

## Relationship of MIC and Bactericidal Activity to Efficacy of Vancomycin for Treatment of Methicillin-Resistant *Staphylococcus aureus* Bacteremia

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We attempted to find a relationship between the microbiological properties of bloodstream isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) and the efficacy of vancomycin in the treatment of bacteremia. Vancomycin susceptibility testing was performed, and bactericidal activity was determined for 30 isolates from 30 different patients with MRSA bacteremia for whom clinical and microbiological outcome data were available. The majority of these patients had been previously enrolled in multicenter prospective studies of MRSA bacteremia refractory to conventional vancomycin therapy. Logistic regression found a statistically significant relationship between treatment success with vancomycin and decreases in both vancomycin MICs ( $\leq 0.5$   $\mu\text{g/ml}$  versus 1.0 to 2.0  $\mu\text{g/ml}$ ;  $P = 0.02$ ) and degree of killing (reduction in  $\log_{10}$  CFU/milliliter) by vancomycin over 72 h of incubation in vitro ( $P = 0.03$ ). For MRSA isolates with vancomycin MICs  $\leq 0.5$   $\mu\text{g/ml}$ , vancomycin was 55.6% successful in the treatment of bacteremia whereas vancomycin was only 9.5% effective in cases in which vancomycin MICs for MRSA were 1 to 2  $\mu\text{g/ml}$ . Patients with MRSA that was more effectively killed at 72 h by vancomycin in vitro had a higher clinical success rate with vancomycin therapy in the treatment of bacteremia ( $\log_{10} < 4.71$  [ $n = 9$ ], 0%;  $\log_{10}$  4.71 to 6.26 [ $n = 13$ ], 23.1%;  $\log_{10} > 6.27$  [ $n = 8$ ], 50%). We conclude that a significant risk for vancomycin treatment failure in MRSA bacteremia begins to emerge with increasing vancomycin MICs well within the susceptible range. Elucidating the mechanisms involved in intermediate-level glycopeptide resistance in *S. aureus* should begin by examining bacteria that begin to show changes in vancomycin susceptibility before the development of obvious resistance. Prognostic information for vancomycin treatment outcome in MRSA bacteremia may also be obtained by testing the in vitro bactericidal potency of vancomycin.

Vancomycin has been the cornerstone of therapy for serious methicillin-resistant *Staphylococcus aureus* (MRSA) infections since the early 1980s, when MRSA emerged as a significant nosocomial pathogen in the United States (2, 11, 25, 32). However, many clinicians believe that the efficacy of vancomycin against MRSA is inferior to that of antistaphylococcal beta-lactams against methicillin-susceptible *S. aureus* (MSSA) infections (11, 14). The basis of this belief comes partly from in vitro data that demonstrate slower bactericidal activity of vancomycin compared to the results seen with antistaphylococcal beta-lactams against *S. aureus* (1, 28) as well as data suggesting slow clinical response (13, 14). In our recent study of evaluating factors associated with clinical failure in 87 patients with MRSA bacteremia, we found evidence of a relationship between vancomycin MIC and clinical vancomycin failure by univariate analysis, although this did not prove to be an independent predictor of failure in multivariate analysis (19). Some view the higher level of mortality seen with MRSA bacteremia compared to the results seen with methicillin-susceptible *S. aureus* bacteremia as another example of the inferiority of vancomycin to beta-lactams (4).

Although the emergence of glycopeptide-intermediate-level-resistant *S. aureus* (GISA) (8, 9, 29) and, most recently, glycopeptide-resistant *S. aureus* (3) are reasons for concern, these cases are quite rare. Nevertheless, vancomycin treatment failures are not uncommon with MRSA infections despite the organism being fully susceptible (vancomycin MIC  $\leq 2$   $\mu\text{g/ml}$ ) by standard methods of testing and criteria (6, 7, 8, 10, 17, 18, 26). Antimicrobial regimens that provide bactericidal therapy have been demonstrated to be superior to bacteriostatic regimens in the treatment of *S. aureus* bloodstream infections, especially with infective endocarditis (5, 22, 23). We performed this study to determine whether the microbiological properties of clinical MRSA in the presence of vancomycin in vitro correlated with the clinical efficacy of vancomycin in the treatment of bacteremia. We used data available to us through patients that had been enrolled previously in prospective studies for the purposes of receiving alternative therapy for MRSA bacteremia because of either intolerance to or failure of conventional vancomycin therapy.

