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SUSCEPTIBILITY

Reduced Vancomycin Susceptibility of Methicillin-Susceptible *Staphylococcus aureus* Has No Significant Impact on Mortality but Results in an Increase in Complicated Infection

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ABSTRACT Methicillin-susceptible *Staphylococcus aureus* (MSSA) bloodstream infections (BSIs) often lead to severe complications despite the availability of effective antibiotics. It remains unclear whether elevated vancomycin MICs are associated with worse outcomes. We conducted a 2-year retrospective cohort study ($n = 252$) of patients with MSSA BSIs at a tertiary care hospital. We defined reduced vancomycin susceptibility (RVS) as a Microscan MIC of 2 mg/liter. All strains were genotyped (*spa*) and assessed for *agr* functionality. Multivariable logistic regression models were used to examine the impact of RVS phenotype and strain genotype on 30-day all-cause mortality and complicated bacteremia (metastatic spread, endovascular infection, or duration ≥3 days). One-third of patients (84/252) were infected with RVS isolates. RVS Infections were more frequently associated with metastatic or embolic sites of infection (36% versus 17%, $P < 0.001$), and endovascular infection (26% versus 12%, $P = 0.004$). These infections occurred more often in patients with fewer underlying comorbidities (Charlson comorbidity index of ≥3 [73% versus 88%, $P = 0.002$]). Genotyping identified 127 *spa*-types and 14 Spa-clonal complexes (Spa-CCs). Spa-CC002 and Spa-CC008 were more likely to exhibit the RVS phenotype versus other Spa-CCs (OR = 2.2, $P < 0.01$). The RVS phenotype was not significantly associated with 30-day mortality; however, it was associated with complicated bacteremia (adjusted odds ratio of 2.35 [range, 1.26 to 4.37]; $P = 0.007$) in adjusted analyses. The association of RVS strains with complicated infection and fewer underlying comorbidities suggests the phenotype as a potential marker of strain virulence in MSSA BSIs. The RVS phenotype itself was not a significant predictor of mortality in this patient cohort. Further studies are necessary to explore this host-pathogen relationship.

KEYWORDS MSSA bacteremia, reduced vancomycin susceptibility

Staphylococcus aureus is a dynamic pathogen causing a broad range of clinical syndromes, from localized skin-and-soft tissue infections to invasive disease (1). *S. aureus* bloodstream infections (SAB) often result in endovascular seeding and are frequently associated with poor outcomes, including 30-day mortality estimated at 20 to 40% (2–6). While bloodstream infections (BSIs) caused by methicillin-resistant *S. aureus* (MRSA) have historically attracted the most attention, methicillin-sensitive *S. aureus* (MSSA) is responsible for the majority of *S. aureus* BSIs (7, 8).

Morbidity and mortality in patients with *S. aureus* BSI are due to a complex interplay between timely and effective treatment, host-pathogen interactions, underlying pa-

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tient characteristics, and pathogen features such as antimicrobial drug resistance (9–12). The mechanism for reduced vancomycin susceptibility (RVS) isolates is poorly understood and is distinct from genetic alterations seen in vancomycin resistance, which involves acquisition of the *vanA* operon (13). Reduced vancomycin susceptibility is thought to be accompanied by thickening of the cell wall, which may alter the ability of vancomycin to bind to its target (14, 15). Whether or not this cell wall thickening translates into altered virulence or is strain dependent has not been determined. A downregulation of *agr*, a quorum-sensing two-component regulatory system, has also been seen in some studies of MRSA VISA/hVISA; however, it is unclear whether *agr* dysfunction is similarly associated with MSSA RVS isolates (13, 16).

Most studies on the RVS phenotype have exclusively focused on MRSA cases; however, RVS remains relevant in MSSA cases because vancomycin is often used empirically in first-line therapy, and RVS may signal a bacterial cell wall alteration that affects clinical course (15, 17). The RVS phenotype in MSSA infection has recently been studied for its effect on clinical outcome. In particular, it has been linked to increased risk of mortality in some studies, although other analyses have failed to show robust associations with clinical outcome (15, 17–23).

However, the contribution of clonal lineage, regulatory system phenotypes, and other phenotypic characteristics has not yet been well defined in outcomes of invasive MSSA infection. Furthermore, the high genetic diversity among MSSA isolates (in contrast to the limited diversity in MRSA) has made it difficult to analyze links between clonal background and clinical outcomes (24). The RVS phenotype, in combination with genotypic information, may prove a more useful predictor of outcome. In this study, we sought to analyze the impact of the RVS phenotype, genetic lineage, and *agr* dysfunction on outcomes in MSSA bloodstream infections.

