Microbiological Features of Vancomycin in the 21st Century: Minimum Inhibitory Concentration Creep, Bactericidal/Static Activity, and Applied Breakpoints to Predict Clinical Outcomes or Detect Resistant Strains

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The results of vancomycin susceptibility tests document that the drug continues to have activity against a wide variety of gram-positive pathogens. The subsequent emergence of vancomycin-resistant enterococci, the persistent failure of vancomycin therapy against strains tested as susceptible, and the more recent discoveries of vancomycin-intermediate or -resistant Staphylococcus aureus strains have compromised the use of vancomycin. Although analyses of surveillance studies fail to demonstrate “minimum inhibitory concentration creep” among populations of wild-type enterococci, streptococci, or staphylococci, enterococci with acquired resistance to vancomycin continue to evolve. The dominantly used automated commercial tests poorly recognize vancomycin-intermediate S. aureus, heteroresistant vancomycin-intermediate S. aureus, and vancomycin-resistant S. aureus isolates, which necessitates the use of expensive supplemental screening tests. Monitoring for appropriate serum levels of vancomycin and determinations of the bactericidal activity of vancomycin appear to best predict clinical outcome, thus creating additional diagnostic burdens for clinical laboratories. Improvements in current test methods with breakpoint criteria and expanded use of the vancomycin bactericidal assays to detect “tolerant” strains will be required to increase the value of vancomycin treatment or to refocus therapy toward the use of newer, alternative agents.

Vancomycin, a glycopeptide antimicrobial (figure 1), is the most widely used agent for the treatment of serious infections caused by gram-positive pathogens. This antimicrobial was obtained from the organism Streptomyces orientalis, and its use predates the origins of the laboratory susceptibility testing standards of the NCCLS (now called the Clinical and Laboratory Standards Institute [CLSI]). Vancomycin was initially used to treat infections with penicillin-resistant Staphylococcus aureus before alternative, less-toxic drugs were introduced. Subsequently, this glycopeptide became vital for the treatment of infections with methicillin (oxacillin)–resistant S. aureus (MRSA) within institutions where the prevalence of MRSA was elevated. According to the Centers for Disease Control and Prevention [1], the prevalence of MRSA in US intensive care units was 57% in 2002, an increase from an incidence of 30%–40% in the middle of the 1990s. As coagulase-negative staphylococci (CoNS), which have high rates of resistance to oxacillin, emerged as important causes of primary or secondary bacteremia among immunocompromised hosts and very young children, the use of vancomycin also accelerated [2].

Several structurally related glycopeptide, lipopeptide, and lipoglycopeptide agents, each with activity or pharmacokinetic-pharmacodynamic features that differentiate it from vancomycin, have been introduced into clinical practice (daptomycin and teicoplanin) or have
entered clinical trials (dalbavancin, oritavancin, ramoplanin, and telavancin). Some of these agents act by inhibiting peptidoglycan synthesis by forming a complex with the terminus of cell wall precursor proteins. Although drugs in this general class are considered to be bactericidal, great variations between them have been recognized with regard to the bacterial species (and, sometimes, strains of the same species) that are susceptible to them.

After the increase in the use of vancomycin in the hospital environment occurred, resistance to vancomycin appeared among the enterococci in the late 1980s [3, 4], followed by continual increases in instances of resistance in the United States, particularly among Enterococcus faecium [4, 5]. Concurrently, the number of alternative agents increased to include linezolid (an oxazolidinone), quinupristin-dalfopristin (a streptogramin combination), teicoplanin (a glycopeptide), and daptomycin (a lipopeptide). In the clinical trials of newer agents, vancomycin was often used as a principal comparator, and critical analyses of the results indicated that the success rates and bactericidal action of treatment with vancomycin appeared to be compromised [6]. This review of the microbiological features of vancomycin was initiated in response to the continuing discovery of vancomycin-resistant gram-positive organisms (particularly staphylococci). The present article will address the changing patterns of susceptibility and emerging resistance to vancomycin, discuss the accuracy of contemporary in vitro diagnostics, and analyze the results of tests, to assist physicians in choosing appropriate parenteral doses of vancomycin.

