




Vancomycin MICs and risk of complicated bacteremia by glycopeptide-susceptible *Staphylococcus aureus*

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Abstract

Vancomycin (VAN) minimum inhibitory concentrations (MICs) at the upper end of the susceptible range for *Staphylococcus aureus* (*S. aureus*), as measured by the Etest method, have been associated with poor clinical outcomes of *S. aureus* bloodstream infections, as has the isolate's genetic background. Here, we assessed the impact of VAN MICs, as determined by a broth microdilution method (BMD) that incorporates incremental VAN concentrations between the conventional log₂ dilutions, isolate susceptibility to killing by human phagocytes, acting as a surrogate marker for bacterial cell wall thickness, and *S. aureus* genetic composition, on the development of complicated *S. aureus* bacteremia (SAB). We carried out a retrospective, observational single-center cohort study of 148 consecutive patients with SAB caused by methicillin-susceptible (MSSA) isolates ($n = 113$) or methicillin-resistant (MRSA) isolates ($n = 35$). *S. aureus* isolates were genotyped using a commercially available DNA microarray. Overall, VAN MICs of *S. aureus* isolates taken from complicated and uncomplicated SAB were comparable, irrespective of the testing method ($P = 0.19$ with BMD, and $P = 0.94$ with Etest). Likewise, *S. aureus* isolates in both comparison groups had the same susceptibility to killing by human phagocytes ($P = 0.5$). Among the genes screened by the *S. aureus* DNA array, only *Sec* and *Sel* were differentially present among *S. aureus* isolates in both groups (overrepresented in those causing complications) and their presence was associated independently with complicated SAB in multivariate models adjusted for potentially relevant clinical covariates. Separate analysis of MSSA SAB episodes yielded similar results.

Keywords Vancomycin · Minimum inhibitory concentration (MIC) · *Staphylococcus aureus* · Broth microdilution method · Complicated bacteremia

Introduction

The debate as to whether or not *Staphylococcus aureus* (*S. aureus*) vancomycin (VAN) minimum inhibitory concentration (MIC) at the upper end of the susceptible range impacts adversely on clinical outcomes of methicillin-resistant

(MRSA) and methicillin-susceptible (MSSA) *S. aureus* bloodstream infections remains unsettled [1–20]. Most studies supporting this assumption used the Etest method for VAN MIC assessment, uniformly showing that bacteremic infections caused by *S. aureus* isolates displaying MICs ≥ 1.5 mg/L are associated with an increased risk of complications, mortality, or both [1, 3, 4, 6, 8, 16, 18]. Nevertheless, *S. aureus* VAN MICs provided by the Etest method are poorly reproducible and lack precision [21], potentially hampering comparison of experiences across centers. In this context, we recently developed a broth microdilution method (BMD) that incorporates incremental VAN concentrations within the susceptible range, not only between the conventional log₂ dilutions, but also between those on the Etest scale, which permits robust and precise measurement of VAN MIC [22], and in our view may contribute toward elucidating the true relevance of this *S. aureus* phenotypic trait in clinical outcomes.

The possibility that high *S. aureus* VAN MICs, as measured by Etest, reflect a phenotypic advantage in terms of

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virulence has been postulated [4, 6]. In this sense, hVISA and *S. aureus* VAN-resistant strains exhibit a thicker cell wall and as a result may be suboptimally phagocytosed [23, 24]. In line with this, we previously found an inverse relationship between the susceptibility of the isolates to killing by human phagocytes and cell wall thickness for VAN-susceptible *S. aureus* isolates [25], thus suggesting that the killing index may be used as a surrogate marker for this structural bacterial phenotypic feature. Furthermore, a number of studies suggest that the ability of *S. aureus* to cause complicated bacteremia is notably influenced by the isolate's genetic background [26–29]. In this context, a number of specific resistance and virulence genes have been linked to elevated VAN MICs in the Etest [26–29]. Here, we assessed the impact of VAN MICs as measured by our BMD method, as well as isolate susceptibility to killing by human phagocytes and bacterial genetic characteristics, on the development of complicated *S. aureus* bacteremia (SAB).

Patients and methods

Study population

We carried out a retrospective, observational single-center cohort study of 148 consecutive patients (median age, 66.5 years; range, 1 to 91 years), of which 139 were adults (≥ 18 years old) and 9 children, with bloodstream infections caused by *S. aureus* within the period January 2013 to May 2018. Only patients with first episodes of SAB were considered. An infectious disease specialist (RO) reviewed all chart information focusing on demographics and relevant clinical data including age, sex, source of bacteremia, co-morbid conditions, antibiotic treatment, and outcome. The study was approved by the Ethics Committee of the Hospital Clínico Universitario INCLIVA, which waived requirement of informed consent.

