) 2000. The gel was made using endonuclease Smal. The 4 isolates (lanes 2–5) were indistinguishable. Lanes 1 and 6, Molecular weight standards.

Figure 2. PFGE of Staphylococcus aureus isolates obtained in February (lane 2), April (2 isolates; lanes 3 and 4), and June (lane 5) 2000. The gel was made using endonuclease Apal. The 4 isolates were indistinguishable. Lanes 1 and 6, Molecular weight standards.

pain, and T11–T12 osteomyelitis and MRSA bacteremia were diagnosed. The patient was again treated with vancomycin (for 6 weeks) and gentamicin. Although vancomycin trough levels were consistently >12 μg/mL, and despite negative results of an intensive workup aimed at finding a persistent focus of infection, the patient had numerous MRSA-positive blood cultures over the next 20 days. Because of the persistent failure of vancomycin and gentamicin to clear the patient's bacteremia, and because isolates from the patient's blood were susceptible to nafcillin, nafcillin was added to the regimen and gentamicin was discontinued. The patient's bacteremia resolved the next day, and all subsequent blood cultures were negative. After 3 weeks of treatment with nafcillin and vancomycin (a total of 6 weeks of vancomycin therapy), treatment with nafcillin as the sole antimicrobial agent was continued for an additional 3 weeks (a total of 6 weeks of nafcillin therapy). In June 2000, the patient died from heart failure and complications of a hemorrhagic stroke. The patient was treated with vancomycin for a total of 26 weeks during April 1999 through April 2000. He received no β-lactam agents until April 2000.

Seventeen S. aureus blood culture isolates obtained from this case patient from January through April 2000 were sent to the Minnesota Department of Health laboratory (Minneapolis). In addition, 7 S. aureus surveillance isolates from specimens from the nares, groin, and axillae that were collected during May through June 2000 (after results of cultures of the patient's blood became negative) were also sent to the Minnesota Department of Health. All 24 S. aureus isolates were viable and available for testing. All S. aureus isolates obtained from the patient were indistinguishable by PFGE subtyping, using both Smal and Apal restriction endonucleases (figures 1 and 2). The pattern yielded by Smal had not been previously identified at the Minnesota Department of Health, despite the fact that >400 distinct MRSA PFGE patterns have been identified there since 1997. The meca gene was present in all isolates.

During the period from January through June 2000, the S. aureus strain found in samples from the case patient exhibited marked variations in vancomycin and oxacillin susceptibility patterns (figure 3). The 3 isolates obtained during January through February were susceptible to vancomycin (MIC ≤2 μg/mL) and resistant to oxacillin (MIC ≥4 μg/mL). However, 3 of 6 isolates obtained in March had reduced susceptibility to vancomycin (MIC of 4–6 μg/mL), and 2 of those 3 isolates were susceptible to oxacillin (MIC ≤2 μg/mL). A total of 8 separate cultures of blood samples obtained in April grew S. aureus. Two isolates obtained on different days had intermediate-level resistance to vancomycin (MIC of 8 μg/mL, by broth microdilution) and 4 isolates had decreased susceptibility to vancomycin (MIC of 4 μg/mL); all 6 isolates were susceptible to
Figure 3. Four isolates of the same *Staphylococcus aureus* strain, from samples obtained at different times from a single patient, were tested for resistance to oxacillin and vancomycin. Left, Oxacillin screening plate (6 μg/mL oxacillin and 4% NaCl). The isolate from February (upper left quadrant) grew on the oxacillin screening agar, confirming methicillin resistance. The 2 vancomycin-intermediate *S. aureus* (VISA) isolates from April (upper and lower right quadrants) did not grow on the screening agar. In the surveillance culture from June (lower left quadrant), of a sample obtained after vancomycin treatment was discontinued and administration of nafcillin had been initiated, the isolate grew on the oxacillin screening agar. Right, Vancomycin screening plate (6 μg/mL vancomycin). The isolate from February (upper left quadrant) did not grow on the vancomycin screening agar. The 2 VISA isolates from April (upper and lower right quadrants) grew on the screening agar. In the surveillance culture from June (lower left quadrant), from a sample obtained after vancomycin was discontinued and administration of nafcillin had been initiated, the isolate did not grow on the vancomycin screening agar.

oxacillin. After vancomycin therapy was discontinued and nafcillin therapy was initiated, all 7 isolates obtained from specimens from the nares, groin, and axillae during May through June were again susceptible to vancomycin and resistant to oxacillin (figure 2). All isolates were susceptible to gentamicin.