**MATERIALS AND METHODS**

**Population analyses of MICs.** To assess the degree of so-called “MIC creep” for vancomycin, the SENTRY Antimicrobial Sur-veilance Program database was searched [5]. This global surveillance program uses centers on 6 continents to monitor key pathogens that cause bacteremia, community-acquired and nosocomial pneumonia, skin and soft tissue infections, urinary tract infections, and various infection types in specific patient populations, such as patients in intensive care units or of certain ages (e.g., neonatal, pediatric, or elderly patients). The SENTRY Program was initiated in 1997, and central monitoring sites (JMI Laboratories, North Liberty, IA, and Women’s and Children’s Hospital, Adelaide, Australia) use reference NCCLS MIC methods to test referred isolates (~500 strains/center/year) [7, 8].

Specifically, the vancomycin MIC results from 1997–2003 were listed by year and were interpreted by use of NCCLS categorical criteria [8]. A total of 75,168 gram-positive organisms were analyzed, including 35,458 S. aureus strains, 5902 CoNS strains, 22,103 Streptococcus pneumoniae strains, 3315 E. faecium strains, and 8390 Enterococcus faecalis strains (not shown here). Percentages of susceptible and resistant strains, proportions of the MIC distributions at each MIC, and the MIC50 and MIC90 values of the population, according to organism group or species, were determined.

**Bactericidal assays and tolerance testing.** A collection of 213 S. aureus strains was used in an experiment to determine minimum bactericidal concentrations (MBCs) and MBC:MIC ratios (i.e., tolerance testing). These strains included 17 vancomycin-intermediate S. aureus (VISA) strains conforming to the Centers for Disease Control and Prevention definition [9], 88 heteroresistant VISA (hVISA) strains confirmed and validated by population analysis profiling [10–12], 3 vancomycin-resistant S. aureus (VRSA) strains obtained from the Network on Antimicrobial Resistance in Staphylococcus aureus program (for more information, see http://www.narsa.net), and 105 wild-type (wt) MRSA strains representing a worldwide collection from >50 medical centers in the SENTRY Program [5]. Each organism was tested for MIC values by reference broth microdilution methods [7] followed by MBC determination via quantitative subcultures of the entire volume of clear wells at concentrations greater than the defined MIC. The methods conformed to those recommended by the NCCLS and early publications by Sherris [13] and Tuomanen et al. [14]. Tolerance to vancomycin was defined as either an MBC:MIC ratio of ≥32 or an MBC:MIC ratio of ≥16 associated with a resistant-level vancomycin MBC (≥32 μg/mL) [8, 13]. These tests were performed for vancomycin and a representative lipopeptide, daptomycin.

**Literature review.** Various references were examined to address the following issues of historical and contemporary vancomycin spectrums and potency:

1. Evidence for change in the distributions of MICs
among the wt population at the global, national, or institutional level;
2. Backgrounds of applied diagnostic susceptibility testing and adjustments instituted for emerging resistance patterns;
3. Accuracy of susceptibility testing methods (reference, commercial, or specialized) for each population with a novel mechanism of vancomycin resistance;
4. Bactericidal activities of vancomycin and newer agents (lipopeptides) tested against contemporary gram-positive pathogens; and
5. Documentation of the predictive values of in vitro tests for vancomycin treatment outcomes.

Each of these issues was considered in the attempt to illustrate the role of microbiological tests (i.e., culture, organism identification, and in vitro susceptibility tests) as a guide to appropriate application of vancomycin in 2005 and beyond.

RESULTS AND DISCUSSION

**Historical overview of clinical results for and in vitro activity of vancomycin.** Contemporary experience with vancomycin as a therapy for a wide variety of infections caused by gram-positive organisms has been provided as a comparison to US Food and Drug Administration–monitored clinical trials of teicoplanin, linezolid, and quinupristin-dalfopristin. These studies were designed and powered statistically to determine equivalence, not superiority. In a comprehensive monograph, Wilson and Gruneberg [6] summarized all published and unpublished clinical trials of teicoplanin. In those 21 studies in which vancomycin was used as the comparison antimicrobial, the study designs were generally blinded and covered 6 major categories of infection or patients: (1) general gram-positive infections (5 studies); (2) patients treated in the hematology/oncology service (6 studies); (3) catheter-associated bacteremia (3 studies); (4) bloodstream infection or endocarditis (3 studies); (5) patients undergoing continuous ambulatory peritoneal dialysis (3 studies); and (6) colitis with vancomycin used orally (1 study). These studies enrolled 879 vancomycin-treated patients whose results could be evaluated, yielding 202 therapeutic failures (23.0%). The success rates (defined as cure and improvements) varied widely between studies of the same type and between the patient groups studied, as follows (ranked from highest to lowest failure rates): for patients treated in the hematology/oncology service, 26% (range, 4%–60%); for patients with bacteremia, infected catheters, or endocarditis, 20% (range, 9%–29%); for patients with colitis, 20%; for patients with general gram-positive infections, 16% (range, 0%–32%); and for patients undergoing continuous ambulatory peritoneal dialysis, 4% (range, 0%–13%). These values were comparable to those recorded for teicoplanin.