Bacterial isolates

A total of 148 *S. aureus* isolates (MSSA, $n = 113$; MRSA, $n = 35$) recovered from first positive blood cultures (BACTEC™ FX Instrument; Beckton Dickinson-BD-, Sparks, MD) from unique patients at the Microbiology Service of the Hospital Clínico Universitario of Valencia were evaluated in this study. *S. aureus* were primarily identified by colony morphology, Gram staining, and a positive slide coagulase test [21]. Bacterial identity was confirmed by MALDI-TOF MS analysis. MRSA were detected by the standard disc diffusion method using a 30-mg cefoxitin disc (BBL Microbiology Systems, Cockeysville, MD, USA). All primary isolates had been cryopreserved at -70 °C in glycerol containing Bacto-Brain Heart Infusion broth-BHI- (BD) and were retrieved for the analyses described herein.

Determining *Staphylococcus aureus* vancomycin MIC values

Frozen stocks were thawed and the isolates were subcultured overnight on trypticase soy agar plates with 5% sheep's blood (BD). VAN MICs were measured using in-house prepared BMD panels that included the following antibiotic concentrations: 0.50, 0.62, 0.75, 0.87, 1.0, 1.25, 1.40, 1.50, 1.60, 1.75, and 2.0 $\mu\text{g/ml}$ in cation-adjusted Mueller-Hinton broth [22], and by Etest, which was performed using a standard procedure as previously reported [21]. All isolates were screened for VAN heteroresistance (hVISA) at time of isolation using the Etest macromethod, performed using a 2.0 McFarland inoculum on BHI agar plates (BD), using vancomycin and teicoplanin Etest strips (bioMérieux) [21]. Heteroresistance by the Etest macromethod was defined as MICs for vancomycin and teicoplanin of ≥ 8 mg/L or a teicoplanin MIC of ≥ 12 mg/L regardless of the vancomycin MIC.

Staphylococcus aureus killing assay

These experiments were performed following a published protocol [23]. Briefly, whole blood was obtained from healthy volunteers by venipuncture using heparinized syringes (Lithium Heparin, BD). An aliquot of heparinized blood was centrifuged 5 min at $3000\times g$ to obtain the plasma fraction. Primary subcultures of the isolates were made on sheep's blood agar plates (BD). Bacterial cells were grown at 37 °C to the exponential growth phase and then collected, washed in phosphate-buffered saline pH, 7.4 (PBS), and resuspended in RPMI 1640 culture medium (Gibco, Barcelona, Spain). Cells (0.5×10^6) were inoculated in parallel into 100 μl of human whole blood or plasma and incubated for 1 h at 37 °C. At the desired times (t_0 and t_1), samples were diluted in pyrogen-free distilled water (pH 4.5–6.5), left for 5 min at room temperature, and vortexed vigorously for ~ 5 s. Viable bacterial cells were determined by plating serial dilutions (in triplicate) on Mueller Hinton agar followed by overnight incubation at 37 °C. The survival percentage was calculated as follows: % CFU in blood = [CFU blood (1 h) / CFU blood (0 h)] $\times 100$; % CFU in plasma = [CFU plasma (1 h) / CFU plasma (0 h)] $\times 100$; % survival = (% CFU in blood / % CFU plasma) $\times 100$; % killing =: $100 - \% \text{ survival}$. Low and high susceptibility to killing were defined as % killing $< 60\%$ and $> 70\%$, respectively [25].

Staphylococcus aureus genetic composition analysis

S. aureus isolates were genotyped using the *S. aureus* DNA microarray from Clondiag (*S. aureus* Genotyping Kit 2.0 Jena, Germany), strictly following the manufacturer's recommendations, and as previously detailed [23]. Extraction of bacterial DNA from primary subcultures on sheep's blood

agar plates (BD) was performed using DNA extraction reagents and the EZ1 automated device (Qiagen, CA, USA).