### MATERIALS AND METHODS

**Clinical isolates.** During the period of July 1998 to November 2001, 87 patients were enrolled in phase III and IV multicenter prospective studies, yielding 122 MRSA isolates from 24 different hospitals across 16 states (19). To further explore the relationship between clinical failure of vancomycin in treatment of MRSA bacteremia and susceptibility to inhibition and killing by vancomycin in

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TABLE 1. Patient characteristics of 30 patients with MRSA infections for whom in vitro testing of a clinical isolate was performed

Characteristic	Value for group <sup>a</sup>		P
	VAN treatment success <sup>b</sup>	VAN treatment failure	
Age in yr (mean $\pm$ SD [median])	63 $\pm$ 15 (62)	59 $\pm$ 17 (60)	0.5
Sex			0.7
Male	4 (57)	9 (39)	
Female	3 (43)	14 (61)	
Located in the ICU	2 (29)	10 (43)	0.7
Log <sub>10</sub> (CFU/ml) of killing (mean $\pm$ SD [median])	6.26 $\pm$ 1.29 (6.27)	4.88 $\pm$ 1.81 (5.10)	0.07

<sup>a</sup> Data are no. (%) of patients.<sup>b</sup> VAN, vancomycin.

vitro, a subset of 30 of these isolates from 30 patients was subjected to additional testing in this study. The majority of patients were enrolled in these studies for the purpose of receiving alternative therapy after unsatisfactory response to conventional therapy. Isolates were stored at  $-70^{\circ}\text{C}$  until the time of in vitro testing. Vancomycin susceptibility was performed under blinded conditions with Mueller-Hinton II agar (Becton Dickinson, Cockeysville, Md.) and an inoculum of  $10^4$  CFU/ml according to recommendations of the NCCLS (20). Growth was evaluated at 24 h at  $35^{\circ}\text{C}$ . Quality control of susceptibility testing was performed using ATCC 33591 (MRSA) reference strains. Bactericidal activity of vancomycin was determined under blinded conditions without knowledge of any clinical outcome. All isolates were susceptible to vancomycin ( $\text{MIC} \leq 2 \mu\text{g/ml}$ ).

**Case definitions.** Vancomycin treatment failure was defined in patients that received at least 5 days of appropriately dosed vancomycin by serum level monitoring (trough, 10 to 15  $\mu\text{g/ml}$ ) when one or more of the following were present in association with persistently positive blood cultures: (i) persistent signs or symptoms of infection (fever, leukocytosis, etc.); (ii) new additional signs or symptoms of infection; or (iii) worsening of signs or symptoms of infection observed at baseline. Serum concentrations of vancomycin were measured by commercially available immunoassay methods employed in clinical laboratories of participating hospitals.

**Vancomycin bactericidal assays.** Overnight (14 to 18 h) cultures of MRSA in brain heart infusion broth (Becton Dickinson, Sparks, Md.) were diluted 1:100 in a 250-ml Erlenmeyer flask to a final volume of 20 ml in fresh brain heart infusion broth containing vancomycin (16  $\mu\text{g/ml}$ ), yielding a starting inoculum of approximately  $10^7$  to  $10^8$  CFU/ml. Flasks were incubated at  $35^{\circ}\text{C}$  with gentle swirling for 30 s once daily. Samples obtained at 0 and 72 h were diluted serially 1:10<sup>0</sup> to 1:10<sup>7</sup>, and 25  $\mu\text{l}$  of each dilution was plated in duplicate on sheep blood agar plates to determine counts of viable bacteria. Vancomycin bactericidal activity for each strain was expressed as log<sub>10</sub> CFU per milliliter (0 h) – log<sub>10</sub> CFU per milliliter (72 h). MRSA ATCC 33591 was used as a control in these experiments. Exposure of this strain to vancomycin (16  $\mu\text{g/ml}$ ) in four separate experiments resulted in a mean ( $\pm$  standard deviation) reduction in viable counts at 72 h of 4.57 ( $\pm$  0.18) log<sub>10</sub> CFU/ml. The maximum and minimum repeats were within 0.4 log<sub>10</sub> CFU/ml.

