THE RELATIONSHIP BETWEEN VANCOMYCIN TOLERANCE AND CLINICAL OUTCOMES IN PATIENTS WITH *STAPHYLOCOCCUS AUREUS* BACTEREMIA

BY

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ABSTRACT

Background: Treatment failure is increasingly common in *Staphylococcus aureus* bacteremia (SAB). Vancomycin tolerance may be playing a role in clinical outcomes in SAB that has yet to be fully explored.

Methods: This was a single-center retrospective cohort study of 166 patients (September 2012 – January 2014) evaluating the relationship between vancomycin tolerance and clinical failure in SAB. Vancomycin minimum inhibitory concentration (MIC) was determined by broth microdilution and Etest. Vancomycin tolerance was defined as a vancomycin minimum bactericidal concentration (MBC)/MIC ≥ 32. Univariable and multivariable analyses were conducted to determine the relationship between vancomycin tolerance and clinical failure after adjusting for other factors.

Results: Of the 166 patients evaluated, 26.5% had vancomycin tolerant clinical isolates. Tolerance to vancomycin was more common in methicillin-susceptible *S. aureus* bacteremia (MSSA-B) than methicillin-resistant *S. aureus* bacteremia (MRSA-B; *n*=29/101 [28.7%] vs. *n*=15/65 [23.1%]), although not significantly (*P*=0.422). Clinical failure was frequently observed (50% overall). Elevated vancomycin MIC by Etest (≥ 1.5 μg/mL) was not associated with clinical failure (*P*=0.50). Vancomycin tolerance was significantly associated with SAB clinical failure in univariable analysis (*P*=0.014). This relationship persisted even when adjusting for other factors in multivariable analysis (adjusted odds ratio [AOR], 2.70; 95% confidence interval [CI], 1.27-5.70; *P*=0.010).

Conclusions: Vancomycin tolerance is a clinically significant predictor of clinical failure in SAB independent of methicillin susceptibility and antibiotic choice. Future research is needed to determine optimal treatment of vancomycin tolerant SAB.

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INTRODUCTION

Staphylococcus aureus is a well-known opportunistic pathogen and the most frequently encountered bacterial species in clinical practice.[1] *Staphylococcus aureus* is implicated in a variety of invasive disease, including bacteremia.[2] *Staphylococcus aureus* bacteremia (SAB) is a life-threatening condition, with an overall mortality of 20%.[3] The incidence of *S. aureus* bacteremia is approximately 20 in 100,000 persons and increases with age.[4,5] While most cases of SAB are caused by methicillin-susceptible strains (MSSA), the incidence of methicillin-resistant *S. aureus* (MRSA) is increasing in recent years and may be associated with increased mortality.[3]

The glycopeptide antibiotic vancomycin has been the mainstay of MRSA treatment since its introduction in the late 1950s.[6,7] Although high-level resistance to vancomycin in MRSA is limited to a few cases worldwide, treatment failure is common – even when the isolate tests susceptible (vancomycin minimum inhibitory concentration (MIC) $\leq$ 2 μg/mL).[8-11] Moreover, the increasing prevalence of elevated vancomycin MIC ("MIC creep") has presented new treatment challenges.[11-13] While the results from studies investigating the clinical implications of elevated vancomycin MIC show conflicting evidence, a recent meta-analysis found that vancomycin MIC $\geq$ 1.5 μg/mL by Etest is associated with treatment failure in MRSA bacteremia (MRSA-B) and vancomycin MIC 2 μg/mL is associated with increased mortality, regardless of methodology..[14] However, elevated vancomycin MIC is not unique to MRSA-B and has also been independently associated with β-lactam failure in MSSA bacteremia (MSSA-B)..[15,16] Thus, although elevated vancomycin MIC is associated with poorer outcomes in SAB, this phenomenon may simply be a marker for some other pathogen-specific factor(s) that have yet to be determined.