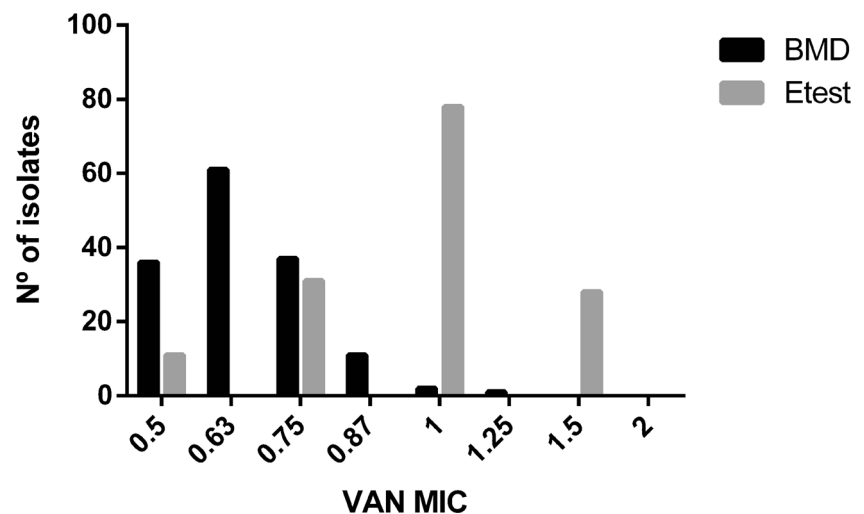
Clinical outcomes

The main outcome variable was the development of complicated SAB, which was defined by the following events, as previously reported [4, 6, 11, 28]: (i) endocarditis, (ii) septic thrombophlebitis, (iii) metastatic infection, (iv) persistent bacteremia for > 72 h after initiating active antimicrobial drug therapy, and (v) recurrent bacteremia, defined as the presence of subsequent blood cultures positive for *S. aureus* within 30 days of the index blood culture after initial clearance of bacteremia. Nosocomial and community SAB were those diagnosed after at least or within 48 h after hospital admission, respectively. Empirical (first-line) treatment was defined as any antibiotic regimen administered within 48 h after bloodstream infection diagnosis in the absence of antimicrobial susceptibility data.

Statistical analysis

The Mann-Whitney *U* test was used to compare continuous variables with non-normal distribution. Correlation between variables was assessed by the Spearman Rank test. The chi-square test was used to compare proportions. All statistical tests were two-tailed and the threshold for statistical significance was $P < 0.05$. To analyze the risk factors for developing complicated bacteremia, we performed a multivariate forward stepwise logistic regression model including all the clinically relevant variables with a *P* value of < 0.05 and possible confounding factors with a *P* value of < 0.1 detected in the univariate analysis. All the statistical analyses were performed using SPSS version 20.0 (SPSS, Chicago, IL, USA).

Fig. 1 *Staphylococcus aureus* (*S. aureus*) vancomycin (VAN) MIC values measured by broth microdilution (BMD) and Etest



Results

Staphylococcus aureus bacteremia episodes

A total of 148 episodes of SAB were included in the current study. Of these, 113 were caused by MSSA and 35 by MRSA. There were 104 community-acquired episodes and 44 nosocomial episodes. The probable sources of SAB were low respiratory tract infection ($n = 34$; 22.3%), skin and soft tissues ($n = 26$; 17.6%), vascular catheter ($n = 25$; 16.9%), intra-abdominal ($n = 15$; 10.1%), urinary tract ($n = 15$; 10.1%), and osteoarticular ($n = 13$; 8.8%) and surgical site infection ($n = 5$; 3.4%). The primary site of infection was unknown in the remaining 15 patients (10.1%). Thirty-seven patients (25%) developed complicated SAB; in detail, 11 patients had suppurative thrombophlebitis, 10 metastatic osteoarticular infection, 7 persistent bacteremia, 4 recurrent bacteremia, 4 endocarditis, and 1 embolic infection.

Staphylococcus aureus vancomycin MIC values

All *S. aureus* isolates were susceptible to vancomycin (≤ 2 mg/L). No isolate was categorized as hVISA. VAN MIC values of the isolates as determined by BMD and Etest are shown in Fig. 1. Modal vancomycin BMD MIC was 0.63 mg/L for both MSSA and MRSA isolates. Modal Etest MIC value was 1 mg/L for both MSSA and MRSA isolates. Poor correlation was seen between VAN MIC values measured by BMD and Etest ($\rho = 0.291$). *S. aureus* isolates in our series were genetically diverse, as inferred by the wide variety of clonal complex (CC) types documented (21 types). The most prevalent clonal types were CC5 ($n = 39$), CC30 ($n = 19$), CC8 ($n = 15$), CC22 ($n = 12$), CC45 ($n = 9$), and CC398 ($n = 8$). As shown in Fig. 2, *S. aureus* VAN MIC values were comparable across the clonal complex types, regardless of the method