**Statistical analysis.** Dichotomous variables were compared using chi-square analysis or Fisher's exact test where appropriate. Continuous variables were analyzed with the Kruskal-Wallis analysis of variance test. We used classification and regression tree modeling (Systat version 10; SPSS Inc., 2000), a form of binary recursive partitioning, to identify breakpoints in log<sub>10</sub> CFU of killing/milliliter at 72 h for analysis of vancomycin response. Tree-based models are useful for both classification and regression problems. This type of modeling identifies what dichotomous split on which predictor variable will maximally improve the predictability of the dependent variable. The predictor variable(s) may be a mixture of nominal and/or ordinal scales. The dependent variable may be quantitative or qualitative (i.e., nominal or categorical). The regression trees parallel regression analysis of variance modeling, and the classification trees parallel discriminant analysis. Finally, logistic regression was used to determine whether a relationship existed between vancomycin treatment success and two explanatory variables: vancomycin MIC and log<sub>10</sub> CFU of killing/ml at 72 h.

## RESULTS

We analyzed 30 MRSA isolates from 30 patients with MRSA bacteremia to determine their susceptibility or resistance to killing by vancomycin. In vitro testing of these isolates

was performed in a blinded fashion without knowledge of any clinical outcomes. Of the patients tested, 23 patients were vancomycin treatment failures and 7 were treated successfully. There were no significant differences in age, sex, and proportion of intensive care unit patients between the two groups (Table 1). We noted marked heterogeneity in the in vitro bactericidal activity of vancomycin against MRSA, with reduction in viable bacteria at 72 h ranging from 0.17 log<sub>10</sub> to 8.16 log<sub>10</sub> CFU/ml. With patients for whom vancomycin treatment was successful, MRSA demonstrated increased killing in vitro at 72 h (mean, 6.26 log<sub>10</sub> CFU/ml) compared to the results seen with those for whom treatment failed (mean, 4.88 log<sub>10</sub> CFU/ml). This difference approached but did not achieve statistical significance ( $P = 0.07$ ).

Classification and regression tree modeling identified two breakpoints resulting in three subgroups with respect to the magnitude of vancomycin log<sub>10</sub> CFU of killing/ml at 72 h for analysis of vancomycin treatment efficacy: group 1,  $<4.71$ ; group 2, 4.71 to 6.26; group III,  $\geq 6.27$ . There were significant differences in the percentages of patients successfully treated with vancomycin in the three groups: 0% for group 1, 23.1% for group 2, and 50% for group 3 (Table 2). There were no statistically significant differences in duration of prior clinical vancomycin exposure prior to obtaining isolates in any of the three groups to account for the differences in killing (Table 2).

We found no significant relationship between the bactericidal activity of vancomycin and the MIC of vancomycin. The values of median vancomycin log<sub>10</sub> CFU of killing/ml at 72 h were 5.94 and 5.40 against MRSA isolates for which vancomycin MICs were  $\leq 0.5 \mu\text{g/ml}$  and 1 to 2  $\mu\text{g/ml}$ , respectively (Table 3).

In a prior study in which we examined the relationship of the accessory gene regulator (*agr*) group II genotype and vanco-

TABLE 2. Rate of vancomycin success and in vitro vancomycin bactericidal activity

Group	Log <sub>10</sub> (CFU/ml) of killing	n	% VAN success <sup>a</sup>	No. of days of VAN Rx prior to isolate procedure <sup>b</sup>
1	$<4.71$	9	0.0	33 $\pm$ 30 (20)
2	4.71–6.26	13	23.1	44 $\pm$ 79 (20)
3	$\geq 6.27$	8	50.0	23 $\pm$ 27 (15.5)

<sup>a</sup> Group 1 versus 3,  $P = 0.029$ ; group 1 versus 2 versus 3,  $P = 0.05$ . VAN, vancomycin.<sup>b</sup> Data are means  $\pm$  SD (median); VAN Rx, vancomycin therapy.

