Evidence for a Continuum of Decreased Vancomycin Susceptibility in Unselected Staphylococcus aureus Clinical Isolates

Farid M. Hussain, Susan Boyle-Vavra, Priya B. Shete, and Robert S. Daum

Department of Pediatrics, University of Chicago, Chicago, Illinois

Some Staphylococcus aureus isolates have glycopeptide minimal inhibitory concentrations (MICs) in the susceptible range but have subpopulations that grow on \( \geq 4 \mu \text{g/mL} \) vancomycin. Clinical laboratory methods for determining susceptibility have proven to be inadequate for detecting these strains. Among methicillin-resistant S. aureus (MRSA) and methicillin-susceptible S. aureus (MSSA) clinical isolates, 149 (66.2%) of 225 and 17 (56.6%) of 30, respectively, grew on brain-heart infusion (BHI) medium containing 2 \( \mu \text{g/mL} \) vancomycin; 17 (7.5%) of the MRSA and 2 (6.6%) of the MSSA isolates grew on BHI screening plates containing 4 \( \mu \text{g/mL} \) vancomycin. One isolate grew on plates containing 6 \( \mu \text{g/mL} \) vancomycin. This isolate escaped detection by routine testing but had a vancomycin MIC of 6 \( \mu \text{g/mL} \) when tested in BHI medium. This isolate also had decreased Triton X-100–induced autolysis and killing when incubated in broth media containing vancomycin, properties accorded to glycopeptide-intermediate S. aureus isolates. These observations suggest that glycopeptide-intermediate–like S. aureus isolates are circulating undetected and that a continuum of decreased susceptibility exists in unselected isolates.
comycin susceptibility among clinical staphylococcal isolates by culturing them on BHI medium containing vancomycin at 4 or 6 μg/mL, in addition to routine nonselective media. However, agar screening plates prepared in-house showed lot-dependent growth of glycopeptide-susceptible \textit{S. aureus} strain ATCC 29213 and, thus, yielded false-positive results [14]. Nevertheless, the BHI screening test can be helpful for screening of isolates growing in pure culture, but because the medium contains glycopeptide, it cannot be used for the direct isolation of specimens [14].

Another reliable technique for assessing glycopeptide resistance, population analysis, is done by culturing an isolate on agar nutrient medium containing varying concentrations of antibiotic to identify and quantify resistant subpopulations. Unfortunately, this method is expensive and time-consuming and, therefore, is not likely to be performed in clinical laboratories.

With these notions in mind, we hypothesized that it is likely that there are GISA (vancomycin MIC, ≥8 μg/mL) or heterogeneously resistant isolates (vancomycin MIC, 4 μg/mL) that have gone unrecognized. Therefore, the purpose of this study was to identify vancomycin-resistant clinical isolates by developing and deploying a sensitive screening technique that uses a relatively high inoculum, BHI medium, and a 48-h incubation.

Materials and Methods

\textbf{Study design.} We prospectively collected 200 consecutive methicillin-resistant \textit{S. aureus} (MRSA) clinical isolates from pediatric and adult patients admitted to University of Chicago Hospitals (UCH; Chicago) during February–August 2000. We also studied a convenience sample of 25 clinical MRSA isolates collected from children during 1995–1997 and 30 methicillin-susceptible \textit{S. aureus} (MSSA) pediatric isolates collected in 1997. To avoid repeated subculturing, which has been shown to result in the loss of the glycopeptide resistance phenotype, original isolates from the Clinical Microbiology Laboratory were subcultured once onto BHI agar, screened for vancomycin resistance, and stored at −70°C in skim milk (Difco Laboratories), as described elsewhere [17].

We used the clinical GISA isolates MI [2] and RN4220, a broadly susceptible host strain derived from isolate NCTC 8325 [18], as control isolates in some experiments. Identification of the isolates as \textit{S. aureus} was confirmed with the use of the Staphaurex latex agglutination test (Abbott Laboratories) and the tube coagulase test.