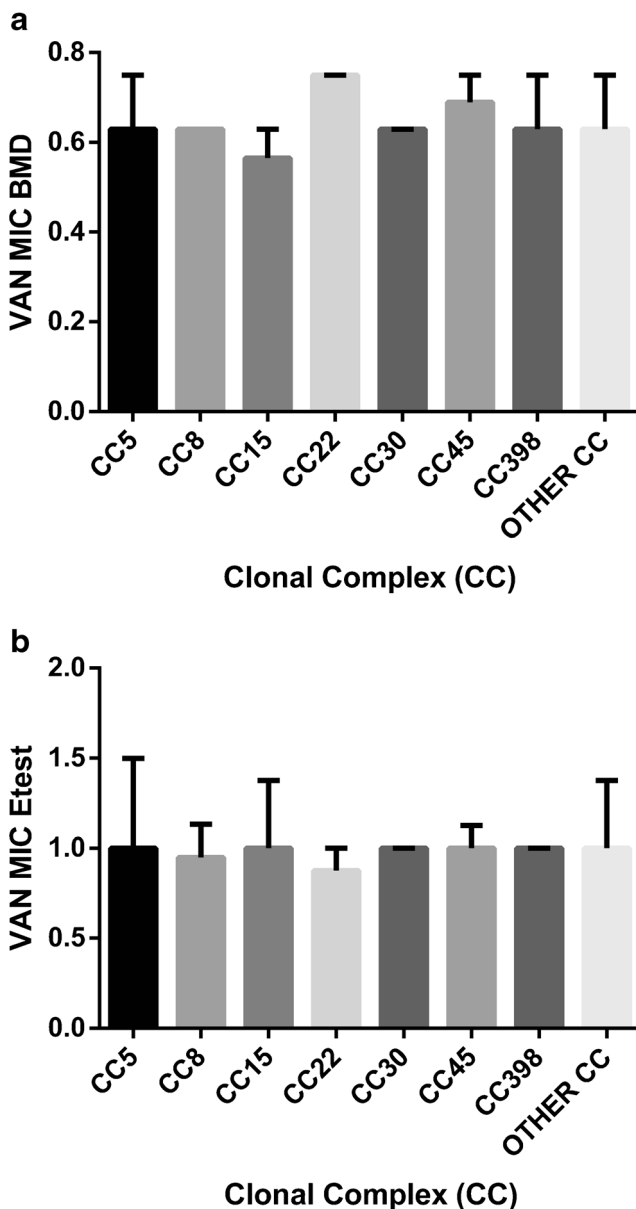


Fig. 2 *Staphylococcus aureus* (*S. aureus*) vancomycin (VAN) MIC values measured by broth microdilution (BMD) (panel a) and Etest (panel b) according to *S. aureus* clonal type (CC)

employed for VAN MIC determination ($P=0.61$ with BMD and $P=0.51$ with Etest).

Phenotypic and genotypic features of *Staphylococcus aureus* isolates and development of complicated bacteremia

Overall, VAN MICs of *S. aureus* isolates recovered from complicated and uncomplicated bacteremia episodes were comparable (Fig. 3), irrespective of the testing method ($P=0.19$ for BMD and $P=0.94$ for Etest). Likewise, as shown in Fig. 4, *S. aureus* isolates in both comparison groups had the same susceptibility to killing by human phagocytes ($P=0.5$). In

fact, ROC curve analyses failed to identify a cut-off value for both parameters to discriminate reliably between *S. aureus* isolates either involved or not involved in complicated bacteremic episodes (not shown). These observations stood when MSSA isolates were analyzed separately ($P=0.68$ with BMD and $P=0.77$ with Etest; $P=0.71$ for the killing index). *S. aureus* isolates in the comparison groups were not significantly different regarding clonal complex type, capsular type, or accessory gene regulator (*agr*) group (Table 1). Among the genes screened by the *S. aureus* DNA array, only *Sec* and *Sel* were differentially present among *S. aureus* isolates in both groups (overrepresented in those causing complications; $P=0.01$) (Table 1 and Supplementary Table 1).

Risk factors for developing complicated *Staphylococcus aureus* bacteremia

In addition to *S. aureus* phenotypic features (VAN MIC value and killing index) and genetic traits found to be associated with complications in frequency comparison analyses (Table 1), several clinical covariates with potential relevance were recorded in order to assess differences between comparison groups, as well as to control for potential confounding factors by logistic regression modeling. Clinical covariates included patient's demographics, acquisition category (community vs. nosocomial) co-morbidities (measured by the Charlson comorbidity index), severity of illness (presence of septic shock at bacteremia onset), source of bacteremia, suitability of first-line antibiotic therapy, overall antibiotic use, and clinical management of the primary source of bacteremia. Note that (i) initial antibiotic therapy was deemed appropriate in 50% of episodes and the median delay in starting it was 1.5 days (IQR, 0–3 days); (ii) β -lactam antibiotics were administered in 86 episodes (52.8%) and glycopeptides in 50 episodes (33.7%). As shown in Table 2, the presence of *Sec* and *Sel* genes was the only factor independently associated with the occurrence of complicated SAB (OR, 3.45; 95% CI 1.26–9.45; $P=0.02$). When considering only MSSA isolates for the analyses, both the presence of *sec* and *sel* genes and the use of non β -lactam agents were associated with increased risk of complicated SAB (Supplementary Table 2).