TABLE 3. Vancomycin treatment success rates and vancomycin bactericidal activity by sensitivity of the MRSA isolate to vancomycin

VAN <sup>a</sup> MIC (μg/ml)	<i>n</i>	Log <sub>10</sub> (CFU/ml) of killing (mean ± SD [median])	Log <sub>10</sub> (CFU/ml) of killing for group:			% VAN success <sup>b</sup>
			<4.71	4.71–6.26	≥6.27	
≤0.5 (5)	9	4.91 ± 2.26 (5.94)	3	3	3	55.6
1.0–2.0	21	5.32 ± 1.59 (5.40)	6	10	5	9.5

<sup>a</sup> VAN, vancomycin.<sup>b</sup> *P* = 0.01 (Fisher's exact test).

mycin treatment failure for MRSA bacteremia, we noted that the vancomycin MIC had an impact on treatment failure in univariate analysis (19). However, when multivariate analysis only was used, patient elevated serum creatinine levels and the *agr* group II genotype were significant predictors of vancomycin treatment failure. When a subset of the same clinical isolates was analyzed in the present study, we again noted a relationship between vancomycin treatment failure and elevated vancomycin MIC. We found a significant decrease in vancomycin treatment efficacy of vancomycin for MRSA isolates with vancomycin MIC values of 1 to 2 μg/ml (9.5% clinical success rate) compared to the results seen with isolates with vancomycin MIC values of ≤0.5 μg/ml (55.6% clinical success rate; *P* = 0.01) (Table 3).

Multivariate analysis using logistic regression showed a statistically significant relationship between the increased therapeutic efficacy of vancomycin and both lower vancomycin MIC and increased killing by vancomycin in vitro (Table 4).

## DISCUSSION

The clinical importance of bactericidal therapy in the treatment of most infections remains controversial. However, in treatment of infective endocarditis, experience during the antibiotic era of the past 6 decades suggests that bactericidal activity is an important determinant of clinical outcome. In one study of 20 patients with serious *S. aureus* infections of whom 80% had endocarditis, patients who received bactericidal therapy (99.9% 24-h killing in vitro) demonstrated a more rapid clinical and microbiological cure and showed a significantly lower mortality of 0% compared to 40% mortality in patients who received bacteriostatic therapy (5). In another study of *S. aureus* endocarditis, the addition of gentamicin to nafcillin resulted in more rapid clinical and microbiological response but had no effect on mortality (12).

More recent studies investigating the importance of bacte-

ricidal activity in the treatment of *S. aureus* bacteremia in an era in which methicillin resistance has emerged are lacking. Despite the recent introduction of linezolid and quinupristin-dalfopristin for use by clinicians in the treatment of serious infections due to gram-positive organisms, vancomycin remains the treatment of choice for serious MRSA infections. These newer agents are generally bacteriostatic against MRSA. However, tolerance to vancomycin among clinical *S. aureus* isolates has been previously described (15, 21, 24, 30, 31). We wanted to investigate whether decreased killing by vancomycin in vitro translated to any clinical effect of treatment.

We investigated the relationship between the degree of vancomycin bactericidal activity in vitro and clinical treatment outcome in MRSA bacteremia. We found a positive correlation with the bactericidal activity of vancomycin in vitro and clinical success in the treatment of bacteremia. MRSA that demonstrated less than 4.7 log<sub>10</sub> killing at 72 h showed 0% successful treatment with vancomycin, whereas isolates that demonstrated ≥6.27 log<sub>10</sub> killing at 72 h demonstrated 50% success.

We found no differences in mortality on the basis of vancomycin bactericidal activity, perhaps a result of the fact that isolates used in this study were obtained from compassionate use studies of linezolid and quinupristin-dalfopristin in which patients who failed vancomycin treatment were offered alternative treatment. This also explains the high rate of vancomycin failure in the sample studied.

Classification and regression tree modeling were used to determine breakpoints of bactericidal killing to provide a fair distribution of the sample into three groups. We chose 72 h as our time point to assess killing in vitro to maximally differentiate isolate characteristics in the setting of the relatively slow bactericidal activity of vancomycin. The killing assay used here did not allow for enough discrimination between isolates for earlier endpoints. Although vancomycin is very stable, longer periods of incubation would have raised questions as to the possibility of breakdown of the compound. Our unpublished observations suggest that failure to achieve a 99.9% bactericidal endpoint after 24 h of incubation with vancomycin at 16 μg/ml is not uncommon in testing recent isolates of MRSA. This concentration is close to what is typically achieved in patients undergoing vancomycin therapy (16). The relatively high-level inoculum was chosen to maximally differentiate killing among the different strains.