Previous studies have demonstrated superiority of antimicrobial regimens featuring a bactericidal agent in the treatment of SAB.[10,17,18] Therefore, it is intuitive that bactericidal activity (≥ 99.9% killing *in vitro* after 24 hours) may be a more clinically relevant predictor of therapeutic effectiveness than measures of inhibitory activity. As expected, reduced bactericidal activity of vancomycin is associated with poor outcomes in MRSA-B, including longer duration of bacteremia and increased vancomycin treatment failure.[10,19] Although vancomycin MIC and minimum bactericidal concentrations (MBC) are highly correlated, there are instances in which there is a large dissociation between these values.[20] Vancomycin tolerance is defined as a MBC/MIC ratio of ≥ 32.[21] Vancomycin tolerance is found in 20% of MRSA isolates overall, although the prevalence is as high as 43% in some institutions.[22] Like elevated vancomycin MIC, vancomycin tolerance is also observed in MSSA and may be even more prevalent in these infections.[23]

We hypothesize that vancomycin tolerance may be associated with clinical failure in SAB. Although the relationship between vancomycin bactericidal activity and clinical outcomes has been explored, the clinical implications of highly dissociated inhibitory and bactericidal activities (i.e., tolerance) remain unclear.[24] Therefore, the objective of this study was to evaluate the relationship between vancomycin tolerance and clinical outcomes in the treatment of SAB.

METHODS

Study Population

This was a retrospective cohort study of hospitalized patients at the University of Kansas Hospital, a tertiary care academic medical center. All adult patients with a positive blood culture for *S. aureus* from September 2012 through January 2014 were eligible for inclusion. Patients

were excluded if they received antimicrobial therapy targeted against SAB for < 48 hours or if they exhibited polymicrobial bacteremia at onset. Clinical data was collected by retrospective review of the electronic medical record. Variables that were collected included basic patient demographics (age, gender), setting of bacteremia onset, comorbidities, Charlson comorbidity index, associated focus of SAB, antimicrobial treatment data, laboratory values, vital signs, and microbiological data. Bacteremia was considered hospital-acquired if all elements of infection were first present on or after the third hospital day. The associated focus of SAB was determined as documented by a treating physician and stratified according to risk for mortality as described by Soriano *et al.*[25] Immunosuppression was defined as neutropenia, leukopenia, chronic steroid (≥ 20 mg prednisone) or antineoplastic use, or as diagnosed by a treating physician. This study was approved by the University of Kansas Medical Center institutional review board.

Outcome Measures

The primary outcome was clinical failure, defined as a composite of: i) 30-day all-cause mortality; ii) non-resolving signs and symptoms of bacteremia (body temperature ≥ 38^{o}C, white blood cell count ≥ 12,000/μL, persistent positive blood cultures) for ≥ 5 days while on antimicrobial therapy; iii) perceived treatment failure, leading to either change of antimicrobial or addition of a second agent targeted against *S. aureus*; iv) recurrent bacteremia within 60 days of the index SAB episode; or v) relapsing bacteremia, defined as a positive blood culture for *S. aureus* following a previous negative culture during the same SAB episode.

Secondary outcomes were 30-day all-cause mortality, duration of bacteremia, and hospital length of stay (LOS). Hospital LOS was defined as the date of first positive *S. aureus* blood culture until date of discharge. Duration of bacteremia was defined as the time from the

first positive *S. aureus* blood culture until the first negative blood culture or complete resolution of signs and symptoms of SAB.

Microbiological Analysis

Clinical *S. aureus* blood isolates were stored at -70^{o}C prior to microbiological testing. Strains were subcultured three times post-freezing to ensure uniform growth and adequate metabolic activity prior to evaluation. Vancomycin MIC was determined by broth microdilution according to Clinical and Laboratory Standards Institute (CLSI) guidelines.[26] Vancomycin MIC was also determined by Etest according to manufacturer recommendations (bioMérieux, Marcy l'Etoile, France). Vancomycin MIC $\geq$ 2 μg/mL by broth microdilution or $\geq$ 1.5 μg/mL by Etest were classified as elevated. Methicillin resistance was confirmed by the presence of penicillin-binding protein 2a (PBP-2a) by latex agglutination. For patients with multiple clinical *S. aureus* blood isolates during the study period, only the first isolate was analyzed.

