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Genetic and Molecular Predictors of High Vancomycin MIC in *Staphylococcus aureus* Bacteremia Isolates

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An elevated vancomycin MIC is associated with poor outcomes in *Staphylococcus aureus* bacteremia (SAB) and is reported in patients with methicillin-susceptible *S. aureus* (MSSA) bacteremia in the absence of vancomycin treatment. Here, using DNA microarray and phenotype analysis, we investigated the genetic predictors and accessory gene regulator (*agr*) function and their relationship with elevated vancomycin MIC using blood culture isolates from a multicenter binational cohort of patients with SAB. Specific clonal complexes were associated with elevated (clonal complex 8 [CC8] [$P < 0.001$]) or low (CC22 [$P < 0.001$], CC88 [$P < 0.001$], and CC188 [$P = 0.002$]) vancomycin MIC. *agr* dysfunction ($P = 0.014$) or *agr* genotype II ($P = 0.043$) were also associated with an elevated vancomycin MIC. Specific resistance and virulence genes were also linked to an elevated vancomycin MIC, including *blaZ* ($P = 0.002$), *sea* ($P < 0.001$), *clfA* ($P < 0.001$), *splA* ($P = 0.001$), and the arginine catabolic mobile element (ACME) locus ($P = 0.02$). These data suggest that inherent organism characteristics may explain the link between elevated vancomycin MICs and poor outcomes in patients with SAB, regardless of the antibiotic treatment received. A consideration of clonal specificity should be included in future research when attempting to ascertain treatment effects or clinical outcomes.

Multiple factors have been implicated in patients with poor outcomes from *Staphylococcus aureus* bacteremia (SAB), including treatment with vancomycin, particularly in methicillin-susceptible *S. aureus* (MSSA) bacteremia (1–4). Elevated vancomycin MIC has also been associated with treatment failure and mortality in a recent meta-analysis of methicillin-resistant *S. aureus* (MRSA) infections (5). We previously reported an association between elevated vancomycin MIC and 30-day mortality in patients with SAB (6). Interestingly, this association was observed even in patients with MSSA bacteremia treated with flucloxacillin, a finding also noted by other authors (7); this persisted despite adjustment for potential clinical confounders (8). Preliminary data on a small subset of our original cohort demonstrated a heterogeneity of genotypes among MSSA and methicillin-resistant *S. aureus* (MRSA) isolates and those with low or elevated vancomycin MICs (6). However, specific organism characteristics, such as resistance or virulence determinants and accessory gene regulator (*agr*) type or function, are increasingly linked with elevated vancomycin MIC. For example, MRSA strains with staphylococcal cassette chromosome *mec* element (SCC*mec*) types II/III typically have higher vancomycin MICs than strains with SCC*mec* types IV/V (9). *agr* type II has been associated with reduced vancomycin susceptibility (10), even in MSSA (11), and *agr* dysfunction has been associated with mortality (12) and other suboptimal outcomes (13). Specific genotypes have also been associated with elevated vancomycin MIC, such as non-CC22/30 clones in a study of MRSA bacteremia (14), or infection syndromes that have increased mortality, such as endocarditis or other invasive disease (14–16). Other organism characteristics, such as the presence of specific enterotoxins and adhesins, have been implicated in invasive infection and may potentially influence clinical outcomes (15).

We therefore performed a detailed microbial genetic characterization on a subset of bacterial isolates from our original cohort in order to identify links between organism factors and elevated

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vancomycin MIC that may in turn explain the increased mortality observed.

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MATERIALS AND METHODS

Study population. The study population was derived from a prospectively collected multicenter cohort of adult and pediatric patients with SAB in Australia and New Zealand, the Australian and New Zealand Cooperative on Outcomes in Staphylococcal Sepsis (ANZCOSS) (4). A substudy was previously created from eight geographically diverse hospitals that contributed to this collaboration, and additional clinical, microbiological, and pharmacological data were collected (6, 8). In brief, basic demographics from the initial cohort and detailed microbiologic testing of stored blood culture isolates (6) were combined with extended clinical data that analyzed detailed comorbidities, comorbidity burden using the Charlson comorbidity index, and disease severity scores using the Acute Physiology and Chronic Health Evaluation (APACHE) II, Simplified Acute Physiology Score (SAPS), and Pitt bacteremia scores (8) measured within 24 h of SAB onset.

Selection of bacterial isolates. The first (index) positive blood culture isolate from each patient in our original substudy was stored at −80°C, and vancomycin MICs using broth microdilution (BMD) and Etest were performed previously (6). Only isolates collected from patients for whom expanded clinical data were available were considered for inclusion in this analysis. We deliberately selected all blood culture isolates with very low vancomycin Etest MICs (0.38 to 0.75 mg/liter, $n = 15$) and very high vancomycin Etest MICs (2.0 to 3.0 mg/liter, $n = 134$) for further characterization. The remaining isolates ($n = 93$) comprised randomly selected isolates with a vancomycin Etest MIC of 1.0 to 1.5 mg/liter.