Vancomycin doses of 1 g or 15 mg/kg were given parenterally every 12 h during the clinical trials of teicoplanin. These doses appear to be adequate, but monitoring has been suggested by some authors for cases of endocarditis, special populations of patients (e.g., drug abusers or patients with renal failure), and cases in which patients are responding poorly. Studies conducted within the past decade have minimized both the concerns regarding vancomycin toxicity (i.e., ototoxicity and nephrotoxicity) and the value of monitoring trough concentrations (>10 μg/mL) to predict clinical success [15, 16].

As greater levels of resistance to numerous older agents emerged among gram-positive cocci, vancomycin was prescribed with greater frequency in Western nations [2]. This increase in use was greatest in the United States, where, by 1996, a total of 10,312 kg of vancomycin was injected, compared with 51–1165 kg for France, Germany, Italy, the United Kingdom, and The Netherlands [2]. However, what has not been appreciated was the disproportionate use of oral vancomycin for colitis therapy in the United Kingdom and The Netherlands, which represented 13.3%–13.8% of all vancomycin use, compared with 7.9% in the United States. A recent study by Currie and Lemos-Filho [17] has also demonstrated higher-than-expected biliary excretion of vancomycin (3.3–94.8 μg/mL) after ≥5 days of treatment with 1 g of vancomycin administered intravenously twice daily. Certainly, continued restrictions of oral vancomycin treatment for colitis appear to be prudent [18], but gastrointestinal levels after parenteral administration may remain significant [17].

Selective pressure resulting from the use of vancomycin has continued since the last year (1996) of the audit by Kirst et al. [2] and has been associated with the discovery of gram-positive cocci, other than enterococci, with diminished susceptibility to vancomycin. However, before these more recent cases were identified, the rapidly increasing use of vancomycin was studied at some university medical centers [19, 20]. Ena et al. [19, 20] noted a 20-fold increase in vancomycin use from 1981 (5.72 g/1000 patient-days) to 1991 (121.25 g/1000 patient-days; P<.0001), with the highest use occurring in the hematology/oncology service. Indications for vancomycin were near equally divided between prophylaxis (35.0%), empirical therapy (31.8%), and directed treatments (33.2%) [19, 20]. Examinations of staphylococci and enterococci isolated from blood during this 11-year period failed to detect evidence of vancomycin MIC creep, but the rapid onset of resistance to ciprofloxacin among MRSA was noted. Similar findings for staphylococci have been reported for 1986–2002 on a national level in Spain [21]. No vancomycin-nonsusceptible S. aureus or CoNS isolates were identified (MIC<, 1 μg/mL); however, 2 teicoplanin-resistant CoNS isolates were found in 1996, and none were found in 2002 [21].

To confirm the absence of MIC creep among wt gram-positive species, findings of the SENTRY Program [5] for 1997–
2003 were analyzed by year, using reference MIC values [7] and interpretations [8]. Table 1 shows the results for 3315 E. faecium strains tested against vancomycin and teicoplanin. At the outset of this global surveillance program, more than one-third of E. faecium isolates were resistant to vancomycin, and nearly 80% had the vanA gene. Over time, the resistance trends were as follows: (1) constant or slight increases in resistance to vancomycin within this prevalent enterococcal species; and (2) gradual increases in the prevalence of the vanA gene worldwide as a cause of vancomycin resistance. However, the MICs of the wt vancomycin-susceptible population remained stable, with MIC<sub>50</sub> and MIC<sub>90</sub> values of 1 μg/mL (table 1).

Vancomycin resistance trends among 22,103 strains of S. pneumoniae are illustrated in table 2. Clearly, no significant trend toward resistance was detected in this collection of isolates from >30 countries and 120 medical centers worldwide. Vancomycin MIC<sub>50</sub> values were always 0.5 μg/mL, and only a rare, reproducible MIC of 2 μg/mL was noted, representing the extreme MIC values of the wt vancomycin-susceptible population.