The MBC was determined according to CLSI recommendations using the microdilution method.[21] Briefly, a 100 μL aliquot of each well with no visible growth after 24 hours of incubation at 35^{o}C was subcultured on tryptic soy agar, allowed to visibly dry at room temperature, and cross-streaked using a sterile cotton-tipped swab to account for antibiotic carryover. Vancomycin MBC was defined as the lowest concentration of drug with $\geq$ 99.9% killing at 24 hours. Clinical isolates with a vancomycin MBC/MIC $\geq$ 32 by broth microdilution were determined to be vancomycin tolerant.

Statistical Analysis

Categorical variables were compared by χ^2 or two-tailed Fisher's exact test and continuous variables were compared by Student's t-test or Mann-Whitney *U* test with a two-

sided *P*-value < 0.05 considered statistically significant. Multivariable logistic regression was performed to determine variables independently associated with clinical failure. All calculations were performed using SPSS statistical software (version 20.0; IBM Corp.: Armonk, NY).

RESULTS

A total of 166 patients met study criteria and were included in the final analysis. Vancomycin tolerance was observed in 44 (26.5%) of the 166 *S. aureus* clinical isolates tested. Although tolerance to vancomycin appeared to be more common in MSSA-B than MRSA-B (28.7%, *n*=29/101 vs. 23.1%, *n*=15/65), this difference was not statistically significant (*P*=0.422). Baseline characteristics of patients with a vancomycin tolerant clinical isolate were compared to those without a vancomycin tolerant isolate, as displayed in Table 1. There were no statistically significant differences observed across baseline characteristics.

The distribution of vancomycin MIC by broth microdilution and corresponding MBC/MIC ratios are displayed in Table 2. As shown, 51.8% of the 166 clinical isolates analyzed had equal vancomycin MIC and MBC values. Elevated MIC by broth microdilution was only observed in 2 of the 166 isolates (1.2%) in this cohort. However, elevated MIC by Etest was observed in 100 of 166 clinical isolates (60.2%). The majority of isolates (56.6%, *n*=94/166) had a vancomycin MIC by Etest of 1.5 μg/mL. Vancomycin MIC of 1 μg/mL by Etest was also commonly observed (35.5%, *n*=59/166). A vancomycin MIC ≥ 2 μg/mL by Etest was rarely encountered in this cohort (3.6%; *n*=6/166). Elevated vancomycin MIC by Etest was not associated with vancomycin tolerance (*P*=0.588, Table 1).

Table 1. Patient characteristics in *Staphylococcus aureus* bacteremia according to vancomycin tolerance

Characteristic	Vancomycin Tolerant (*n*=44)	Non-Vancomycin Tolerant (*n*=122)	*P* value
Age (years), mean ± SD	58.1 ± 16.5	59.0 ± 14.9	0.725
Age > 65 years, *n* (%)	15 (34.1)	44 (36.1)	0.815
Age > 85 years, *n* (%)	3 (6.8)	5 (4.1)	0.438[a]
Female gender, *n* (%)	19 (43.2)	42 (34.4)	0.302
Methicillin resistance, *n* (%)	15 (34.1)	50 (41.0)	0.422
Hospital-acquired, *n* (%)	15 (34.1)	33 (27.0)	0.377
Intensive care unit, *n* (%)	12 (27.3)	39 (32.0)	0.563
Sepsis, *n* (%)	28 (63.6)	76 (62.3)	0.875
Septic shock, *n* (%)	7 (15.9)	20 (16.4)	0.941
Immunosuppression, *n* (%)	13 (29.5)	40 (32.8)	0.693
High-risk focus, *n* (%)	12 (27.3)	34 (27.9)	0.940
S. aureus pneumonia, *n* (%)	8 (18.2)	23 (18.9)	0.922
S. aureus endocarditis, *n* (%)	6 (13.6)	12 (9.8)	0.487
Medium-risk focus, *n* (%)	24 (54.5)	64 (52.5)	0.812
S. aureus osteomyelitis, *n* (%)	5 (11.4)	10 (8.2)	0.546[a]
Low-risk focus, *n* (%)	8 (18.2)	23 (18.9)	0.922
Central line-associated, *n* (%)	10 (22.7)	25 (20.5)	0.755
S. aureus bacteruria, *n* (%)	4 (9.1)	13 (10.7)	1.000[a]
Diabetes mellitus, *n* (%)	20 (45.5)	52 (42.6)	0.745
Hemodialysis, *n* (%)	7 (15.9)	27 (22.1)	0.381
Charlson com. index, median (IQR)	6 (4-8)	6 (4-9)	0.477[b]
Vancomycin MIC ≥ 1.5 μg/mL[c], *n* (%)	25 (56.8)	75 (61.5)	0.588