Outcome measures. The primary outcome was vancomycin Etest MIC, categorized into low (≤1.5 mg/liter) or elevated (>1.5 mg/liter) groups, as defined by our previous findings (6). Secondary outcomes included persistent bacteremia (defined as blood cultures positive for *S. aureus* taken ≥7 days after the index positive blood culture), and recurrent bacteremia (defined as a new episode of SAB after having documented negative blood cultures occurring within 30 days of the index positive blood culture) (18).

Delta-hemolysin assay for *agr* function. The *S. aureus* accessory gene regulator (*agr*) activity was measured by delta-hemolysin production on Trypticase soy agar with 5% sheep blood using *S. aureus* strain RN4220, as previously described (13, 19, 20). Evidence of enhanced hemolysis between RN4220 and a test isolate was considered positive for delta-hemolysin production (20). The presence of *agr* dysfunction was defined as an absence or severe depression of delta-hemolysin production (21). The positive control was JKD6159, a fully sequenced *S. aureus* reference isolate known to have a functional *agr* operon, and the negative control was TPS3105, a sequenced *S. aureus* isolate with defective *agr* function (22).

DNA microarray. The isolates were characterized using the StaphyType *S. aureus* DNA microarray (Alere Technologies, Jena, Germany). This microarray detects 334 target sequences from 185 genes and allelic variants, including resistance determinants and pathogenicity markers, and it infers the genotype according to clonal complex (CC) or sequence type (ST). The microarray was performed as per the manufacturer's instructions, with the arrays and reagents obtained from Alere (11, 23–25).

Genotyping. A phylogram was constructed with the microarray data using SplitsTree version 4.13.1 (26). The tree was constructed as an unrooted tree without any reference isolates in order to visualize the represented genotypes, and no inference of phylogenetic evolutionary direction was made.

Statistical analysis. Statistical analyses were performed using Stata version 11.1 (StataCorp, College Station, TX, USA). Categorical variables were compared using the χ^2 test or Fisher's exact test, while the Mann-Whitney U test was used for continuous variables. A P value of <0.05 was considered to be statistically significant. Statistical corrections for multiple comparisons were not performed, as this was a hypothesis-generating study to ascertain possible organism characteristics.

Ethics. Human ethics committee approval was obtained at each participating site.

RESULTS

Clinical characteristics. Two hundred forty-two isolates were tested, representing almost half (45.5%) of the 532 isolates collected from the original multicenter cohort. The demographics of the tested patient population (Table 1) revealed no statistically significant differences between patients in the low- or elevated-vancomycin MIC groups. There were no differences between the vancomycin MIC group and common clinical manifestations. The proportion of MRSA was overrepresented in the elevated-vancomycin MIC group compared with the low-vancomycin MIC group (52.2% versus 31.5%, $P = 0.001$).

Genotype. There were 25 different CCs among the 242 isolates. The DNA microarray was unable to assign a CC for one isolate. Greater clonal diversity was noted among the MSSA isolates than with the MRSA isolates (24 versus 10, respectively). Figure 1 shows the unrooted phylogenetic relationship between the isolates. CC8 was the most common genotype ($n = 69$), followed by CC5 ($n = 26$), CC22 ($n = 20$), CC45 ($n = 18$), and CC30 ($n = 15$). The isolate with an unassigned CC on the microarray clustered phylogenetically with the CC188 isolates. Two isolates clustered within the livestock-associated *S. aureus* CC398 (ST398 and ST291). There were differences in the vancomycin MIC groups according to CC (Fig. 2), with seven CCs having a geometric mean vancomycin MIC of >1.5 mg/liter (CC8, CC15, CC6, CC5, CC20, CC45, and CC1). Table 1 also identifies specific clones associated with low (CC22, CC88, and CC188) or elevated vancomycin MICs (CC8 and CC5), and Table 2 shows the vancomycin MIC values for the more common clonal complexes. Clones with higher geometric mean vancomycin MICs also had higher MIC_{50}s and MIC_{90}s (see Fig. S1 in the supplemental material). Notably, CC22 was predominantly composed of ST22-MRSA-IV (known as epidemic MRSA [EMRSA]-15) (19/20) and was exclusively associated with a low vancomycin MIC. CC22 was also negatively associated with persistent bacteremia ($P = 0.020$). The majority of the isolates within CC8 were ST239-MRSA-III (60/69), an epidemic health care-associated MRSA clone, most of which had elevated vancomycin MIC. These ST239-MRSA-III isolates originated from five different hospitals in diverse geographic regions in Australia and New Zealand. Staphylococcal chromosomal cassette (SCC) *mec* type II/III was associated with a high vancomycin MIC ($P < 0.001$) and was exclusively found in the isolates from CC8, whereas SCC*mec* type IV/V was associated with a low vancomycin MIC ($P < 0.001$) and predominantly comprised ST22-MRSA-IV isolates. CC8 was associated not only with a high vancomycin MIC (Table 1) but also persistent bacteremia (8/13, $P = 0.014$), recurrent bacteremia (9/17, $P = 0.025$), and sepsis syndrome ($P = 0.04$). CC188, a clone with a low vancomycin MIC, was associated with reduced 30-day mortality (0/72, $P = 0.026$) and community-onset infections (10/120, $P = 0.005$) and it comprised MSSA only. There were 11 different clones that caused endocarditis, and no single clone predominated.