\textbf{Screening clinical MRSA isolates for vancomycin resistance.} Each isolate was screened on 4 BHI agar plates. Three contained vancomycin at 2, 4, and 6 μg/mL and were prepared in our laboratory. The fourth was a commercially available BHI screening plate containing vancomycin at 6 μg/mL (BBL Microbiology Systems). Isolates stored at −70°C were plated on BHI agar, incubated overnight at 35°C, and subcultured once onto fresh BHI agar. A bacterial suspension of ≥3 colonies was adjusted by dilution in 0.85% NaCl to a turbidity equivalent to a 0.5 McFarland standard (~10⁴ cfu/mL). Aliquots (10 or 100 μL) of this suspension were spotted onto the agar surface with a calibrated pipettor; the plates were incubated at 35°C and examined for growth at 24 and 48 h, as described elsewhere [14].

### MIC determinations.

\textbf{The vancomycin MIC for any isolate growing on the screening plates was determined by a broth dilution method with use of arithmetic dilutions of antibiotics, as described elsewhere [15].} Broth microdilution MIC testing was done according to the NCCLS method with cation-adjusted MHB (Difco Laboratories) and read after an incubation of 24 and 48 h [19]. Broth microdilution MIC testing was also done in BHI medium [15]. The oxacillin MIC was determined by a broth microdilution method according to NCCLS guidelines [19].

Testing was done according to the manufacturer’s instructions (AB BIODISK). In brief, an overnight culture of the test isolate was suspended in 0.85% NaCl to a turbidity equivalent to a 0.5 McFarland standard. The surface of a BHI agar plate was inoculated with a sterile cotton swab dipped into the bacterial suspension. After drying, a vancomycin Etest strip was applied to the plate, which was then incubated at 35°C. The vancomycin MIC was read at 24 and 48 h.

\textbf{Population analysis.} Quantitation of vancomycin heteroresistance was done by population analysis or “efficiency of plating” analysis on medium containing vancomycin, as described elsewhere [15]. In brief, strains were grown overnight in BHI broth, serially diluted in BHI broth, and plated on BHI agar containing vancomycin at various concentrations.

Susceptibility to Triton X-100 autolysis was assessed as described elsewhere [15]. In brief, a midlogarithmic phase culture grown in BHI broth medium was centrifuged. The resulting bacterial cell pellet was washed twice in ice-cold water and resuspended in lysis buffer (0.05 M Tris-HCl [pH 7.2] and 0.05% Triton X-100; Sigma Chemical). To assess lysis, the decrease in absorbance at 620 nm was monitored at 30-min intervals for 4 h.

Vancomycin kill curves were determined according to the method of May et al. [20], except that BHI broth was the medium used. In brief, 0.1 mL of an overnight culture was inoculated into 10 mL of BHI broth and allowed to grow to midlogarithmic phase in a shaking incubator. Vancomycin was then added at a concentration of 20 μg/mL and then incubated again as described above. Cultures were sampled hourly for 6 h; 100-μL samples were serially diluted in BHI broth and plated for viability counts on BHI agar.

\textbf{Vancomycin gradient plate analysis.} Molten BHI agar was introduced onto a square Petri dish and allowed to harden while tilted at 20°C. After hardening, the agar was overlaid with molten BHI containing vancomycin (4 μg/mL). After the second BHI agar solution hardened, a vancomycin gradient was created with vancomycin concentrations of 0–4 μg/mL. To inoculate the gradient plate, a sterile cotton swab was introduced into a bacterial suspension of the isolate to be examined adjusted to 0.5 McFarland density. The plate was then incubated (24 h at 37°C), and growth was evaluated on the next day.

\textbf{Clinical correlate.} After screening of the \textit{S. aureus} isolates was complete, we reviewed the charts of selected patients from whom some of the MRSA isolates were obtained. The Clinical Microbiology Laboratory at the UCH serves patients at the UCH and one other institution, Weiss Memorial Hospital. For convenience, we reviewed the charts of all patients at the UCH who had an MRSA isolate that grew on medium containing vancomycin at ≤4 μg/mL (Vim0 isolates). For each of these isolates, we selected a patient for whom an MRSA isolate was obtained that did not grow on a medium containing vancomycin (Vim+ isolates) without a
priori knowledge of the patients’ clinical situations. For this comparison, we chose patients with MRSA Vm0 isolates who most closely matched, by age and sex, those patients with Vm4 isolates. We compared the patients with Vm4 and Vm0 isolates for site of MRSA culture, clinical syndrome, resistance of the MRSA isolate to other antibiotics, previous antibiotic exposure, response to antibiotic therapy, duration of antibiotic treatment, and clinical outcome. We also reviewed the medical record of the patient from whom the single isolate was obtained that grew on media containing 6 μg/mL vancomycin (isolate 23; Vm6 isolate).