In vitro comparisons of clinical isolates are very difficult to control for prior exposure of the organism to antibiotic. We tried to control for vancomycin exposure of the tested organism within the patient from whom it was isolated to address

TABLE 4. Multivariate analysis of factors associated with vancomycin treatment success

Factor	OR (95% CI) <sup>a</sup>	<i>P</i>
Increased VAN killing <sup>b</sup>	10.73 (1.24–92.95)	0.031
Decreased VAN MIC <sup>c</sup>	35.46 (1.76–715.95)	0.020

<sup>a</sup> OR, odds ratio; CI, confidence interval.<sup>b</sup> The odds ratio for increased vancomycin killing represents the incremental increased chance of treatment success between the groups identified by regression tree modeling (log<sub>10</sub> [CFU/ml] of killing at 72 h: group 1, <4.71; group 2, 4.71 to 6.26; group 3, ≥6.27). VAN, vancomycin.<sup>c</sup> The odds ratio for decreased vancomycin MIC represents the increased chance of success for treatment of MRSA infection with vancomycin MIC ≤0.5 μg/ml versus MIC 1.0 to 2.0 μg/ml.

confounding by vancomycin exposure in the individual patient. However, we were unable to control for any exposure that the organism may have had to vancomycin in any previously colonized or infected patient with the same strain.

Despite the fact that hospital-associated MRSA isolates in the United States share a fairly homogeneous genetic background (27), our findings demonstrated significant heterogeneity in the bactericidal activity of vancomycin against MRSA, with 72-h killing ranging from 0.17 to 8.16 log<sub>10</sub>. This may be a possible explanation for the variable success seen in patients with MRSA bacteremia treated with vancomycin (8).

The vancomycin killing assay used in this investigation allowed us to demonstrate that increased bactericidal activity of vancomycin against MRSA may predict a higher probability of clinical success in the treatment of MRSA bacteremia. However, it should be pointed out that employing such methods in the clinical laboratory is impractical because they are too time consuming. In addition, although our data confirm a relationship between susceptibility to inhibition and killing by vancomycin in vitro and response to vancomycin treatment of MRSA bacteremia, the utility of these methods for testing individual isolates is doubtful. For example, we identified individual MRSA bloodstream isolates from patients who failed to respond to vancomycin therapy which demonstrated greater killing than other isolates from patients who were treated successfully. However, our findings of decreased efficacy of vancomycin in MRSA bacteremia with isolates for which vancomycin MICs were 1 to 2 µg/ml suggests that useful clinical information may be extrapolated from a clinical microbiology susceptibility report. While decreased vancomycin efficacy may be expected in isolates for which vancomycin MICs are higher, as observed for hetero-GISA and GISA (MIC, 4 to 16 µg/ml) (8), we found it noteworthy that the efficacy of vancomycin began to decline for isolates with vancomycin MICs that lie well within the susceptible range. Despite the high rate of treatment failure with vancomycin in this study, we found no MRSA isolates for which the vancomycin MIC was >2 µg/ml. While we used agar dilution susceptibility testing in this study, there is no reason to suspect that similar findings could not be anticipated with susceptibility reports from automated systems employed in many hospital laboratories. However, although MIC measurements are readily obtained in the clinical microbiology laboratory, a more precise measurement of how useful MIC data can be to clinicians and confirmation of these findings would require a larger study of patients in a group perhaps not weighted as heavily towards vancomycin treatment failures.

It is important to point out that because our collection of isolates was drawn from a group of patients of whom most had failed vancomycin therapy, it would be inappropriate to extrapolate from the results of this paper a quantitative prediction of vancomycin treatment failure rate with each incremental increase in vancomycin MIC.

A multivariate analysis using logistic regression showed a statistically significant relationship between increased vancomycin efficacy and both decreased vancomycin MIC (≤0.5 µg/ml) and increased vancomycin killing. A sample size of only 30 patients was sufficient to demonstrate the statistical significance of these variables. However, the small sample size resulted in extremely large confidence intervals when calculating odds ratios; therefore, the magnitudes of the odds ratios are

difficult to interpret. A more quantitative analysis would require a larger study.

A final extrapolation of these findings is this: for investigators to fully elucidate the multiple-step genetic pathways involved in the development of intermediate-level glycopeptide resistance in *S. aureus*, studies should begin with the analysis of isolates that show subtle microbiological changes in the presence of glycopeptides before the development of overt resistance.

In summary, we demonstrated that vancomycin-susceptible clinical MRSA isolates demonstrate considerable heterogeneity in vitro with respect to vancomycin MIC and vancomycin killing. These differences appear to affect the clinical efficacy of vancomycin and the probability of successful treatment of MRSA bacteremia.

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