***agr* dysfunction.** *agr* dysfunction was detected in more than half of the isolates (124/242 [51.2%]), with high rates in *agr* type III (34/48 [70.83%], $P = 0.002$) and lower rates in *agr* type II

TABLE 1 Demographics and clinical features of patients with SAB and microbial characterization of bacterial isolates according to low versus elevated vancomycin MIC

Variable[a]	Low MIC ($n = 108$)	Elevated MIC ($n = 134$)	P value[b]
Demographics			
Age (median [IQR]) (yr)	64 (44.5–77.5)	67.5 (50–76)	0.886
Male sex	81 (75.0)	91 (67.9)	0.227
Caucasian ethnicity	91 (84.3)	110 (82.1)	0.655
Hospital onset	48 (44.4)	74 (55.2)	0.095
Device-associated (no./total evaluable[c] [%])	47/103 (45.6)	65/129 (50.4)	0.471
MRSA	34 (31.5)	70 (52.2)	0.001
Vancomycin treatment	51 (47.2)	79 (59.0)	0.069
Relapse (no./total infected [%])	8/103 (7.77)	9/130 (6.9)	0.806
Persistent bacteremia (no./total evaluable[c] [%])	5/82 (6.1)	8/115 (7.0)	0.811
Clinical features			
DNR order (no./total evaluable[c] [%])	34/95 (35.8)	51/122 (41.8)	0.368
ICU admission (no./total evaluable[c] [%])	34/107 (31.8)	40/132 (30.3)	0.807
Infective endocarditis	8 (7.4)	8 (6.0)	0.655
Sepsis syndrome	14 (13.0)	18 (13.4)	0.915
Pneumonia	7 (6.5)	7 (5.2)	0.677
Osteoarticular	7 (6.5)	9 (6.7)	0.942
Skin and skin structure	13 (12.0)	23 (17.2)	0.265
Disease severity			
APACHE II score (median [IQR])	15 (10–19)	15.5 (11–20)	0.577
SAPS II score (median [IQR])	26 (19–35)	27 (20–37)	0.354
Charlson comorbidity index (median [IQR])	1 (0–2)	2 (1–3)	0.097
Pitt bacteremia score (median [IQR])	3 (1–3)	3 (1–3)	0.845
Genotype (n)[d]			
CC8 (69)	11 (10.2)	58 (43.3)	<0.001
CC5 (26)	7 (6.5)	19 (14.2)	0.055
CC22 (20)	20 (18.5)	0 (0)	<0.001
CC45 (18)	9 (8.3)	9 (6.7)	0.634
CC30 (15)	8 (7.4)	7 (5.2)	0.484
CC1 (14)	6 (5.6)	8 (6.0)	0.891
CC15 (11)	2 (1.9)	9 (6.7)	0.071
CC88 (11)	11 (10.2)	0 (0)	<0.001
CC188 (11)	10 (9.3)	1 (0.7)	0.002
agr type (n)			
I (146)	64 (59.3)	82 (61.2)	0.760
II (45)	14 (13.0)	31 (23.1)	0.043
III (48)	29 (26.9)	19 (14.2)	0.014
IV (3)	1 (0.9)	2 (1.5)	0.692
agr dysfunction present	45 (41.7)	79 (59.0)	0.007
Polysaccharide capsule			
cap5	42 (38.9)	27 (20.1)	0.001
cap8	64 (59.3)	107 (80.0)	<0.001
Antimicrobial resistance gene (antibiotic)			
blaZ (penicillin)	84 (77.8)	124 (92.5)	0.002
ermA (erythromycin)	10 (9.3)	63 (47.0)	<0.001
aacA-aphD (gentamicin)	3 (2.8)	58 (44.3)	<0.001
mupR (mupirocin)	0 (0)	1 (0.8)	0.368
tetK (tetracycline)	2 (1.9)	39 (30.0)	<0.001
fosB (fosfomycin)	42 (38.9)	107 (79.9)	<0.001
qacA (quaternary ammonia compounds)	12 (11.3)	50 (37.3)	<0.001
Leukocidins or exotoxins			
PVL	5 (71.4)	2 (28.6)	0.148
tst	7 (46.7)	8 (53.3)	0.870

(Continued on following page)

TABLE 1 (Continued)

Variable[a]	Low MIC ($n = 108$)	Elevated MIC ($n = 134$)	P value[b]
Enterotoxins			
sea	19 (19.4)	79 (80.6)	<0.001
seb	11 (10.9)	4 (3.0)	0.015
sec	23 (21.3)	2 (1.5)	<0.001
seg	50 (46.7)	40 (30.1)	0.008
Exfoliative toxin *etA*	2 (1.9)	1 (0.8)	0.435
Adhesion factors			
clfA	22 (27.9)	57 (72.2)	<0.001
cna	63 (41.2)	90 (58.8)	0.216
Miscellaneous			
ACME locus	5 (21.7)	18 (78.3)	0.020
sak	99 (46.1)	116 (53.9)	0.210
CHIPS	61 (57.0)	46 (43.0)	0.001
isaB	68 (54.4)	57 (45.6)	0.002
splA	67 (37.0)	114 (63.0)	0.001

[a] Data are represented as number (%), unless otherwise indicated. IQR, interquartile range; MRSA, methicillin-resistant *S. aureus*. DNR, do not resuscitate; ICU, intensive care unit; APACHE, Acute Physiology and Chronic Health Evaluation; SAPS, Simplified Acute Physiology Score; ACME, arginine catabolic mobile element; CHIPS, chemotaxis inhibiting protein of *S. aureus*.