Discussion

Elucidation of the true impact of glycopeptide-susceptible *S. aureus* VAN MIC values on SAB outcomes is of indisputable relevance, as it may critically influence therapeutic management of this clinical entity. VAN MIC values at the upper end of the susceptibility range (≥ 1.5 mg/L), using the Etest method, have been associated with higher morbimortality rates than those caused by highly susceptible strains (MIC ≤ 1 mg/mL) [1, 3, 4, 6, 8, 16, 18], although this assumption has

Fig. 3 *Staphylococcus aureus* (*S. aureus*) vancomycin (VAN) MIC values measured by broth microdilution (BMD) (panel **a**) and Etest (panel **b**) in isolates either involved or not involved in complicated bacteremia episodes (SAB). Data restricted to methicillin-susceptible (MSSA) isolates are shown in panels **c** and **d**

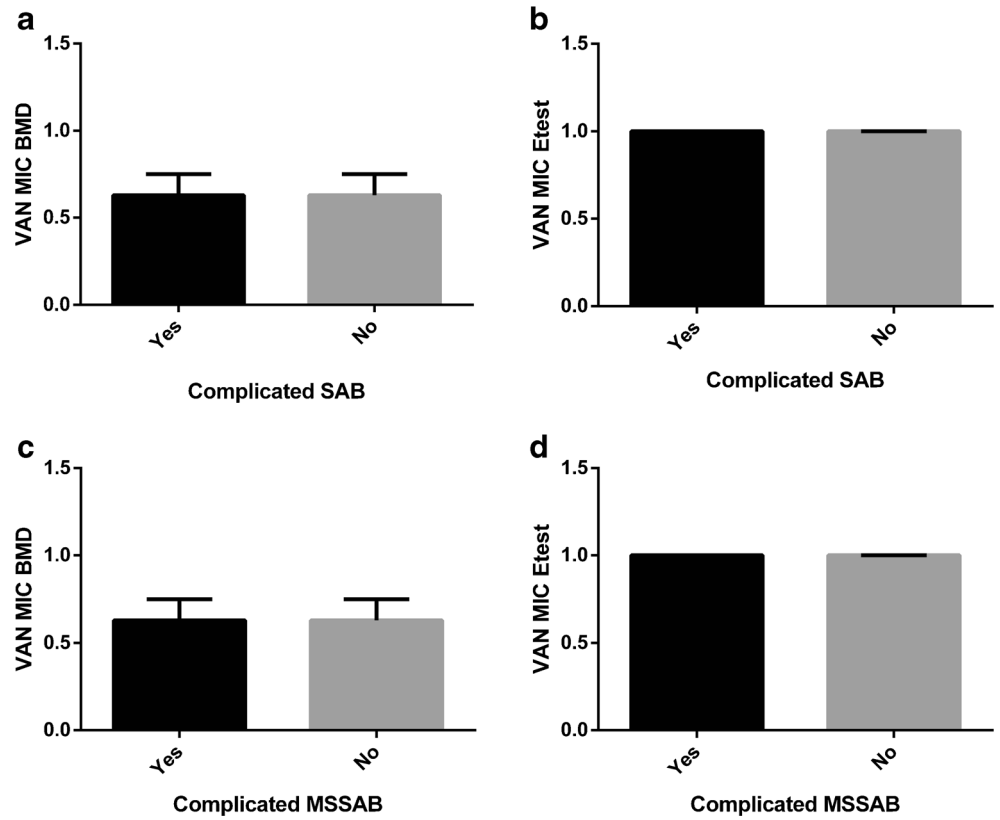


Fig. 4 Distribution of killing indices in *Staphylococcus aureus* isolates (**a**) and **b** killing indices of *Staphylococcus aureus* isolates either involved or not involved in complicated bacteremia episodes (SAB). Data restricted to methicillin-susceptible (MSSA) isolates are shown in panels **c** and **d**