In table 3, the vancomycin MIC results for >41,000 staphylococcal strains are listed. Among the CoNS isolates, reference vancomycin MIC values remained constant (MIC<sub>50</sub>, 2 μg/mL), and the proportion of strains at the extreme of the breakpoint (vancomycin MIC, 4 μg/mL) varied from 0.8% to 2.7%, with the highest percentage noted in 1998. Teicoplanin, which is less active than vancomycin against some CoNS isolates (Staphylococcus epidermidis and Staphylococcus haemolyticus) [5], also showed no evidence of MIC creep. Similarly, S. aureus (table 3) had stable vancomycin MIC distributions over the course of the 6-year monitoring interval. The proportions of S. aureus with MIC values for vancomycin concentrations of 2, 4, and 8 μg/mL and for teicoplanin concentrations of ≥8 μg/mL were not significantly changed over time. Only the emergence of VISA strains in Hong Kong was detected in 2000.

VISA strains were first widely recognized in articles published in 1997 [22–24], but retrospective literature searches and testing of earlier isolates clearly show a long-term presence of VISA strains [25, 26], even among CoNS isolates [27, 28]. Since publication of the initial reports, VISA isolates have been recovered from patients in Europe (Belgium, France, Germany, Italy, Poland, and the United Kingdom) [12, 29–33], South America (Brazil) [34], East Asia (South Korea) [35], and the United States [36–39]. VISA strains have developed while patients were receiving vancomycin therapy [40] and can be spread within the hospital environment; therefore, compliance with infection-control principles will be critical [18, 41].

VISA strains have a lower growth rate and thicker cell walls than do vancomycin-susceptible S. aureus isolates [9]. These strains include modestly heterogenous subpopulations that have MICs of 5–14 μg/mL (figure 2). Nearly a decade before Japanese cases of VISA infection [22–24], Reynolds [42] postulated that the mechanism of increased glycopeptide resistance...

<table>
<thead>
<tr>
<th>Organism, year</th>
<th>No. of isolates tested</th>
<th>MIC50, μg/mL</th>
<th>MIC90, μg/mL</th>
<th>Percentage of isolates, according to MIC</th>
<th>Isolates with a teicoplanin MIC of ≥8 μg/mL, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>CoNS</td>
<td></td>
<td></td>
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<td></td>
<td>2 μg/mL</td>
</tr>
<tr>
<td>1998</td>
<td>848</td>
<td>2</td>
<td>2</td>
<td>55.7 2.7a 0.0 8.4</td>
<td></td>
</tr>
<tr>
<td>1999</td>
<td>1034</td>
<td>2</td>
<td>2</td>
<td>53.5 1.0a 0.0 8.7</td>
<td></td>
</tr>
<tr>
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<td>1045</td>
<td>2</td>
<td>2</td>
<td>63.3 1.5a 0.0 7.6</td>
<td></td>
</tr>
<tr>
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<td>1155</td>
<td>2</td>
<td>2</td>
<td>50.3 1.1a 0.0 4.0</td>
<td></td>
</tr>
<tr>
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<td>914</td>
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<td>2</td>
<td>43.5 0.9a 0.0 2.0</td>
<td></td>
</tr>
<tr>
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<td>906</td>
<td>1</td>
<td>2</td>
<td>53.4 0.8a 0.0 4.1</td>
<td></td>
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<tr>
<td>S. aureus</td>
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<td></td>
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<tr>
<td>1998</td>
<td>5966</td>
<td>1</td>
<td>1</td>
<td>5.3 0.1 0.0 0.1b</td>
<td></td>
</tr>
<tr>
<td>1999</td>
<td>5011</td>
<td>1</td>
<td>1</td>
<td>4.8 &lt;0.1 0.0 0.1b</td>
<td></td>
</tr>
<tr>
<td>2000</td>
<td>6346</td>
<td>1</td>
<td>1</td>
<td>7.8 &lt;0.1 &lt;0.1 &lt;0.1b</td>
<td>0.1b</td>
</tr>
<tr>
<td>2001</td>
<td>5907</td>
<td>1</td>
<td>1</td>
<td>6.5 0.1 0.0 0.1b</td>
<td></td>
</tr>
<tr>
<td>2002</td>
<td>7046</td>
<td>1</td>
<td>1</td>
<td>6.4 0.0 0.0 0.1b</td>
<td></td>
</tr>
<tr>
<td>2003</td>
<td>5182</td>
<td>1</td>
<td>1</td>
<td>4.7 0.1 0.0 0.0b</td>
<td></td>
</tr>
</tbody>
</table>

* There were 7–23 cases/year.

b There were 0–7 cases/year.

c Vancomycin-intermediate S. aureus strains from Hong Kong.

among S. haemolyticus isolates was the thickened cell walls, which either reduced access to the target sites or promoted excessive nonspecific binding (trapping), thus limiting active, free glycopeptide concentrations. These assumptions were subsequently confirmed [43–46]. Some investigators also described reduced bactericidal action of vancomycin against such VISA isolates as strain Mu50 [26]. These VISA strains have also been associated with poorer clinical outcomes in a variety of infectious syndromes [47–49].