In the 24 *S. aureus* isolates from this patient, there was an inverse relationship between the vancomycin MICs and the oxacillin MICs. Specifically, isolates found to have a vancomycin MIC ≥4 μg/mL by Etest were more likely to have an oxacillin MIC ≤2 μg/mL than were isolates with a vancomycin MIC <4 μg/mL (8 of 10 vs. 1 of 14 isolates; OR, 52.0; *P* < .001, by 2-tailed Fisher's exact test).

The *S. aureus* strain found in samples from this patient was heteroresistant (i.e., it had subpopulations with varying resistance) to vancomycin and to teicoplanin. When Etest with a high inoculum and increased incubation time was used to detect heteroresistance, vancomycin MICs ≥8 μg/mL were observed for 5 *S. aureus* isolates. Four isolates were found by this method to have teicoplanin MICs ≥12 μg/mL, compared with no isolates when a standard protocol was used.

**Discussion.** This case illustrates the adaptive ability of *S. aureus*. To our knowledge, this was the first clinical VISA strain in which phenotypic oxacillin susceptibility was seen despite the presence of the *mecA* gene in all isolates. The evolution of vancomycin and oxacillin MICs of this strain was striking. Early isolates were resistant to oxacillin and susceptible to vancomycin. After several months of treatment with vancomycin and no treatment with β-lactam antimicrobials, *S. aureus* isolates from the patient were found to be susceptible to oxacillin and intermediately resistant to vancomycin. After nafcillin was added to the regimen and administration of vancomycin subsequently discontinued (3 weeks later), *S. aureus* isolates from surveillance cultures of specimens from the nares and axillae were resistant once again to oxacillin and susceptible to vancomycin (figure 3). Overall, there was a statistically significant inverse relationship between the MICs of vancomycin and oxacillin.

The evolution of this patient’s *S. aureus* strain in vivo is similar to findings for VISA strains in vitro. For example, suppression of the *mecA* gene (resulting in phenotypic oxacillin resistance) in vancomycin-resistant laboratory strains of *S. aureus* has been described elsewhere [10]. In addition, an inverse relationship between vancomycin and oxacillin MICs has been noted in laboratory experiments [11]. Other work has shown that MRSA isolates grown in the presence of concentrations of vancomycin lower than the MIC become more susceptible to...
oxacillin over time and that glycopeptides and oxacillin have synergistic action against MRSA [12–14].

The inverse relationship observed between oxacillin and vancomycin MICs in the laboratory and in this patient suggests that using vancomycin-oxacillin combinations to treat MRSA infections might improve treatment efficacy in some cases and might prevent the development of intermediate resistance to vancomycin in MRSA strains. At a minimum, this case suggests that nafcillin can help treat oxacillin-susceptible S. aureus infections, at least initially, even in the presence of the mecA gene and in the context of apparent vancomycin treatment failure. It is important to note that these considerations are not relevant to vancomycin-resistant MRSA strains, for which the mechanism of resistance differs from that of VISA strains.

To our knowledge, this was the fifth case of VISA infection reported in the United States [15]. As with other US patients with VISA infections, this patient was undergoing hemodialysis and had been treated extensively with vancomycin. As with other cases, vancomycin failed to effectively treat the patient’s infection, despite achievement of an appropriately high serum concentration of vancomycin and despite negative results of initial evaluations for metastatic foci of infection. VISA isolates from this case also shared features of VISA isolates from cases reported elsewhere [15]. The VISA strain was derived from an MRSA strain (i.e., an S. aureus strain with the mecA gene). In addition, initial determinations of vancomycin MICs (obtained by the referring hospital, using commercial broth microdilution panels) were inconsistent. This reinforces the Centers for Disease Control and Prevention recommendation that S. aureus isolates be tested with a vancomycin MIC ≥4 μg/mL [5].

Heteroresistance to vancomycin was observed in this strain. In heteroresistant strains, the measured MIC represents the resistance profile of a majority, but not all, organisms. The increase in vancomycin MIC among heteroresistant strains is likely due to the selection of progressively more-resistant subpopulations of S. aureus. This phenomenon may have special relevance for patients undergoing hemodialysis, in whom peak levels of vancomycin in serum are infrequently achieved, compared with patients with normal renal function, even though the 2 groups of patients may have similar average vancomycin levels. If only peak levels of vancomycin were bactericidal for S. aureus subpopulations with relatively high MICs, then patients undergoing hemodialysis would be at increased risk of persistence of heteroresistant S. aureus strains beyond the duration of antimicrobial therapy. This, in turn, would put such patients at increased risk for recurrent S. aureus infections and for the subsequent development of VISA organisms.

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References