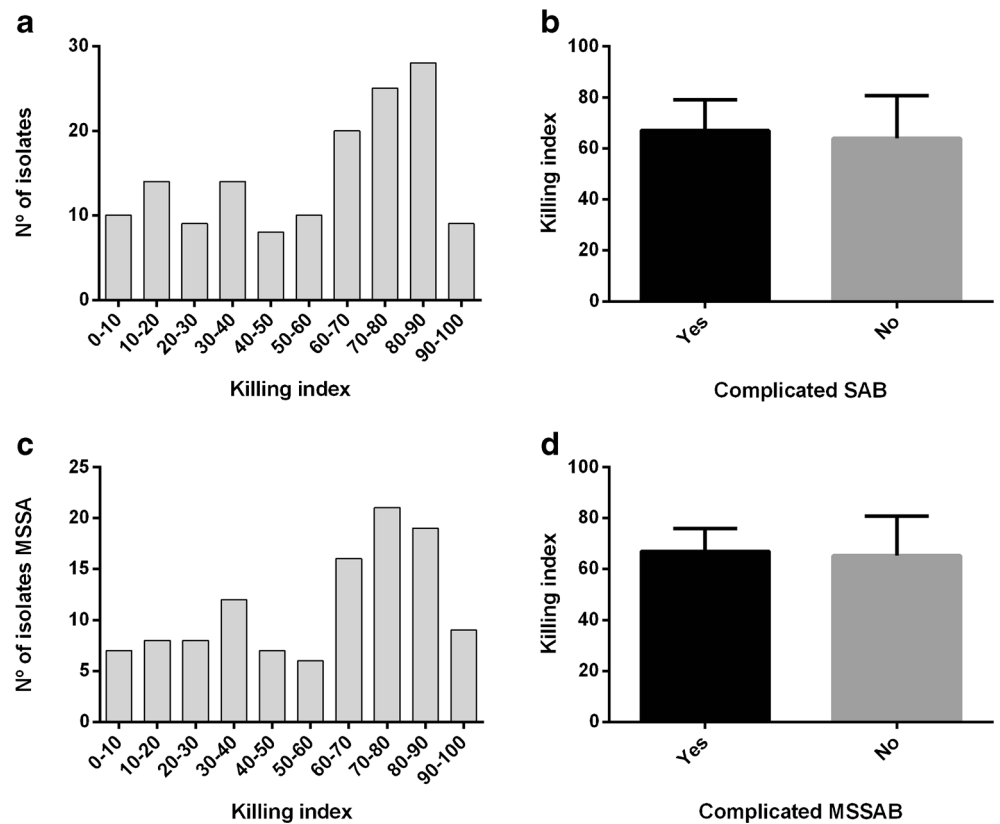


Table 1 Genetic characteristics of *Staphylococcus aureus* isolates recovered from patients with or without complicated bacteremia

<i>Staphylococcus aureus</i> genetic characteristics	Complicated bacteremia. Number of isolates (%) (<i>n</i> = 37)	Uncomplicated bacteremia. Number of isolates (%) (<i>n</i> = 111)	<i>P</i> value
Accessory gene regulator (<i>agr</i>) group ^a			
I	15 (40.5)	43 (38.7)	0.97
II	13 (35.1)	40 (36.6)	0.78
III	6 (16.2)	19 (17.1)	0.81
IV	2 (5.4)	2 (1.80)	0.25
Clonal Complex (CC) ^b			
CC5	11 (29.7)	28 (25.2)	0.69
CC8	6 (16.2)	10 (9.0)	0.26
CC15	0 (0)	4 (3.6)	0.23
CC22	4 (10.8)	8 (7.20)	0.54
CC30	4 (10.8)	15 (13.5)	0.61
CC45	3 (8.1)	7 (6.3)	0.76
CC398	3 (8.1)	5 (4.5)	0.44
Other CC	6 (16.2)	30 (27.0)	0.31
Genes ^c			
<i>Sec</i> and <i>Sel</i>	10 (27.0)	11 (9.9)	0.01

Sec and *sel*, Enterotoxin C and L.

^a The *agr* group could not be determined for 8 isolates

^b The clonal complex type could not be determined for 5 isolates

^c Only genes differentially expressed in isolates linked to complicated *Staphylococcus aureus* bacteremia are shown

been disputed [11, 12]. In our view, the Etest method is inappropriate for evaluating the attributable impact of *S. aureus* VAN MICs on outcomes in any clinical setting, owing to the method's low reproducibility and lack of precision [21]; in fact, *S. aureus* VAN MIC values measured by Etest correlate poorly with those determined by BMD and agar dilution assays, the methods of choice according to international agencies (i.e., Clinical Laboratory Standard Institute-CLSI, and the European Committee on Antimicrobial Susceptibility Testing-EUCAST-) [30–32] and are uniformly reported to be higher. We recently developed a BMD method incorporating several “inter log₂ dilution” concentrations of VAN, which permits a robust and precise determination of VAN MICs with intra- and inter-assay CVs below 20%, which could be instrumental for shedding light on the above issue [22]. Here, we found that *S. aureus* VAN MIC values within the susceptible range measured with our BMD method were not predictive of complicated SAB as defined herein, either as a continuous or dichotomous variable, in a series including mostly community-acquired episodes originating from a highly heterogeneous source and caused by clonally diverse isolates. In our experience, *S. aureus* VAN MIC values measured by Etest (≥ 1.5 mg/L vs. ≤ 1 mg/mL) were also not associated with developing SAB complications, in line with previous reports [11, 12]. The limited number of death events in this series

precluded any statistically meaningful analysis as to the potential effect of *S. aureus* VAN MIC values on mortality.