RESULTS

MSSA study cohort. During the 2-year study period, 252 adult patients had bloodstream infections caused by MSSA. Of these, 84 patients (33%) were infected by MSSA isolates with vancomycin MICs $\geq$ 2 mg/liter (RVS phenotype), and 168 (67%) were infected with isolates with MICs $<$ 2 mg/liter (non-RVS group). The majority of patients in the cohort were male (148/252 [59%]), and patients had an average age of 60.2 years (median, 60; interquartile range [IQR], 18 to 98; Table 1). The average Charlson comorbidity index (CCI) score was 5.4 (median, 5; range, 3 to 7), and 83% of patients (209/252) had a CCI of $\geq$3. The severity of illness at onset of bacteremia was low since patients had an average Pitt bacteremia score (PBS) of 2.3 (median, 2.3; IQR, 0 to 3), and 22% (56/252) had a score of $\geq$4. The most common primary sources of bacteremia were catheter-related infection (21%), pneumonia (16%), and skin-and-soft tissue infection (13%), while the source was unknown in 21% of cases. Overall, 42 (16%) patients developed endocarditis or deep endovascular infection, as determined by the clinical team and based on imaging studies (echocardiogram). Of the 53 catheter-related infections, 62% were central line-associated (33/53), and 38% were related to peripheral intravenous treatment (20/53). Surgical site infection (5%) and device-associated infection (3%) accounted for a small portion of the infections.

The majority of patients had health care-associated infections (n = 204, 82%) with 26% (n = 66) being community-onset infections [HA-CO] and 55% (n = 138) hospital-onset infections ([HA-HO]). Thirty patients (12%) were admitted directly from a long-term care facility, 14% (n = 36) were receiving hemodialysis, 52% (n = 131) had been hospitalized in the past 6 months, and 34% (n = 86) had been hospitalized in the past 1 month. At the time of bacteremia, 13% (n = 34) had a temporary central venous catheter (CVC), and 17% (n = 43) had a permanent CVC in place. 10% (n = 24) had percutaneous devices, and 6% (n = 15) underwent surgery in the 72 h preceding bacteremia. Only 18% (n = 46) of infections were community associated (CA).

The 30-day mortality for the cohort was 18% (n = 45), and the 90-day mortality was 26% (n = 66). The average duration of bloodstream infection was 2.3 days (median, 1 day; IQR, 1 to 3 days), and 19% (n = 48) of the patients had a bloodstream infection $>$3

TABLE 1 Baseline characteristics of BSI study population and bacterial isolates

Variable	Total (n = 252)	No. (%) of patients[a] with a vancomycin MIC of:		P[b]
		<2 mg/liter (n = 168)	2 mg/liter (n = 84)	
Demographics				
Female sex	104 (41)	72 (43)	32 (38)	0.47
Mean age in yrs (IQR)	60 (49–73)	62 (52–75)	56 (45–67)	0.01*
Health care risk factors				
Admission from NH/LTCF	30 (12)	23 (14)	7 (8)	0.22
Hemodialysis within past yr	36 (14)	23 (14)	13 (15)	0.70
Hospital admission, <1 mo	86 (34)	56 (33)	30 (36)	0.71
Hospital admission, <6 mo	131 (52)	89 (53)	42 (50)	0.66
Surgery, <72 h	15 (6)	10 (6)	5 (6)	1
Surgery, <1 yr	71 (28)	46 (27)	25 (30)	0.69
Central venous catheter (temporary)	34 (13)	23 (14)	11 (13)	0.90
Central venous catheter (permanent)	43 (17)	28 (17)	15 (18)	0.81
Percutaneous device	24 (10)	15 (9)	9 (11)	0.65
Primary site of infection by category				0.47
Bacteremia only	53 (21)	38 (23)	15 (18)	
Catheter	53 (21)	34 (20)	19 (23)	
Pneumonia	40 (16)	30 (18)	10 (12)	
SSTI	34 (13)	23 (14)	11 (13)	
Other[c]	72 (29)	43 (26)	29 (35)	
Secondary site of infection				0.0011*
Metastatic or embolic	59 (23)	29 (17)	30 (36)	
No secondary infection	193 (77)	139 (83)	54 (64)	
Comorbidity and disease severity scores				
Mean Charlson comorbidity index (IQR)	5.4 (3, 7)	5.5 (4, 7)	5.1 (2, 8)	0.35
Score ≥3	209 (83)	148 (88)	61 (73)	0.002*
Mean Pitt bacteremia score (n = 246) (IQR)	2.3 (0–3)	2.2 (0–3)	2.4 (0–4)	0.12
Score ≥4	56 (23)	35 (21)	21 (26)	0.37
Infection category[d] (n = 250)				
Hospital associated (HA)	204 (82)	136 (81)	68 (83)	
Health care onset (HA/HO)	138 (55)	95 (57)	43 (52)	0.59
Community onset (HA/CO)	66 (26)	41 (24)	25 (30)	
Community associated (CA)	46 (18)	32 (19)	14 (17)	
Treatment and clinical course				
Initial treatment (n = 245)				
Vancomycin only	72 (29)	43 (27)	29 (35)	0.20
Vancomycin with other agent	91 (37)	56 (35)	35 (42)	0.29
Beta-lactam with or without nonvancomycin agent	67 (27)	53 (33)	14 (17)	0.007*
Other antistaphylococcal agent	10 (4)	6 (4)	4 (5)	0.74
Ineffective or absent therapy	5 (2)	3 (2)	2 (2)	1
Treatment with ≥1 vancomycin dose (n = 245)	177 (72)	108 (67)	69 (82)	0.01*
Treatment alteration (n = 245)				
Altered within 2 days of initiation	54 (22)	34 (21)	20 (24)	0.63
Altered within 7 days of initiation	88 (36)	53 (33)	35 (42)	0.18
ID consult	148 (59)	91 (54)	57 (68)	0.04*
Mean bacteremia duration (IQR)	2.3 (1–3)	2.4 (1–3)	2.1 (1–3)	0.30
>1 day	95 (38)	69 (41)	26 (31)	0.12
>3 days	48 (19)	32 (19)	16 (19)	1
>5 days	20 (8)	13 (8)	7 (8)	0.87
All-cause mortality				
30 day	45 (18)	31 (18)	14 (17)	0.73
60 day	59 (23)	41 (24)	18 (21)	0.60
90 day	66 (26)	47 (28)	19 (23)	0.36
Death while bacteremic	14 (6)	11 (7)	3 (4)	0.40