SD, standard deviation; IQR, interquartile range; APACHE II, Acute Physiology and Chronic Health Evaluation II; MIC, minimum inhibitory concentration

[a] Calculated by Fisher's exact test; all other categorical variables compared by χ^2 test

[b] Calculated by Mann-Whitney *U* test; all other continuous variables compared by Student's t-test

[c] Determined by Etest

Table 2. Vancomycin minimum inhibitory concentrations and minimum bactericidal concentration/minimum inhibitory concentration ratios by broth microdilution

		Vancomycin MBC/MIC					
Vancomycin MIC (μg/mL)	No. isolates (%) (*N*=166)	1	2	4	8	16	≥32
0.25	2 (1.2)	---	1	---	---	---	1
0.5	57 (34.3)	19	17	1	2	---	18
1	105 (63.3)	65	7	2	4	2	25
2	2 (1.2)	2	---	---	---	---	---

Clinical failure was common, occurring in 50.0% of cases overall. Univariable comparisons according to antimicrobial clinical success or failure are displayed in Table 3. As shown, clinical failure was more frequent among those with a vancomycin tolerant isolate compared to those without a vancomycin tolerant isolate (65.9% vs. 44.3%) and this difference was statistically significant (*P*=0.014). Other variables that were significantly associated with clinical failure on univariable analysis ($P < 0.05$) were hospital-acquired infection (odds ratio [OR], 2.04; 95% confidence interval [CI], 1.03-4.07; *P*=0.042), intensive care unit (ICU) admission (OR, 2.38; 95% CI, 1.20-4.72, *P*=0.012), sepsis (OR, 2.31; 95% CI, 1.21-4.41; *P*=0.010), septic shock (OR, 4.35; 95% CI, 1.65-11.43; *P*=0.002), high-risk focus of infection (OR, 2.09; 95% CI, 1.04-4.20, *P*=0.037), and *S. aureus* pneumonia (OR, 2.57; 95% CI, 1.08-5.65, *P*=0.028). Elevated vancomycin MIC by Etest was not significantly associated with clinical failure (*P*=0.526).

Table 3. Patient characteristics in *Staphylococcus aureus* bacteremia according to clinical failure

Characteristic	Clinical Failure (*n*=83)	Clinical Success (*n*=83)	*P* value
Age (years), mean ± SD	59.4 ± 16.6	58.1 ± 13.8	0.588
Age > 65 years, *n* (%)	31 (37.3)	28 (33.7)	0.627
Age > 85 years, *n* (%)	7 (8.4)	1 (1.2)	0.064[a]
Vancomycin tolerance, *n* (%)	29 (34.9)	15 (18.1)	0.014
Female gender, *n* (%)	33 (39.8)	28 (33.7)	0.421
Methicillin resistance, *n* (%)	33 (39.8)	32 (38.6)	0.874
Hospital-acquired, *n* (%)	30 (36.1)	18 (21.7)	0.040
Intensive care unit, *n* (%)	33 (39.8)	18 (21.7)	0.012
Sepsis, *n* (%)	60 (72.3)	44 (53.0)	0.010
Septic shock, *n* (%)	21 (25.3)	6 (7.2)	0.002
Immunosuppression, *n* (%)	22 (26.5)	31 (37.3)	0.134
High-risk focus, *n* (%)	30 (36.1)	19 (22.9)	0.061
S. aureus pneumonia, *n* (%)	21 (25.3)	10 (12.0)	0.028
S. aureus endocarditis, *n* (%)	9 (10.8)	9 (10.8)	1.000
Medium-risk focus, *n* (%)	38 (45.8)	47 (56.6)	0.162
S. aureus osteomyelitis, *n* (%)	8 (9.6)	7 (8.4)	0.787
Low-risk focus, *n* (%)	14 (16.9)	17 (20.5)	0.550
Central line-associated, *n* (%)	17 (20.5)	18 (21.7)	0.849
S. aureus bacteruria, *n* (%)	11 (13.3)	6 (7.2)	0.201
Diabetes mellitus, *n* (%)	35 (42.2)	37 (44.6)	0.754
Hemodialysis, *n* (%)	16 (19.3)	18 (21.7)	0.701
Charlson com. index, median (IQR)	6 (4-8)	6 (4-9)	0.595[b]
Vancomycin MIC ≥ 1.5 μg/mL[c]	48 (57.8)	52 (62.7)	0.526