A few studies have assessed the relationship between *S. aureus* VAN MICs, as measured by BMD methods, and complicated SAB, most failing to find an association. Specifically, Rojas and colleagues [11] found no correlation between VAN MIC values (high, > 1 mg/L; low, ≤ 1 mg/L), measured by an in-house BMD method, and the development of metastatic infection or persistent bacteremia caused by MRSA isolates. Adani et al. [15] reported that VAN MIC values (high levels 2 mg/L; low levels ≤ 2 mg/L), as determined by a commercially available automated BMD method (MicroScan; Beckman Coulter, West Sacramento, CA, USA) were not predictive of persistent or recurrent bacteremia due to MRSA. Baxi et al. [33] reported that high (2 mg/L vs. low, ≤ 2 mg/L) VAN MIC values determined by Microscan were not associated with greater risk of a composite outcome of mortality, readmission, and recurrence in a series including cases due to MSSA and MRSA. Likewise, San-Juan et al. [18], in a study including catheter-related MSSA bacteremia episodes, found no significant differences between isolates either involved or not involved in the development of endocarditis when VAN susceptibility testing was performed using commercial BMD panels (ESTEN 2009, Sensititre™, Izasa, Barcelona, Spain), irrespective of whether VAN MIC values

Table 2 Risk factors for *Staphylococcus aureus* complicated bacteremia: univariate and multivariate analysis

Factor	Univariate		Multivariate	
	OR (95%CI)	P value	OR (95%CI)	P value
Age (> 70 vs. ≤ 70 years)	0.45 (0.21–0.994)	0.048	–	NS
Sex (male vs. female)	1.08 (0.505–2.30)	0.85		
Charlson Index (≥ 3 vs. < 3)	0.858 (0.35–2.10)	0.74		
Comorbidity				
Diabetes mellitus	0.85 (0.33–2.16)	0.73		
Chronic pulmonary disease	1.80 (0.50–6.59)	0.37		
Malignancy	0.57 (0.20–1.61)	0.29		
Chronic renal disease	0.80 (0.28–2.36)	0.69		
Acquisition (community vs. hospital)	0.84 (0.38–1.88)	0.68		
Inappropriate first-line (empirical) therapy ^a	0.98 (0.36–2.74)	0.89		
Acquisition (community vs. hospital)	0.84 (0.38–1.88)	0.68		
Antibiotic use				
Glycopeptides	1.08 (0.49–2.37)	0.84		
β-lactams	0.93 (0.44–1.97)	0.85		
Other agents ^b	2.472 (1.11–5.48)	0.03	–	NS
Septic shock	3.18 (0.61–16.48)	0.17		
Inadequate clinical management ^c	4.81 (0.77–29.98)	0.09	–	NS
Meticillin resistance	2.20 (0.97–4.98)	0.06	–	NS
Vancomycin MIC (mg/L) ^d	1.00 (0.10–9.92)	1.00		
BMD (≥ 0.5 mg/L vs. < 0.5 mg/mL)	1.00 (0.42–2.38)	1.00		
BMD ≥ 0.75 mg/mL vs. < 0.75 mg/mL)	1.11 (0.39–3.12)	0.85		
Etest (≥ 1.5 mg/mL vs. < 1.5 mg/mL)	1.25 (0.5–3.15)	0.63		
Killing index (%) ^e	1.63 (0.66–4.12)	0.30		
Killing index (< 60% vs. 70%)	1.18 (0.53–2.63)	0.68		
<i>Sec</i> and <i>Sel</i> (presence vs. absence)	3.20 (1.23–8.33)	0.02	3.45 (1.26–9.45)	0.02

BMD broth microdilution, OR odds ratio, *Sec* and *Sel* Enterotoxin C and L, respectively

^a Use of beta-lactam agents for MRSA bacteremia, use of ciprofloxacin, rifampin, or piperacilin/tazobactam, or no empirical treatment

^b Ciprofloxacin, clindamycin, daptomycin, gentamicin, levofloxacin, linezolid, meropenem, and rifampin

^c Inadequate clinical management: (i) lack of catheter removal within 72 h of bacteremia onset ($n = 2$); (ii) lack of drainage of primary infection site ($n = 3$)

^d VAN MIC values analyzed as a continuous variable

^e Killing index values analyzed as a continuous variable

were analyzed as a continuous or dichotomized (> 1.0 ≤ 1.0 mg/L) variable. In contrast, Sullivan et al. [20] reported that SAB due to reduced VAN susceptibility, as defined by Microscan MIC of 2 mg/L, was associated with increased risk of complications in adjusted analyses. The above studies used either commercially available or in-house prepared panels, and all employed conventional BMD methods using serial twofold doubling dilutions of antimicrobials. Between- and within-run variation of 1 log₂ dilution is considered acceptable for BMD methods by international agencies (CLSI and EUCAST). Thus, the lack of margin for VAN MIC thresholds defining low vs. high susceptibility in these studies makes the possibility of isolate miscategorization relatively high.