Acknowledged precursors to VISA strains are the hVISA isolates [9], which comprise a heterogeneous population of organisms that have MICs of 2 to ~11 μg/mL (figure 2). The MICs of these strains are positioned between the MICs of wt MRSA and VISA populations and often have susceptible-level vancomycin MICs. In numerous tabulated hVISA prevalence studies (n = 14), Liu and Chambers [9] found, among 7920 S. aureus strains screened, an overall prevalence of hVISA strains of 1.67%; the prevalence of hVISA strains was highest among MRSA isolates (2.16%) and some isolates dating from the early 1990s. These strains appeared after exposure to antimicrobials (including vancomycin), but some isolates have been observed in drug-naive patients, thus representing an intrinsic feature of the strains that occurs only rarely [9].

To date, the highest level of resistance to vancomycin (figure 2) has occurred in the 3 VRSA isolates found in Michigan, New York, and Pennsylvania [50–52]. The high level of resistance to vancomycin (MIC, ≥32 μg/mL) has been attributed to plasmid-based vanA genes of E. faecalis origin [53–56]. The expression of the gene may vary with operon heteroexpression, as described by Perichon and Courvalin [57], resulting in a vancomycin MIC of only 32 μg/mL, compared with MICs several-fold higher for the Michigan and New York isolates. One strain also emerged in the absence of prior vancomycin treatment [58]. Although the plasmid acquisition of the vanA gene by MRSA was predicted from in vitro passaging and transfer experiments reported by Noble et al. [59] in 1992, only 3 VRSA strains have appeared, each of which has not been associated with dissemination [60], and the strains remain susceptible to several older agents and newer compounds [50, 52, 61].

Clearly, threats by organisms to the continued role of vancomycin have emerged. VISA, hVISA, and VRSA strains remain very uncommon, but their MICs or zone diameters may be indistinguishable from those of wt MRSA when some in vitro tests are used. This results in little proof of vancomycin MIC creep (tables 1, 2, and 3) among monitored gram-positive species, but incidences of VRE have continued to increase in some geographic areas. As stated by Bush [60], our problem may not be the treatment of these mutants but, rather, the initial accurate detection by available, commonly used susceptibility testing methods.

History of vancomycin susceptibility testing and wandering breakpoints. Earliest records of the NCCLS consensus process for vancomycin susceptibility testing indicate that a susceptible breakpoint of ≥12 mm was selected (MIC correlate, ≤5 μg/
The emergence of VRE [3, 4] results in various levels of resistance to vancomycin and small populations of vancomycin-intermediate resistant cells that grow at a vancomycin concentration of 4 μg/mL and also with significant subpopulations that grow at a vancomycin concentration of 6 μg/mL. hVISA strains demonstrate heterogeneous resistance and include both subpopulations of cells with various levels of resistance to vancomycin and small populations of vancomycin-intermediate resistant cells that grow at a vancomycin concentration of 8 μg/mL. Reprinted from Liu and Chambers [9] with permission from the American Society for Microbiology.

The limitations of the in vitro detection methods for strains with reduced susceptibility to vancomycin have been discussed widely [38, 66, 67]. Problems with the disk diffusion method emanate from the poor diffusion characteristics of this glycopeptide, which has a diffusion coefficient of 0.72 (i.e., only 72%–74% of disk content enters the agar after 6 h) [68]. Barry et al. [69, 70] reported the use of vancomycin scattergrams (figure 3) as controls during teicoplanin studies that illustrate the monomodal population of vancomycin MICs and zones of inhibition. Figure 3 shows the current vancomycin breakpoints superimposed on this 20-year-old scattergram. Correlation coefficients were extremely poor (P<.5; data not shown), and the teicoplanin disk diffusion test also performed poorly [69, 70]. These characteristics of the glycopeptide disk diffusion method encouraged the use of reference-quality MIC methods or well-defined agar-screening tests [8, 9, 38, 66, 67]. Alternate methods, such as Etest (AB BIODISK), have been used effectively in detecting VISA, hVISA, and VRSA isolates [9, 66], but the population analysis profiling method (figure 2) remains the preferred, yet very cumbersome, technique for defining elevated vancomycin MIC results. The commercial automated systems (Vitek and Vitek 2 [bioMérieux] and MicroScan WalkAway [Dade Behring]) have performed with unacceptable accuracy for VISA, hVISA, and even VRSA strains [71].