[b] *P* values are uncorrected for multiple analyses (as described in Materials and Methods).

[c] "Total evaluable" represents the total number of patients for whom the indicated variable was recorded.

[d] The most common genotypes are recorded. The remaining isolates belonged to CC6 (5), CC7 (2), CC9 (2), CC12 (5), CC20 (6), CC25 (2), CC50 (1), CC59 (3), CC78 (4), CC80 (1), CC93 (3), CC97 (3), CC101 (5), CC361 (2), CC398 (2), unassigned CC on DNA microarray (1; mapped to CC188 on phylogram).

(12/45 [26.7%], $P < 0.001$). Of note, *agr* dysfunction was more common in the isolates with elevated vancomycin MICs (Table 1, $P = 0.007$). We also noted that *agr* dysfunction was present in 55.7% (68/122) of the hospital-onset SAB ($P = 0.158$) and 65.4% (68/104) of the MRSA ($P < 0.001$) isolates. Among the commonly encountered clinical presentations, *agr* dysfunction was associated with pneumonia (12/14, $P = 0.008$). *agr* dysfunction was not associated with 30-day mortality ($P = 0.662$), persistent bacteremia ($P = 0.563$), or recurrent bacteremia ($P = 0.530$). *agr* function according to each clone is represented in Fig. 3, and *agr* dysfunction was frequently observed in CC8 (50/69, $P < 0.001$) and CC30 (15/15, $P < 0.001$). Interestingly, the majority of the CC30 isolates were MSSA (13/15), and *agr* dysfunction in these isolates was present in both community- and hospital-onset infections.

Virulence determinants. There was significant heterogeneity of resistance genes, toxin profiles, and other virulence determinants among the isolates. The frequency of genes encoding Panton-Valentine leukocidin (*lukF/lukS-PV*) and staphylococcal toxic shock syndrome toxin 1 (*tst*) were low (7/242 and 15/242, respectively). Selected genes within the most common bacteremia clones in our cohort are shown in Table 3, and there were some striking differences between the clones. Capsule type 5 was restricted to CC5 and CC22. These same clones also carried enterotoxin gene cluster (*egc*) genes (e.g., *seg*), which were not seen in other dominant clones. CC8 contained multiple antimicrobial resistance genes and adhesion factors frequently compared with other clones, and it accounted for the majority of isolates with the arginine catabolic mobile element (ACME) locus (18/21), as well as all of the isolates containing clumping factor A (*clfA*). However, compared with the other clones associated with a high vancomycin MIC, hospital-onset bacteremia, or MRSA, CC8 isolates rarely contained the chemotaxis inhibitory protein (CHIPS). There were few resistance genes present among the MSSA and MRSA non-CC8 clones apart from *fosB*. Although the prevalence of *tst* was low, it was almost exclusively linked with CC30 (12/15, $P < 0.001$). The gene encoding enterotoxin A (*sea*) was common among the isolates with a high vancomycin MIC, such as CC8 (50/69) and CC5 (15/26), and it was completely absent among the isolates with a low vancomycin MIC, such as CC22, CC88, and CC188. Genes encoding exfoliative toxins, such as *etA*, were present infrequently. *agr* type II was associated with an elevated vancomycin MIC (Table 1) and was predominantly found in CC5 (26/45) and CC15 (11/45), whereas *agr* type III was associated with a low vancomycin MIC (Table 1) and was predominantly found in CC1 (14/48) and CC30 (15/48). Although the gene encoding serine protease-like protein A (*splA*) was found in a range of isolates (Table 3), its presence was associated with elevated vancomycin MIC (Table 1). The virulence determinants according to methicillin susceptibility can be found in Table S1 in the supplemental material, although our focus was on clonal origin and vancomycin MICs, irrespective of methicillin susceptibility, in accordance with the association identified in our original manuscript (6).

DISCUSSION

Elevated vancomycin MIC is associated with poor outcomes in MRSA bacteremia (5) and may relate to difficulties in achieving pharmacodynamic targets. However, the association between elevated vancomycin MICs and poor outcome in patients with MSSA bacteremia in the absence of vancomycin therapy (6, 7) is more challenging to explain. In this study, we explored the hypothesis that elevated vancomycin MIC may be a signal of or marker for other intrinsic bacterial characteristics that directly lead to poor patient outcomes in SAB. Our data support this hypothesis. A high vancomycin MIC in *S. aureus* (MSSA or MRSA) predicts infection with particular *S. aureus* clones that harbor a specific repertoire of virulence and antibiotic resistance genes, as well as *agr* genotypes and phenotypes. The combination of these