Based on previous observations [23, 24], Aguado and colleagues hypothesized that high *S. aureus* VAN MIC levels, as determined by Etest, may reflect certain structural modifications (i.e., thicker cell wall), which may result in increased bacterial pathogenicity [4]. In this respect, we previously showed that *S. aureus* isolates' susceptibility to killing by human phagocytes in a relatively simple in vitro assay may be used as a surrogate marker for *S. aureus* cell wall thickness, which requires a rather elaborate measurement protocol involving the use of electronic microscopy [25]. We specifically found the cell wall to be significantly thicker in isolates displaying low killing indices (< 60%) than in ones exhibiting high killing indices (> 70%), irrespective of their

susceptibility to methicillin [25]. In this study, we found no association between the killing group to which the isolates belonged and the development of complicated SAB, thus indirectly suggesting that cell wall thickness might not be a relevant pathogenetic factor accounting for increased risk of these clinical events. Naturally, the true relevance of cell wall thickness as a virulence trait in this clinical setting can only be elucidated by electronic microscopy assessment.

The isolate's genetic background has been suggested as modulating the ability of *S. aureus* to cause complicated bacteremia; namely, *S. aureus* isolates belonging to certain genotypic (clonal) types, such as CC5, CC8, CC12, CC15, CC20, CC30, or CC45, have been shown to exhibit a significant trend toward increasing levels of persistent bacteremia or endocarditis [17, 26, 28, 29, 34–38]. Nevertheless, in line with previous reports [12, 19, 27, 39, 40], we failed to find any such associations. Of interest, CCs associated with complicated SAB in the aforementioned studies were well represented in our panel (almost 50%). In addition to substantial differences among cohorts in terms of patients' clinical characteristics and distribution of MSSA and MRSA isolates, a variety of genotypic typing methods were used in these studies, which may account, at least in part, for the discrepant findings.

Conflicting data have been published on the potential association between *S. aureus* genotypic traits including the *agr* type, capsular type, and a wide variety of bacterial genes and the development of complicated SAB [11, 12, 17, 19, 26–29, 34, 35, 37, 38, 40]. Here, only *sec* and *sel* genes (coding for enterotoxin C and L, respectively) were found to be differentially present among *S. aureus* isolates depending on whether they caused complicated SAB or not (overrepresented in the former isolates). Furthermore, the presence of *Sec* and *Sel* genes was the only factor independently associated with complicated SAB in multivariate models adjusted for potentially clinical relevant covariates including patient's demographics and co-morbidities, severity of illness, source of bacteremia, management of the primary source of bacteremia, suitability of first-line antibiotic therapy, and overall antibiotic use. As for the latter variable, as expected, use of antibiotics other than β -lactams and glycopeptides was also independently associated with complicated SAB caused by MSSA. Enterotoxins C and L exhibit superantigenic properties [41]. Superantigens bind simultaneously to MHC class II (MHC-II) and TCRs, outside their antigen-binding groove, resulting in an excessive inflammatory response that can lead to toxic shock, multiorgan failure, and death [42]. The pathogenetic relevance of *S. aureus* enterotoxins in the development of endocarditis has also recently been suggested [43]. Notably, *S. aureus* VAN MIC values were significantly lower in isolates harboring the *sec* and *sel* genes than ones in which these genes were absent (not shown), further reinforcing our assumption that VAN MIC values within the susceptibility range may not impact on the risk of complicated SAB.

The current study has a number of limitations deserving mention: (i) clinical data were retrospectively collected and thus possibly subject to information bias; (ii) our single-center design precludes generalizing the study's conclusions to other communities; (iii) the relatively scarce number and heterogeneity of SAB complications in our series may have resulted in a high probability of type II errors and prevented us from performing robust multivariate analyses; (iv) different sources of SAB; (v) occurrence of recurrent SAB was only assessed through inpatient hospitalization at our center; (vi) conclusions regarding the impact of antibiotic therapy should be taken cautiously due to lack of control over choice of antibiotics and lack of data on vancomycin serum levels; (vii) cryopreserved isolates were used for *S. aureus* VAN MICs testing; in this sense, we are not certain how long-term storage may impact on MIC values measured by our BMD method. In summary, the data presented herein argue against a potential impact of VAN MIC values on risk of complicated bacteremia due to glycopeptide-susceptible *S. aureus*, regardless of isolate susceptibility to methicillin. In addition, although firm conclusions on the relevance of *sec* and *sel* genes as pathogenetic factors predisposing to complicated SAB cannot be drawn here, our data warrant further studies to explore the possibility.

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Compliance with ethical standards

Transparency declarations None to declare

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