Results

Of the 225 clinical MRSA isolates screened, 149 (66.2%) grew on medium containing vancomycin (≥2 μg/mL), and 17 (7.6%) grew on the BHI screening plate containing 4 μg/mL vancomycin after a 48-h incubation (table 1); 1 (0.4%) of the 225, called isolate 23, also grew on the plates containing 6 μg/mL vancomycin. Notably, isolate 23 had a weakly positive result of Staphaurex testing, although the tube coagulation test result was positive after a 1-h incubation.

Of the 17 isolates that grew on medium containing 4 μg/mL vancomycin, 12 had a vancomycin MIC of 1–2 μg/mL, 4 had an MIC of 4 μg/mL, and isolate 23 had an MIC of 6 μg/mL after a 48-h incubation in BHI broth (table 2). When MIC testing of isolate 23 was done in MHB according to NCCLS guidelines, which specify a 24-h incubation, the MIC was only 2 μg/mL. The teicoplanin MIC for this isolate was 3 μg/mL (table 2) in MHB.

From the BBL plate containing 6 μg/mL vancomycin that was inoculated with isolate 23, a single colony was chosen at random and subcultured onto a fresh BHI agar plate. After a 24-h incubation, the growth from this plate was scraped and stored at −70°C. We refer to this subclone as isolate 23A. Isolate 23A had a vancomycin MIC of 8 μg/mL after a 48-h incubation in BHI broth. After a 24-h incubation, the MIC was 6 μg/mL (table 2). When determined in MHB according to NCCLS guidelines, the MIC was 4 μg/mL at 24 h and 6 μg/mL at 48 h. The oxacillin MICs of isolates 23 and 23A were both ≥256 μg/mL (table 2).

Figure 1 shows the population analyses for isolate 23, vancomycin-susceptible S. aureus strain RN4220, and GISA isolate MI. As could be anticipated from the screening plates, isolate 23 contained a subpopulation of ~10^6 and 10^9 bacteria that grew on medium containing 5 and 6 μg/mL vancomycin, respectively. Thus, this isolate was more “heteroresistant” than the susceptible control isolate RN4220, which had a much smaller percentage of the bacterial population that grew on media containing 2 μg/mL vancomycin. RN4220 colonies were not recovered from media containing vancomycin at a concentration of >2 μg/mL. Isolate 23 also had decreased Triton X-100–induced autolysis, compared with vancomycin-susceptible strain RN4220, and the magnitude of the Triton X-100–induced

Figure 1. Population analysis profile of Staphylococcus aureus isolates RN4220, 23, and MI. Strains grown overnight were serially diluted and plated on varying concentrations of vancomycin-containing agar medium.
effect on isolate 23 was similar to that occurring with GISA isolate MI (figure 2).

Vancomycin kill-curve data (figure 3) demonstrated that incubation of isolate 23 in media containing vancomycin at 20 μg/mL produced no substantial killing in the 6-h test period. In contrast, vancomycin at 20 μg/mL produced an ∼2 log (99%) kill for isolate 76, which at initial screening had not grown on BHI agar containing vancomycin and had a vancomycin MIC of 1 μg/mL.

Of the 30 clinical MSSA isolates screened, 20 (66%) grew on the BHI screening plates containing ≥2 μg/mL vancomycin, and 2 (6%) grew on plates containing 4 μg/mL vancomycin (table 1). These results closely resembled those obtained for the MRSA isolates, although none of the MSSA isolates grew on plates containing 6 μg/mL vancomycin.

At an inoculum of 10^6 cfu, the percentage of MSSA isolates that grew on media containing vancomycin at any concentration was significantly (χ^2, 26.6; P < .001) higher than the percentage of MRSA isolates that did (table 1). This difference was not apparent when an inoculum of 10^6 cfu was used. Furthermore, the increase in the percentage of MRSA isolates that grew on media containing vancomycin increased significantly when 10^7 cfu was the inoculum, compared with 10^6 cfu. A smaller, insignificant increase occurred with the MSSA isolates when the larger inoculum was used.