Figure 4 shows the vancomycin MIC and zone diameter scatterplots for 213 contemporary MRSA isolates. Clear differences were noted between the wt MRSA and VRSA populations; however, results for the VISA and hVISA strains, as well as the current vancomycin breakpoint of ≥4 μg/mL, overlap results for the wt MRSA strains. Nearly all zone diameters of VISA and hVISA strains remain indistinguishable from those of wt MRSA, with zone diameters of ≥17 mm [8]. Because wt MRSA strains with a vancomycin MIC of 4 μg/mL are very unusual (table 3 and figure 4), a further modification of the CLSI breakpoints for staphylococci should be considered. If the breakpoint for susceptibility was lowered to ≈2 μg/mL, the strains with MICs of 4 or 8 μg/mL (intermediately resistant) would be recognized, thus minimizing the requirement for routine screening tests and for the referral of strains with MIC values of 4 μg/mL to reference laboratories, as recommended by the CLSI [8]. Modification of the disk diffusion test zone diameters to ≥17 mm should also be required [8, 65]. However, the disk test would continue to be unable to routinely distinguish hVISA or VISA isolates from wt MRSA (figure 4).

As these in vitro susceptibility testing parameters are adjusted for the CLSI reference or standardized tests [7, 8, 65], the manufacturers of the dominant commercial systems (Vitek and MicroScan) must improve vancomycin test accuracy for staph-

Figure 2. Population analysis profile of vancomycin-resistant Staphylococcus aureus (VRSA), vancomycin-intermediate S. aureus (VISA), heteroresistant VISA (hVISA), and vancomycin-susceptible S. aureus (VSSA) strains. The population analysis shows how many cells in a fixed number of cells (usually ~10^7 cfu) of each strain are resistant to various concentrations of vancomycin. VRSA strains are highly resistant and homogeneously resistant, with 100% of the population growing at each of the vancomycin concentrations tested. VISA strains are intermediately resistant, with 100% of the population growing at a vancomycin concentration of 4 μg/mL and also with significant subpopulations that grow at a vancomycin concentration of 6 μg/mL. hVISA strains demonstrate heterogeneous resistance and include both subpopulations of cells with various levels of resistance to vancomycin and small populations of vancomycin-intermediate resistant cells that grow at a vancomycin concentration of 8 μg/mL. Reprinted from Liu and Chambers [9] with permission from the American Society for Microbiology.
Figure 3. Scattergram demonstrating the relationships of vancomycin MICs and zones of inhibition around 30-µg disks using an organism collection dating from the middle of the 1980s (53 methicillin-resistant *Staphylococcus aureus* strains had small zone diameters). Current breakpoints for enterococci (solid lines) and staphylococci (broken lines) are shown. Reprinted from Barry et al. [70] with permission from the American Society for Microbiology.

Figure 4. Contemporary vancomycin scattergram comparing MICs and zone diameters for vancomycin-resistant *Staphylococcus aureus* (circled numbers), vancomycin-intermediate *S. aureus* and heteroresistant vancomycin-intermediate *S. aureus* (boxed numbers), and wild-type methicillin-resistant *S. aureus* isolates sampled from the SENTRY Antimicrobial Surveillance Program collection worldwide. Broken horizontal and vertical lines denote possible breakpoint adjustments to maximize detection of staphylococcal strains with decreased susceptibility to vancomycin.

Bacterial action and tolerance to glycopeptides. Clearly, the vancomycin MIC results failed to differentiate the potentially responsive cases and organisms from those that may have
a higher likelihood of causing clinical failure (e.g., VISA or some strains of wt MRSA). Past experience dictates that failures of vancomycin therapy for serious infections (enterococcal or bacteremias) may occur even when combination regimens are used against “susceptible” strains [72]. Early studies [73–75] recognized the phenomenon of “bacterial tolerance” among laboratory strains and clinical isolates, as well as their association with suppression of the autolytic system. A wide variety of agents were judged to be devoid of bactericidal activity in tolerant strains that included relatively high numbers of wt S. aureus [75, 76]. Nearly a decade of controversy and experiments culminated in excellent “state-of-the-art” reviews [13, 14, 77] that presented the complex technical problems for measuring tolerance and the evidence for its influence on clinical cases of serious infection or in animal models. These initial reports were significantly related via mechanisms, but clinical response information remained inconclusive, with conflicting results from human case studies and animal models [13, 14, 77].