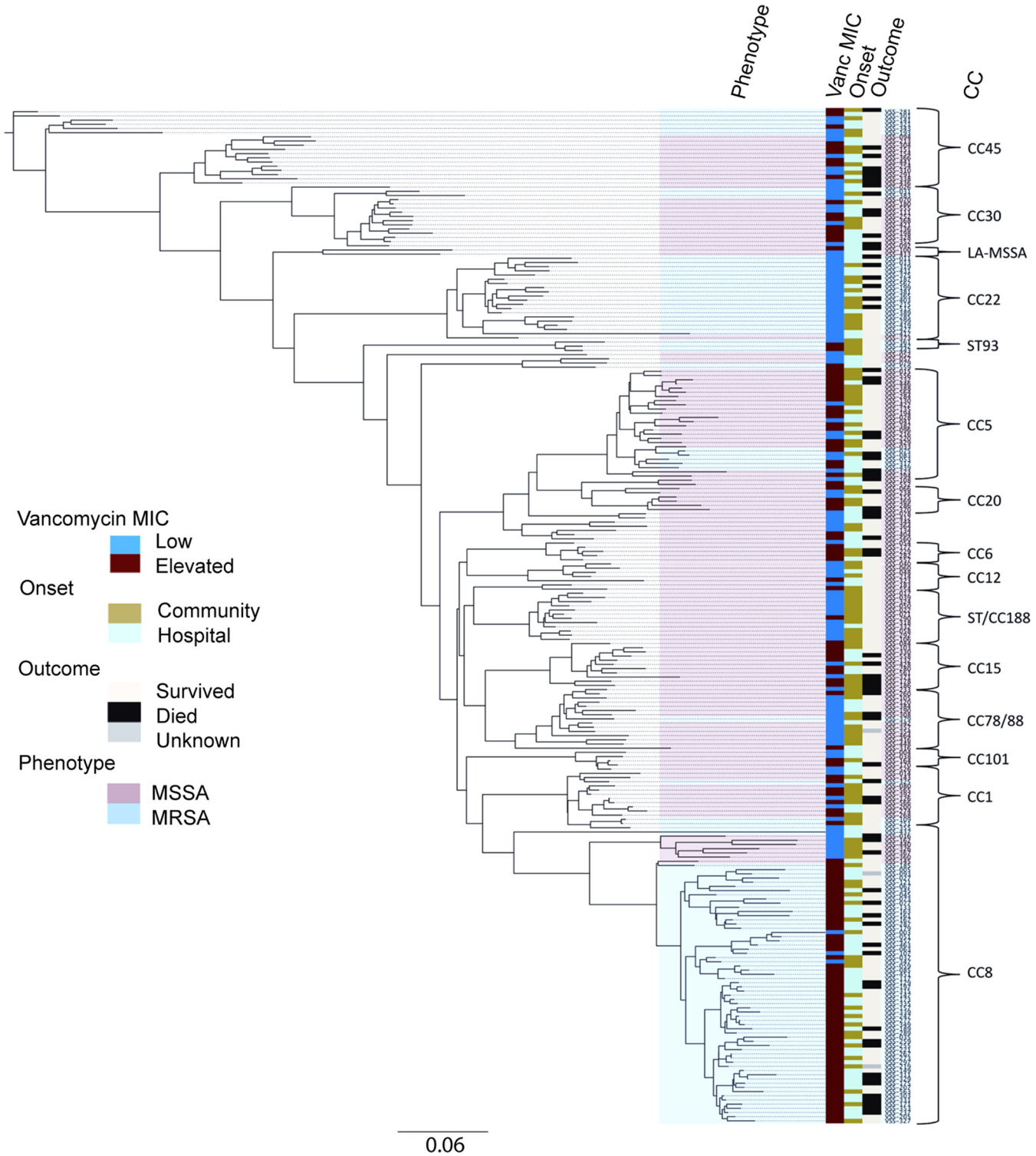

FIG 1 Unrooted phylogram of 242 MSSA and MRSA bacteremia isolates and relationship to vancomycin MIC group, onset of SAB, methicillin susceptibility, and 30-day mortality. CC, clonal complex; ST, sequence type; LA-MSSA, livestock-associated MSSA.

clone-specific features may be critical in determining clinical outcomes irrespective of the vancomycin MIC of the isolate and may explain the observation in high-vancomycin MIC MSSA infections not treated with vancomycin.

Methicillin resistance was strongly linked with elevated vancomycin MICs, as was demonstrated in a recent meta-analysis (5). However, within MRSA isolates, there was clonal variation in vancomycin MICs. MRSA clones with SCC*mec* type II/III were asso-