SD, standard deviation; IQR, interquartile range; APACHE II, Acute Physiology and Chronic Health Evaluation II; MIC, minimum inhibitory concentration

[a] Calculated by Fisher's exact test; all other categorical variables compared by χ^2 test

[b] Calculated by Mann-Whitney *U* test; all other continuous variables compared by Student's t-test

[c] Determined by Etest

Multivariable logistic regression was conducted to evaluate the relationship between vancomycin tolerance and clinical failure after adjusting for potential confounders. All variables that were associated with vancomycin tolerance or clinical failure in univariable analysis ($P < 0.20$) were eligible for inclusion in the explanatory model. As displayed in Table 4, the association between vancomycin tolerance and clinical failure persisted when adjusting for other factors in multivariable logistic regression (adjusted odds ratio [AOR], 2.67; 95% CI, 1.26-5.64;

P=0.010). Septic shock was also independently associated with clinical failure (AOR, 4.34; 95% CI, 1.60-11.74; *P*=0.004) in this model. Although ICU admission and sepsis were associated with clinical failure in univariable analysis, these factors were not included in the final model due to shared variance with septic shock, which is an established predictor of poor outcomes in SAB.[3]

Table 4. Multivariable logistic regression model of variables associated with clinical failure in *Staphylococcus aureus* bacteremia

Variable (*N*=166)	Adjusted Odds Ratio (95% CI)	*P* value
Age (years)	1.00 (0.98-1.03)	0.678
Septic shock	4.34 (1.60-11.74)	0.004
S. aureus pneumonia	2.23 (0.95-5.47)	0.065
Vancomycin tolerance	2.67 (1.26-5.64)	0.010

CI, confidence interval

All of the patients in this study received appropriate empiric treatment within 24 hours of positive *S. aureus* blood culture. Following empiric vancomycin therapy in cases of MSSA-B (*n*=101), 46 patients (45.5%) were treated primarily with a penicillin (nafcillin or piperacillin-tazobactam), 27 patients (26.7%) were treated with a cephalosporin, and 26 patients (25.7%) were treated with vancomycin. The vast majority (90.8%) of MRSA-B cases were treated with vancomycin. The other 9.2% of cases were treated with daptomycin. Treatment with vancomycin was not associated with clinical failure in SAB overall (*P*=0.277). When restricting our analysis to patients with MSSA-B, vancomycin tolerance was associated with clinical failure of not only vancomycin therapy (100%, *n*=4/4 vs. 42.9%, *n*=9/21), but β-lactam therapy as well (64.0%, *n*=16/25 vs. 38.3%, *n*=18/47; *P*=0.038).

A summary of all clinical outcomes measured is included in Table 5. Overall, 30-day all-cause mortality was observed in 15.1% of cases. Mortality was lower in the vancomycin tolerant

group (11.4% vs. 16.4%), although this difference was not statistically significant (*P*=0.424). Overall median hospital LOS was 9 days (interquartile range [IQR], 5-16 days). As shown, the observed difference in clinical failure between groups was driven primarily by non-resolving signs and symptoms of SAB (36.3% vs. 15.6%; *P*=0.004). Median LOS was longer in the vancomycin tolerant group (10.0 days vs. 9.0 days), although this relationship was not statistically significant (*P*=0.342). The median duration of SAB for this cohort was 66.0 hours (IQR, 38.5-97.0 hours). There was not a significant association between vancomycin tolerance and median duration of SAB (*P*=0.725).