The recent development of newer classes of antimicrobial agents directed against gram-positive cocci (e.g., glycopeptides, lipopeptides, streptogramin combinations, oxazolidinones, and glycyclines) afforded opportunities to resume investigations of the efficacy of and outcome measurements associated with the use of vancomycin. Several reports by Sakoulas et al. [78–80] and Moise-Broder et al. [81], which were later validated by Verdier et al. [82], again affirm the relationship of vancomycin bactericidal activity and the competence of the bacterial autolytic mechanism. These investigations significantly expanded our knowledge by recognizing the accessory gene regulator (agr) locus of S. aureus as an important mechanism in susceptibility to vancomycin. Other findings included determinations that (1) agr types I and II were associated with evolution toward reduced vancomycin susceptibility (hVISA and VISA); (2) agr type II polymorphism was associated with vancomycin therapy failures and reduced bacterial killing due to diminished autolysis; and (3) decreased agr function promotes organism survival (e.g., intracellularly, in biofilms, and via cell binding), especially in the hospital environment [78–83]. Exposure of agr II–null organisms to low concentrations of vancomycin also rapidly escalated the risk of the emergence of hVISA strains [83], and studies in animal models again confirmed that vancomycin MIC values do not predict clinical efficacy (endocarditis model with VISA strain) and that glycopeptide killing decreases significantly (mouse peritonitis model) with slight increases in MICs [84, 85]. All these findings led to the realization that determination of vancomycin bactericidal action through an MBC, serum bactericidal assay, or kill-curves, as a guide to successful treatment, was critical in conjunction with the use of appropriate dosing regimens [86]. If the drug is not determined to be bactericidal, an alternative to vancomycin with significant killing should be considered [87].

The importance of this long-held concept of selecting a bactericidal agent for the treatment of serious staphylococcal infections [86] can be illustrated by examining the vancomycin MBC results. Table 4 lists the MBC distribution for vancomycin among 4 MRSA organism groups. Only 18% of hVISA strains and none of the VISA or VRSA strains had vancomycin MBCs of ≤4 µg/mL (susceptible), and vancomycin MBCs at the resistant level (≥32 µg/mL) were identified for 64 (73%) of 88

### Table 4. Vancomycin minimum bactericidal concentrations (MBCs) for contemporary strains of vancomycin-resistant Staphylococcus aureus (VRSA), vancomycin-intermediate S. aureus (VISA), heteroresistant VISA (hVISA), and wild-type (wt) methicillin-resistant S. aureus (MRSA) (n = 213 strains).

<table>
<thead>
<tr>
<th>Strain</th>
<th>No. of isolates tested</th>
<th>No. of isolates, according to vancomycin MBC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤0.5 µg/mL</td>
<td>1 µg/mL</td>
</tr>
<tr>
<td>wt MRSA</td>
<td>105</td>
<td>2</td>
</tr>
<tr>
<td>hVISA</td>
<td>88</td>
<td>...</td>
</tr>
<tr>
<td>VISA</td>
<td>17</td>
<td>...</td>
</tr>
<tr>
<td>VRSA</td>
<td>3</td>
<td>...</td>
</tr>
</tbody>
</table>

* The numbers in parentheses denote the percentage of isolates at or below NCCLS susceptible breakpoint concentrations for vancomycin [7, 8].

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**Figure 5.** Kill-curve results of selected antimicrobials against a vancomycin-intermediate Staphylococcus aureus strain (Mu50 Hiramatsu) when tested against growth control ( ), vancomycin ( ), teicoplanin ( ), linezolid ( ), and daptomycin ( ). cfu, colony-forming units. Adapted from Rybak et al. [26] with permission from Elsevier.
hVISA isolates. These tolerant isolates would be risks for clinical failure [47–49] and would conform to nonbactericidal kill-curve results for vancomycin published for hVISA and VISA isolates [26] (figure 5). These results also comply with the definition of bacterial tolerance found in previous reviews [13, 14], reference procedures [88], and the present article. Table 5 shows that 15.2% of wt MRSA met the definition of tolerance, whereas all or the vast majority of hVISA (73.9%), VISA (100.0%), and VRSA (100.0%) strains were either tolerant or frankly resistant (MBC, $\geq 32 \mu g/mL$). These findings, which were derived from a contemporary collection of MRSA, show that a significant subset of strains remains at risk for clinical failures, regardless of appropriate dosing or the susceptibility level of reported MICs.