Results of the vancomycin gradient plate test are shown in figure 4. In this assay, more-resistant isolates have a longer streak of growth on the gradient plate, as demonstrated by taller bars. The growth pattern of the test isolates—76 (Vm0), 65 (Vm2), 98 (Vm4), and 23 (Vm6)—indicated that a continuum of vancomycin resistance exists.

The Vm4 MRSA isolates from 7 patients at the UCH were obtained from bone (n = 1), respiratory tract (n = 3), neck wound (n = 1), urine in the presence of an indwelling Foley catheter (n = 1), foot ulcer (n = 1), and tip of a removed, previously indwelling venous catheter (n = 2). Six of the 7 patients had clinically apparent infectious syndromes, including bacteremia with an indwelling prosthetic valve (presumed endocarditis), aortic valve abscess, sternal wound infection after thoracic surgery, clinical evidence of pneumonia with MRSA respiratory tract isolates, infected cervical fistula, and urinary tract infection associated with an indwelling Foley catheter. Four of these patients required aggressive or prolonged therapy, 2 were discharged with continuing vancomycin treatment, and 2 others had surgical debridement in association with antibiotic treatment.

The medical records of 8 patients at the UCH with Vm0 MRSA isolates were also reviewed. These isolates were obtained from a joint (n = 2), respiratory tract (n = 2), skin (n = 1), breast abscess (n = 1), arm wound (n = 1), and blood (n = 1). Four of these 8 patients had a clinically apparent infectious syndrome, all of which resolved with antimicrobial treatment before discharge from the hospital.

The Vm4 MRSA isolates (n = 9) were uniformly resistant to ciprofloxacin, clindamycin, and erythromycin. Some isolates were also resistant to gentamicin (n = 5) and trimethoprim-sulfamethoxazole (n = 3). Of the 9 Vm0 isolates analyzed, 8 were resistant to ciprofloxacin and erythromycin. Resistance to clindamycin (n = 7), gentamicin (n = 4), and trimethoprim-sulfamethoxazole (n = 3) was also noted.

Of the 9 Vm4 MRSA isolates analyzed, 6 were obtained after the patients had received vancomycin therapy. Other previous therapies include cephalosporins (n = 6), ciprofloxacin (n = 5), tetracycline (n = 1), clindamycin (n = 7), azithromycin (n = 3), and piperacillin-tazobactam (n = 3). The 9 Vm0 MRSA isolates were obtained after the patients were treated with vancomycin (n = 3), a cephalosporin (n = 5), ciprofloxacin (n = 3), tetracycline (n = 1), clindamycin (n = 3), azithromycin (n = 2), erythromycin (n = 1), or piperacillin-tazobactam (n = 2).

**Table 2.** Characterization of *Staphylococcus aureus* isolate 23 and 23A subclone derivative.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Vitek vancomycin&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Etest vancomycin, 48 h</th>
<th>MHB vancomycin, 24 h</th>
<th>BHI broth vancomycin, 24 h</th>
<th>BHI broth teicoplanin, 24 h</th>
<th>MHB oxacillin, 24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>23</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>&gt;268</td>
</tr>
<tr>
<td>23A</td>
<td>ND</td>
<td>6</td>
<td>4</td>
<td>4</td>
<td>8</td>
<td>3</td>
</tr>
</tbody>
</table>

<sup>a</sup> The Vitek MIC was read at a machine-determined time, always <24 h.

![Figure 2. Triton X-100-induced whole cell autolytic activity of *Staphylococcus aureus* isolates RN4220, 23, and MI.](https://academic.oup.com/jid/article-abstract/186/5/661/2191214)
Figure 3. Effect of vancomycin on rate of killing of *Staphylococcus aureus* isolates 23 (one of a group of isolates that grew on media containing 6 μg/mL vancomycin [Vm6]) and 76 (a methicillin-resistant *S. aureus* isolate with vancomycin MIC of 1 μg/mL; one of a group of isolates that did not grow on medium containing vancomycin [Vm0]). Vancomycin (20 μg/mL) was added to broth cultures in midlogarithmic phase of growth (∼10⁷ cfu/mL). Bacterial viability was assessed by quantitative subculture at hourly intervals for 6 h.

We recorded the number of antibiotics that were used to treat patients with Vm0 and Vm4 isolates in the same hospitalization before the date that the Vm0 or Vm4 isolate was obtained. On average, patients with Vm4 isolates had a mean (±SD) of 3.88 ± 1.8 antibiotic courses, compared with 2.44 ± 1.8 courses for the patients from whom Vm0 isolates were obtained (P = .035, 1-tailed unpaired t test).