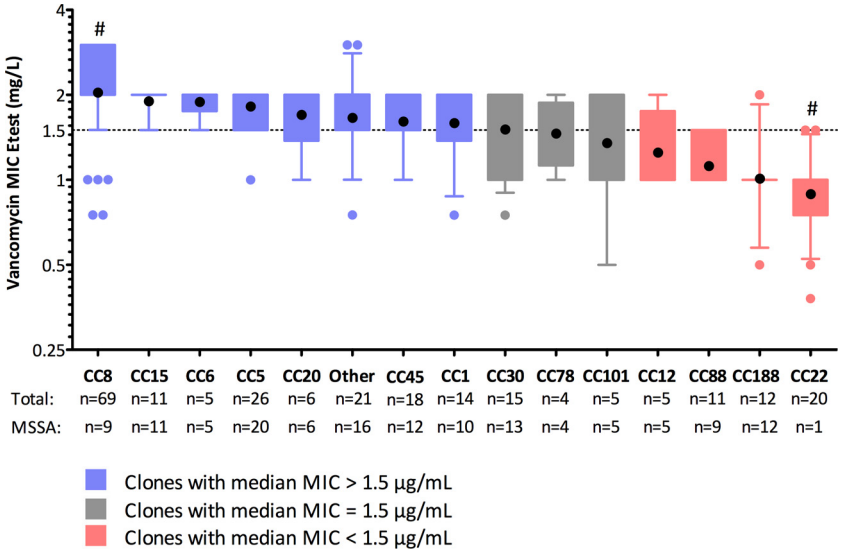

FIG 2 Vancomycin Etest MIC according to clonal complex. Black circles indicate geometric mean MICs, boxes indicate interquartile range, error bars represent range between 10th and 90th percentiles, colored circles represent values outside the 10th to 90th percentiles, and the dotted line indicates vancomycin Etest MIC of 1.5 mg/liter. The *y* axis is on a $\log_2$ scale. "Other" indicates the remaining clones ($n = 21$): CC7, CC9, CC25, CC50, CC59, CC80, ST93, CC97, CC361, CC398. CC188 includes one isolate for which a clonal complex was unassigned on microarray but clustered with CC188 on phylogenetic analysis. #, $P < 0.001$ for comparison of geometric mean MIC for CC8 versus CC22.

ciated with elevated vancomycin MICs, whereas clones with SCC*mec* types IV/V had lower vancomycin MICs, similar to the results reported by Jang et al. (9). This is consistent with the high rates of heterogeneous VISA (hVISA) observed in ST239-MRSA-III, whereas hVISA is less frequent in MRSA harboring SCC*mec* types IV/V (9, 27).

CC8 was strongly associated with elevated vancomycin MICs. It predominantly was found to contain ST239-MRSA-III, a successful multiresistant epidemic health care-associated MRSA clone. Not surprisingly, this clone frequently contained other determinants that may impact vancomycin MIC and mortality, such as the presence of multiple resistance genes or adhesion factors. As our CC8 isolates also comprised community-onset *S. aureus* isolates distinct from hospital-onset ST239-MRSA-III, this also supports the observations by Miller et al. (28) that CC8 may carry genetic features supporting its persistence in the community as well as in the hospital environment. In contrast, CC22 was associated with low vancomycin MICs and was almost exclusively EMRSA-15, an epidemic health care-associated MRSA clone originally seen in the United Kingdom. Low vancomycin MICs have been reported in CC22 isolates from the United Kingdom, and Hope et al. (29) reported no association between vancomycin Etest MICs and mortality in their MRSA bacteremia cohort. Unlike Miller et al. (14), we found no CC22 isolates with high vancomycin MICs. Despite the common clonal ancestry of these isolates, the clone itself is clearly not the only factor determining vancomycin MIC or clinical outcomes and may suggest an additional effect of geographic adaptation. Interestingly, our CC30 isolates had a much higher median vancomycin MIC than did those from the Miller cohort (14). Importantly, some CCs had elevated vancomycin MICs even though they were predominantly MSSA, for example, CC5, CC6, CC15, and CC20. Different clones have also been associated with invasive disease, e.g., CC30 and CC45 (15, 16), and this potentially explains the differences in clinical outcomes and elevated vancomycin MICs.

We confirmed previous findings that *agr* type II and *agr* dysfunction were associated with elevated vancomycin MIC and reduced vancomycin susceptibility (10, 13, 27), and these have also been linked to poor outcomes (12). However, the association between elevated vancomycin MIC and mortality may not necessarily be explained by *agr* dysfunction alone, as these isolates have been shown to be less virulent in an invertebrate model (30). The different clonal backgrounds of isolates within the same *agr* type or with *agr* dysfunction may provide alternative hypotheses. For example, common clones containing *agr* type II in our cohort were CC5 and CC15; both of these clones exhibited a high geo-

TABLE 2 Vancomycin Etest MICs among the common MSSA and MRSA bacteremia clones in the cohort

CC[a]	Vancomycin Etest MIC (mg/liter)								
	Geometric mean	0.38	0.5	0.75	1	1.5	2	3	Total
8	2.04			2	3	6	40	18	69
15	1.90					2	9		11
6	1.89					1	4		5
5	1.82				1	6	19		26
20	1.70				1	1	4		6
45	1.61				3	6	9		18
1	1.59			1	2	3	8		14
30	1.51			1	3	4	7		15
101	1.35		1			2	2		5
12	1.25				3	1	1		5
88	1.12				8	3			11
188	1.01		1	1	7	1	1		11
22	0.89	1	1	5	11	2			20

[a] Clones with ≥5 isolates are represented.