Table 5. Comparison of clinical outcomes by vancomycin tolerance in *Staphylococcus aureus* bacteremia

Outcome	Vancomycin Tolerant (*n*=44)	Non-Vancomycin Tolerant (*n*=122)	*P* value
Clinical failure, *n* (%) [a]	29 (65.9)	54 (44.3)	0.014
30-day all cause mortality	5 (11.4)	20 (16.4)	0.424
Non-resolving signs/symptoms ≥ 5 d	16 (36.3)	19 (15.6)	0.004
Persistent bacteremia	7 (15.9)	20 (16.4)	0.941
Relapsing bacteremia	2 (4.5)	1 (0.8)	0.172[b]
60-day recurrence	0 (0.0)	1 (0.8)	1.000[b]
Hospital LOS (days), median (IQR)	10.0 (6.0-14.0)	9.0 (5.0-18.0)	0.342
Bacteremia duration (hrs), median (IQR)	64.0 (41.0-90.0)	66.0 (38.0-105.25)	0.725

LOS, length of stay; IQR, interquartile range

[a] Composite endpoints may not add up if multiple outcomes contributed to clinical failure

[b] Calculated by Fisher's exact test

DISCUSSION

The purpose of this study was to evaluate the relationship between vancomycin tolerance and clinical outcomes in SAB. After adjusting for host factors, we found an independent association between vancomycin tolerance and clinical failure in SAB, irrespective of methicillin susceptibility and antibiotic choice. While previous researchers have detailed the importance of

vancomycin bactericidal activity in this setting, data supporting the clinical relevance of vancomycin tolerance, particularly the ≥ 32 MBC/MIC breakpoint, are limited to a single case series.[17] Antibiotic tolerance is well-documented in the literature and data from previous studies in other settings suggest that tolerance is likely not just an *in vitro* phenomenon, but may have clinical implications.[17,18] Notably, Rahal *et al.* noted an association between β-lactam tolerance and increased duration of clinical symptoms of infection, despite patients not requiring additional antimicrobial agents to eventually achieve cure.[18] This is similar to our findings in that microbiological cure was achieved relatively quickly in both the vancomycin tolerant and non-tolerant group, but clinical signs and symptoms persisted longer when tolerance was observed *in vitro*, ultimately leading to a higher rate of clinical failure.

The "paradoxical effect" is an *in vitro* phenomenon commonly encountered in bactericidal activity testing for cell wall-active agents.[21] Observation of this phenomenon is credited to Eagle and colleagues, in which they observed that *S. aureus* was paradoxically killed more slowly at higher concentrations of benzylpenicillin than at concentrations slightly above the MIC.[27] A similar effect has been observed with vancomycin in MRSA *in vitro* and represents a potential mechanism of vancomycin tolerance.[28] It is believed that at lower inhibitory concentrations, vancomycin acts simply by binding to C-terminal D-alanyl-D-alanine residues, blocking the transglycosylation reaction required for cell wall synthesis.[28] However, at concentrations of 12 μg/mL or greater, a transient vancomycin intermediate *S. aureus* (VISA)-like phenomenon occurs in which vancomycin binding consequently blocks access of murein hydrolases to substrates, leading to inhibition of the cell wall autolytic system and vancomycin tolerance.[28] If tolerance can be induced at vancomycin concentrations that would be observed *in vivo*, it is plausible that this may have clinical implications in the treatment of staphylococcal

infections, including SAB.[29,30] The observed increase in clinical failure associated with the vancomycin tolerant phenotype described in the present study support this hypothesis.

It is important to note that although vancomycin tolerance was associated with increased clinical failure in this cohort, a difference in 30-day all-cause mortality was not observed. Rather, it appears that mortality may even be lower when vancomycin tolerance is observed *in vitro*. The heteroresistant VISA (hVISA) phenotype has been independently associated with increased treatment failure and persistent bacteremia, yet decreased mortality in SAB.[3,31] This phenomenon appears to be due to alterations in the accessory gene regulator (*agr*) controlling for virulence in *S. aureus.*[32] In a study of clinical MRSA blood isolates, the *agr* group II genotype was associated with reduced vancomycin bactericidal activity (MBC/MIC $\geq$ 8 μg/mL) in time-kill assays.[24] Although we did not perform *agr* genotyping in this study, the lack of an observed mortality increase despite high rates of clinical failure suggest a possible interplay between *agr* genotype, virulence, and vancomycin tolerance that needs to be further explored.