Vm6 MRSA strain 23 was isolated from a 94-year-old woman, a resident of a nursing home for elderly persons. She was hospitalized for evaluation of jaundice, diarrhea, decreased appetite, and weakness. She had a history of multiple medical problems, including coronary artery disease, hypertension, cholelithiasis, carcinoma of the breast, peripheral vascular disease, Bell palsy, depression, and urinary incontinence. *S. aureus* isolate 23 was obtained from eye drainage associated with conjunctivitis that resolved without specific therapy. In the previous 5 years, she was hospitalized in the same institution 3 times. A review of her medical records revealed no previous vancomycin administration. Samples for culture from the eyes, nares, and axilla, in response to the isolation of strain 23, were obtained ~2 months later and no longer yielded *S. aureus*. She was discharged without any systemic infectious event.

Discussion

We identified isolate 23 by screening 225 unselected MRSA and 30 MSSA isolates obtained from our tertiary-care University Hospital Clinical Microbiology Laboratory, which had determined the vancomycin MIC of isolate 23 to be in the susceptible range (2 μg/mL) according to the Vitek automated procedure and by broth MIC testing according to the NCCLS protocol. However, in BHI broth, the vancomycin MIC was 6 μg/mL by our arithmetic dilution testing method, a value that would correspond to 8 μg/mL had the traditional geometric dilution method been used. Thus, this isolate escaped detection by routine Vitek and MIC testing but, uniquely among heterogeneously resistant isolates described to date, had a vancomycin MIC when tested in BHI broth that would have classified it in the GISA group.

Isolate 23 had several phenotypic characteristics demonstrated in GISA isolates that have a vancomycin MIC of ≥8 μg/mL when determined according to NCCLS methodology. For example, isolate 23 has the decreased lytic activity, demonstrated by Triton X-100 incubation, that has been a feature of laboratory-derived GISA isolates [21] and attributed to most clinical GISA isolates as well [22]. Moreover, GISA isolates have often manifested decreased cell death and lytic activity when incubated in vancomycin [21]. Isolate 23 shared this property as well.

Thus, although isolate 23 has many features common to GISA isolates, MIC testing in our clinical microbiology laboratory did not render it noteworthy. Although this isolate is therefore similar to those termed heterogeneously resistant [9–12], because of its MIC testing in the susceptible range and

Figure 4. Vancomycin gradient plate. Isolates tested included glycopeptide-intermediate *Staphylococcus aureus* clinical isolates MI and RN4220, a broadly susceptible strain. In screening procedure, methicillin-resistant *S. aureus* (MRSA) isolate 76 grew only on medium lacking vancomycin. MRSA isolate 65 grew on medium containing vancomycin at 2 μg/mL. MRSA isolate 98 grew on medium containing vancomycin at 4 μg/mL, and MRSA isolate 23 grew on medium containing vancomycin at 6 μg/mL.
its vancomycin-resistant subpopulation, its GISA-like properties reinforce the notion that isolates like this go undetected among unselected *S. aureus* strains.

Although other researchers performed screening studies of unselected *S. aureus* isolates [9, 12, 23, 24], no GISA isolates (vancomycin MIC of \( \geq 4 \) \( \mu \)g/mL) were identified. Thus, the identification of an isolate such as isolate 23 by a screening strategy such as ours is novel and may reflect our strategy of minimal passage of the clinical isolates before testing, our method of inoculating the agar media, or luck.

With a single exception [24], all screening reports by other researchers identified *S. aureus* strains that had heterogeneously resistant subpopulations that grew on medium containing vancomycin at \( \geq 4 \) \( \mu \)g/mL. Moreover, the results of some studies [8–11] documented that the vancomycin MIC of such subpopulations was as high as 8 \( \mu \)g/mL, although substantially lower values were also reported [25]. Some isolates identified by these screening studies received additional characterization, and properties accorded to GISA isolates were sometimes identified. For example, cell-wall thickening of 1 isolate [11], derived from a resistant subpopulation of a vancomycin-susceptible clinical isolate, resembled thickened cell walls described in most GISA isolates to date [17]. An increase in glutamine nonamidated muropeptides demonstrated by analysis of cell-wall composition by high-performance liquid chromatography was found in another heterogeneously resistant isolate [12]. A similar change was also found in GISA isolate Mu50 from Japan [26], although this change has been shown to be neither necessary nor sufficient for the GISA phenotype [25].