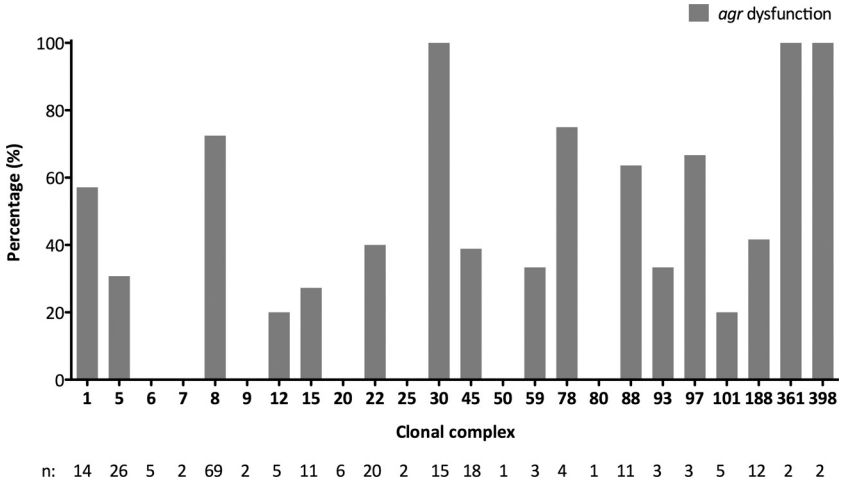

FIG 3 *agr* dysfunction (as measured by delta-hemolysin assay) according to clonal complex. n, number of isolates from each CC. CC188 includes one isolate for which a clonal complex was unassigned on microarray but clustered with CC188 on phylogenetic analysis.

metric mean vancomycin MIC (Table 2) and may possess other characteristics that contribute to virulence and impact vancomycin susceptibility or clinical outcomes. *agr* dysfunction was prevalent in CC8 and CC30 and was previously reported in other studies (11, 31). Although these are successful endemic MRSA clones (32), *agr* dysfunction also occurred in the community-onset and MSSA isolates, and this suggests that other features of these genotypes must contribute to reduced vancomycin susceptibility and clinical outcomes. Unlike Chong et al. (31), we did not find a significant proportion of CC5 isolates with *agr* dysfunction. We were unable to find an association between *agr* dysfunction and persistent bacteremia, as was previously reported (33); however, this may be due to the low frequency of persistent bacteremia in our cohort (6.6%).

Isolates containing the *sea* gene were associated with elevated vancomycin MICs, whereas those containing *egc* were associated with low vancomycin MICs. These findings are similar to those previously reported, in which the presence of the *sea* gene was associated with the severity of infection (34) or invasive disease (15), and *egc* was inversely related to septic shock (35). This suggests that reduced vancomycin susceptibility may indeed be a marker of other organism characteristics that are implicated in inferior clinical outcomes, even in patients not receiving vancomycin. *tst* was almost exclusively associated with the CC30 MSSA isolates, and this was also noted by Blomfeldt et al. (36). Although *clfA* has been shown to be important in nasal colonization (37) and endocarditis (38), the *S. aureus* isolates in our cohort containing this gene all belonged to CC8; it is therefore uncertain whether elevated vancomycin MIC is due to *clfA* itself or other features of this clonal complex. The high frequency of ACME carriage in CC8 ST239-MRSA-III (39) may also explain the association between the ACME locus and elevated vancomycin MIC in our cohort. *splA* is essential for dissemination after initial colonization (40) and was found in the high-vancomycin MIC genotypes CC8 and CC5; the presence of this gene may be associated with poor clinical outcomes as a result of metastatic infection.

Antimicrobial resistance genes were frequently associated with elevated vancomycin MICs. The high prevalence of *blaZ* is not unexpected, as *S. aureus* remains penicillin susceptible in approximately 10% of isolates (4, 41, 42). Although *blaZ* was statistically associated with elevated vancomycin MICs, this may reflect the higher proportion of MRSA among these isolates. Other antimicrobial resistance genes, such as *ermA*, *aaC-aphD*, *tetK*, and *qacA*, which were also associated with elevated vancomycin MICs, were found mostly in the CC8 isolates; the association may therefore reflect the underlying clonal background rather than individual resistance determinants such as these. Three MRSA isolates did not have the *mecA* gene detected by microarray; these isolates were confirmed to be methicillin resistant by susceptibility testing and belonged to typical MRSA clones (ST239-MRSA-III, EMRSA-15, and Panton-Valentine leukocidin [PVL]-positive ST93). We do not know if these isolates lost the *mecA* cassette or if there is an alternative explanation, and this is currently under further investigation and is beyond the scope of this paper. In a study of this size, it was not practical to test every gene allele for multiple individual clinical conditions due to the large number of variables, so instead, we focused on a smaller number of grouped alleles (as represented by CC or ST type) and the relationship with vancomycin MIC, an important predictor of clinical outcome. The increasing availability of whole-genome sequencing will allow for more detailed analysis of the isolates and may help to identify features of specific clones that lead to an elevated vancomycin MIC. Significant heterogeneity exists, even within a clonal complex, in organism factors, such as resistance genes and virulence determinants. It is difficult to ascertain if different genetic or organism factors directly influence vancomycin MIC, or if an elevated vancomycin MIC is a consequence of various virulence factors that allow subsequent antibiotic selection pressure. We believe that an elevated vancomycin MIC may be a proxy for organism factors that may explain our previous association between elevated vancomycin MICs and mortality in patients with SAB. This is particularly relevant, as it highlights the importance of bacterial factors and potential host-pathogen interactions in determining clinical outcomes, and it may also explain the results seen in patients with MSSA bacteremia treated with flucloxacillin. Future clinical trials and research may require considerations of