Previous researchers have hypothesized that conflicting results from studies examining the relationship between elevated vancomycin MIC and clinical outcomes in SAB may be partially explained by unmeasured phenotypic variation or reduced vancomycin bactericidal activity.[20,24] The observation that vancomycin tolerance, but not elevated vancomycin MIC by Etest was significantly associated with clinical failure in SAB is a novel and intriguing finding that supports this hypothesis. This observation may also be attributed to geographic variation in vancomycin MIC distributions and tolerance rates. Importantly, the frequency of vancomycin MIC $\geq$ 2 μg/mL by Etest that we observed was significantly less than other studies in which a difference in treatment failure was noted.[14] Therefore, although the relationship between elevated vancomycin MIC and clinical outcomes is well-established in the literature, it is also

possible that vancomycin tolerance may be a more reliable of clinical failure in SAB at some institutions.[14,33] As no association between vancomycin tolerance and elevated MIC was observed in this cohort, these phenotypes appear to be caused by distinct mechanisms. We observed vancomycin tolerance in 26.5% of clinical isolates, which is consistent with percentages described at some other institutions (range 10% to 43%; mean 20.1%).[22] Of note, we included patients with MSSA-B in our analyses due to the high prevalence of vancomycin tolerance in this population. Additionally, we used an enhanced methodology for MBC testing. Specifically, the cross-streaking technique described by Pelletier and Baker was used to reduce false negatives caused by antibiotic carryover, which is most commonly encountered at drug concentrations ≥4 x MIC.[21,34]

The finding that vancomycin tolerance is more prevalent among MSSA isolates is consistent with previous research.[23] This result is not surprising, as the mechanism of vancomycin tolerance is believed to result from phenotypic changes in the cell wall autolytic mechanism, independent of the *mecA* gene.[28,35] Holmes *et al.* recently described an association between elevated vancomycin MIC and increased 30-day mortality in patients with SAB; however, elevated vancomycin MIC was also predictive of mortality in those who received antistaphylococcal penicillin (flucloxacillin) therapy for MSSA-B.[15] As vancomycin and β-lactams both act at similar sites in the bacterial cell wall, it is not overly surprising that pathogenic changes resulting in elevated vancomycin MIC may also lead to decreased efficacy of other cell wall-active agents. Our finding that vancomycin tolerance was independently predictive of clinical failure regardless of methicillin susceptibility and antibiotic choice is suggestive of a similar effect with vancomycin tolerance.

This study was limited by its retrospective nature and relatively small sample size. In SAB, the definition of clinical failure is arbitrary and inconsistent across studies. We chose to use an inclusive definition which was a composite of multiple negative outcomes . The observed difference in clinical failure was driven primarily by persisting signs and symptoms of SAB ≥ 5 days while on antimicrobial therapy; however, this didn't translate into significant increases in mortality or length of stay in this cohort. It is not known whether this would hold true if adequate power was achieved to test these secondary outcomes. We believe the inclusion of both MSSA-B and MRSA-B cases and the use of an enhanced methodology for MBC testing that accounted for *in vitro* antibiotic carryover were important strengths of this study. Given the high prevalence of elevated vancomycin MIC and vancomycin tolerance in MSSA-B, we believe these cases should be included in future investigations of SAB.[15]

In summary, vancomycin tolerance, but not elevated vancomycin MIC, was significantly associated with clinical failure in SAB regardless of methicillin susceptibility or antibiotic choice. This association persisted even when adjusting for host factors in multivariable analysis. This finding adds to the growing body of evidence demonstrating the importance of bactericidal activity in SAB. Tolerance to vancomycin occurs irrespective of methicillin susceptibility and represents a clinically significant bacterial phenotype that warrants continued investigation. Future research is needed to determine optimal treatment of vancomycin tolerant *S. aureus* infections.

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