One relevant question has concerned whether heterogeneously resistant isolates such as isolate 23 are ancestors of a more obvious GISA phenotype. A recent case we described was instructive in this regard. A series of MRSA isolates was obtained from blood from a 63-year-old patient receiving dialysis in Illinois who received vancomycin therapy but died of GISA infection [15]. The initial MRSA blood isolate we studied was susceptible to glycopeptide antimicrobials by routine testing. As bacteremia continued, despite vancomycin therapy, the vancomycin MIC among the subsequent, clonally related blood isolates increased to 12 \( \mu \)g/mL, an intermediate level. We found that the initial isolate had substantial subpopulations that could grow on BHI agar containing vancomycin. We also found that this isolate had decreased Triton X-100 autolysis and could survive during incubation in low-level vancomycin. We termed this the “pre-GISA” phenotype. Identification of isolates such as these reinforce the prevailing view that in vivo development of vancomycin resistance occurs in a stepwise fashion. A similar phenomenon was documented by Sugino et al. [27], who demonstrated a decrease in vancomycin susceptibility by population analysis of serial isolates obtained from 2 patients from Japan during the course of vancomycin therapy. Hiramatsu et al. [9] also described a heterogeneously resistant clinical isolate, Mu3, from a Japanese patient who had failure of vancomycin therapy. Interestingly, this isolate was clonally related to GISA isolate Mu50 [9]. Plating Mu3 on 8 \( \mu \)g/mL vancomycin agar yielded clones with resistance identical to that of the resistant Mu50 [28]. This interrelationship between Mu3 and Mu50 and, more directly, the sequence of isolates from a patient in Illinois [15] and another in Port Chester, New York [3, 4], provides further evidence that there is a pre-GISA state and that several genetic and biochemical changes probably occur in sequence to produce a GISA isolate such as Mu50.

By our screening technique, we categorized the 225 MRSA and 30 MSSA isolates into 4 categories on the basis of their ability to grow on screening plates containing different concentrations of vancomycin. The interesting observation that these isolates could be stratified in this way reinforces the notion that unselected *S. aureus* isolates may differ in their predisposition to become GISA isolates. Surprisingly, our data show that MSSA isolates are more likely to grow on media containing vancomycin; the explanation for this interesting observation is under investigation.

Our review of clinical data regarding the patients was done after screening of the isolate on medium containing vancomycin was complete. Thus, our study was not designed to directly assess the clinical importance, if any, of the continuum of decreased susceptibility we found. It also remains uncertain at what point the decreased susceptibility we observed would translate into clinical resistance; none of the isolates we screened would qualify as a GISA isolate by NCCLS definitions. Nevertheless, it is interesting that patients with Vm4 isolates had received more recent antimicrobial therapy before the Vm4 isolate was obtained and often required more intensive therapies after the infection caused by the Vm4 isolate was recognized.

Our screening technique allowed stratification of unselected isolates by a highly reproducible assay and suggests that a continuum of ability of *S. aureus* isolates to survive on media containing vancomycin exists among unselected isolates. Is such a phenomenon new? Other researchers [10, 11] have suggested that MRSA (and MSSA) strains with heterogeneous resistant subpopulations may have been circulating for many years. Indeed, preliminary analysis of our screening data suggests that isolates have manifest a continuum of decreased susceptibility similar to that we demonstrated here for at least 6 years (authors’ unpublished data). To extend this view, we speculate that the relatively recently recognized GISA isolates associated with treatment failure may thus represent a “tip of the iceberg” phenomenon. In this scenario, we hypothesize that the isolation of clinical GISA isolates represents an increase in the stability of the GISA phenotype and a slight increase in the vancomycin MIC measured in MHB over and above that of isolate 23 that allowed recognition by assays routinely performed in a clinical microbiology laboratory.
Acknowledgments

We thank Cindy Bethel and Scott Matushek for assistance in collection of samples and Fred Tenover (Centers for Disease Control and Prevention) for providing glycopeptide-intermediate Staphylococcus aureus isolate MI.

References