TABLE 3 Comparison of selected genes detected by microarray among the common MSSA and MRSA bacteremia clones in the cohort[a]

Variable[b]	Data by clonal complex: 8	5	22	45	30	1	15	88	188[d]	Total[c]
No. (%)	69 (35.4)	26 (13.3)	20 (10.3)	18 (9.2)	15 (7.7)	14 (7.2)	11 (5.6)	11 (5.6)	11 (5.6)	195
agr type										
I	69 (100)	0	20 (100)	16 (88.9)	0	0	0	0	11 (100)	116
II	0	26 (100)	0	0	0	0	11 (100)	0	0	37
III	0	0	0	0	15 (100)	14 (100)	0	11 (100)	0	40
IV	0	0	0	2 (11.1)	0	0	0	0	0	2
Polysaccharide capsule										
cap5	8 (11.6)	26 (100)	20 (100)	0	0	0	0	0	0	54
cap8	61 (88.4)	0	0	17 (94.4)	15 (100)	14 (100)	11 (100)	11 (100)	11 (100)	140
Antimicrobial resistance										
blaZ	66 (95.7)	22 (84.6)	19 (95)	14 (77.8)	15 (100)	10 (71.4)	10 (90.9)	10 (90.9)	10 (90.9)	176
mecA	59 (85.5)	6 (23.1)	18 (90)	6 (33.3)	2 (13.3)	4 (28.6)	0	2 (18.2)	0	97
ermA	61 (88.4)	0	0	1 (5.6)	5 (33.3)	0	0	3 (27.3)	0	70
aacC-aphD	58 (84.1)	0	0	0	0	0	0	0	0	58
mupR	1 (1.4)	0	0	0	0	0	0	0	0	1
tetK	34 (49.3)	0	0	5 (27.8)	0	0	0	0	0	39
fosB	69 (100)	26 (100)	0	0	15 (100)	0	11 (100)	1 (9.1)	0	122
qacA	48 (69.6)	3 (11.5)	0	0	0	1 (7.1)	0	5 (45.5)	0	57
Leukocidins or exotoxins										
PVL	0	0	0	0	2 (13.3)	1 (7.1)	0	0	1 (9.1)	4
tst	1 (1.4)	2 (7.7)	0	0	12 (80)	0	0	0	0	15
Enterotoxins										
sea	50 (72.5)	15 (57.7)	0	0	9 (60.0)	10 (71.4)	0	0	0	84
seb	1 (1.4)	2 (7.7)	2 (10.0)	0	0	1 (7.1)	0	1 (9.1)	1 (9.1)	8
sec	0	0	14 (70.0)	5 (27.8)	1 (6.7)	0	0	5 (45.5)	0	25
seg[e]	2 (2.9)	26 (100)	20 (100)	18 (100)	14 (93.3)	0	0	0	0	80
Exfoliative toxin *etA*	0	0	0	0	0	0	1 (9.1)	2 (18.2)	0	3
Adhesion factors										
clfA	59 (85.5)	0	0	0	0	0	0	0	0	59
cna	61 (88.4)	0	20 (100)	18 (100)	14 (93.3)	14 (100)	0	1 (9.1)	11 (100)	139
Miscellaneous										
ACME locus	18 (26.1)	0	3 (15.0)	0	0	0	0	0	0	21
sak	62 (89.9)	25 (96.2)	17 (85.0)	18 (100)	15 (100)	13 (92.9)	1 (9.1)	11 (100)	11 (100)	173
CHIPS	4 (5.8)	17 (65.4)	17 (85.0)	18 (100)	13 (86.7)	0	11 (100)	0	11 (100)	91
isaB	8 (11.6)	26 (100)	0	0	1 (6.7)	14 (100)	11 (100)	11 (100)	11 (100)	82
splA	68 (98.6)	25 (96.2)	0	0	0	14 (100)	11 (100)	11 (100)	11 (100)	140
agr dysfunction present[f]	50 (72.5)	8 (30.8)	8 (40.0)	7 (38.9)	15 (100)	8 (57.1)	3 (27.3)	7 (63.6)	5 (45.5)	111

[a] MSSA, methicillin-susceptible *S. aureus*; MRSA, methicillin-resistant *S. aureus*.
[b] Data are represented as number (%), unless otherwise indicated. PVL, Panton-Valentine leukocidin; ACME, arginine catabolic mobile element; CHIPS, chemotaxis inhibitory protein of *S. aureus*.
[c] These isolates represent 80.6% of the total tested cohort (195/242 isolates).
[d] This does not include the isolate for which clonal complex was unassigned on microarray but clustered with CC188 on phylogenetic analysis.
[e] Part of enterotoxin gene cluster (*egc*).
[f] As measured by delta-hemolysin assay.

the clonal origin of *S. aureus* isolates, especially when trying to establish the magnitude of treatment effect or assess clinical outcomes.

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