These discoveries contrast with those for daptomycin, a lipopeptide for which MBC:MIC ratios (table 6) remain at 1 or 2 (bactericidal); however, some MBCs can be $>1 \mu g/mL$ (non-susceptible) but $\leq 4 \mu g/mL$ for VISA (64.7%), hVISA (2.3%), and wt MRSA (0.9%) strains. Because the current susceptibility breakpoint for daptomycin is $\leq 1 \mu g/mL$ for staphylococci and $\leq 4 \mu g/mL$ for enterococci [8], a uniform susceptibility MIC breakpoint would be desirable to predict the utility of daptomycin against hVISA, VISA, or even VRSA strains [26, 87, 89, 90]. A uniform breakpoint could rectify the perceived inability of the daptomycin MIC to detect less-susceptible strains, such as those isolates considered to be hVISA or VISA [91]. The current VRSA strains (3 documented) have daptomycin MICs of $\leq 0.5 \mu g/mL$ [50–52].

**SUMMARY**

Vancomycin has achieved the very worthy reputation as the “drug of choice” for the most troublesome gram-positive infections. The physical-chemical characteristics of this glycopeptide and its mode of action give vancomycin an excellent spectrum of potency, but, unfortunately, those same characteristics cause the drug to perform poorly according to in vitro susceptibility testing methods. Early and contemporary investigations outline the limited bactericidal activity for vancomycin, which is attributed to genetic variations in the targeted species, such as staphylococci. Recently reported hVISA, VISA, and VRSA isolates have been poorly recognized or not sufficiently differentiated from fully susceptible S. aureus isolates when disk diffusion tests or rapid, automated commercial systems (Vitek or MicroScan) were used. To address these shortcomings, resistance surveillance programs must use reference broth microdilution methods to adequately monitor emerging resistance to vancomycin. Data presented here from the SENTRY Program (1997–2003) show that true resistance to vancomycin remains rare and, apparently, has a stable prevalence among staphylococci and streptococci (tables 2 and 3), although the prevalence of acquired resistance has slightly increased among enterococci (table 1).

To better recognize glycopeptide-resistant gram-positive organisms, the CLSI has consistently modified interpretive criteria for susceptibility or resistance and has introduced novel (and expensive) screening methods to enhance standardized disk diffusion or dilution test methods [7, 8, 63–65]. However, even in an era of emerging hVISA, VISA, and VRSA strains, current CLSI recommendations for testing vancomycin still lack acceptable levels of performance accuracy. This situation has been complicated by the inability of reference vancomycin MIC tests to predict therapeutic success or failure, but the MBC and other bactericidal assays appear to be better positioned for expanded clinical use against serious staphylococcal infections (i.e., bacteremia and endocarditis) [9, 13, 14, 78–81, 84, 86, 88]. Because vancomycin MBCs can indicate bacterial tolerance in a significant proportion of wt MRSA (15.2% of strains) (table 5) and in nearly all hVISA and VISA strains, clinical microbiology laboratories must acquire competency in bactericidal procedures to effectively guide chemotherapy with vancomycin or alternative bactericidal agents (e.g., daptomycin) (table 6) [88]. Until such procedures become commercially viable in auto-

<table>
<thead>
<tr>
<th>Strain</th>
<th>No. (%) of isolates, according to daptomycin MBC:MIC ratio</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>wt MRSA</td>
<td>81 (77.1)</td>
</tr>
<tr>
<td>hVISA</td>
<td>69 (78.4)</td>
</tr>
<tr>
<td>VISA</td>
<td>12 (70.6)</td>
</tr>
</tbody>
</table>

**Table 6. Distribution of minimum bactericidal concentration (MBC):MIC ratio results for daptomycin for a collection of 210 wild-type (wt) methicillin-resistant Staphylococcus aureus (MRSA), vancomycin-intermediate S. aureus (VISA), and heteroresistant VISA (hVISA) strains.**
mated formats, MBCs for vancomycin must be determined by use of acceptable, published techniques [88] using broth subcultures in reference-quality microdilution trays. These methods, supplemented by vancomycin MIC screens to maximize detection of staphylococci with elevated MICs (e.g., VISA, hVISA, and VRSA strains), and the development of simpler tests for regulator (agr) expression or presence of important virulence factors, could lead to a new era of maximized glycopeptide therapy.

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