(Continued on next page)

TABLE 1 (Continued)

Variable	Total (n = 252)	No. (%)[a] with a vancomycin MIC of:		P[b]
		<2 mg/liter (n = 168)	2 mg/liter (n = 84)	
Strain characteristics				
Spa clonal complex				0.02*
Spa-CC002	49 (19)	27 (16)	22 (26)	
Spa-CC008	36 (14)	21 (13)	15 (18)	
Spa-CC012	35 (14)	30 (18)	5 (6)	
Other	132 (52)	90 (54)	42 (50)	
SpaCC, dichotomized				0.01*
Spa-CC002 or Spa-CC008	85 (34)	48 (29)	37 (44)	
Other	167 (66)	120 (71)	47 (56)	
agr deficient	20 (8)	12 (7)	8 (10)	0.51
Antibiotic nonsusceptibility				
Fluoroquinolone (levofloxacin)	30 (12)	18 (11)	12 (14)	0.41
Macrolides (erythromycin)	93 (37)	68 (40)	25 (30)	0.10
Tetracycline	14 (6)	6 (4)	8 (10)	0.07
Penicillin	157 (83)	99 (59)	58 (69)	0.54
Bactrim	9 (4)	6 (4)	3 (4)	1

[a]Except as indicated otherwise in column 1.
[b]*, Statistically significant ($P < 0.05$) as determined by chi-square, Fisher exact, two-sample t, or Wilcoxon rank sum test, where appropriate.
[c]"Other" includes UTI, device, surgical site, endovascular, CNS, and osteoarticular primary sites.
[d]Comparison of HA/CO versus HA/HO versus CA infection categories.

days in length. A total of 6% (n = 14) of the patients died while still actively bacteremic. Endovascular infection was a complication of 17% (n = 42) of bloodstream infections, and metastatic or embolic spread of infection was documented in 23% of the cases overall (n = 59).

Molecular and phenotypic characterization of MSSA strains. The collection of BSI isolates was highly genetically diverse: 127 *spa* types were identified among 252 clinical isolates. A total of 32 isolates (13%) were *spa* type t002, and 16 isolates were *spa* type t008 (6%). Another 100 isolates (40%) had *spa* types that occurred only once. Spa-CC002 was the most prevalent clonal cluster (CC), or group of related strain types, with 49 isolates (19%). Spa-CC008 and Spa-CC012 each accounted for 14% (n = 36 and n = 35, respectively). Regarding antibiotic nonsusceptibility, 12% (n = 30) of the isolates were resistant to fluoroquinolones, 37% (n = 93) to were resistant macrolides, 6% (n = 14) were resistant to tetracyclines, 83% (n = 157) were resistant to penicillin, and 4% (n = 9) were resistant to trimethoprim-sulfamethoxazole. A small proportion (n = 20, 8%) of isolates were *agr* deficient. We also carried out vancomycin Etests on 99% (250/252) of our isolates to ascertain the RVS phenotype by an alternative method. We found that a majority (n = 194 [78%]) were in agreement, having automated MIC values that were ≤1 dilution different from the Etest values (Fig. 1). In addition, applying the Spearman correlation test showed that the Etest and Microscan results correlated (Spearman correlation coefficient = 0.126, P = 0.046). Of note, all 56 isolates that were not in agreement had higher Microscan MICs than Etest MICs. The study was not powered to assess the Etest-defined RVS phenotype as the exposure variable of interest.

Predictors of RVS phenotype. Univariable analyses comparing the groups infected by Microscan RVS and non-RVS phenotype isolates showed that patients in the RVS group were significantly younger (average age, 56 years versus 62 years; P = 0.01). However, there was no significant difference in gender distribution between the RVS and non-RVS groups (Table 1). Patients in the two groups had similar frequencies of health care-associated exposures, and there was no significant difference in the severity of illness at bacteremia onset, as assessed by the PBS (average PBS, 2.2 versus 2.4; P = 0.12). Although the average CCI scores were similar, fewer patients in the RVS group

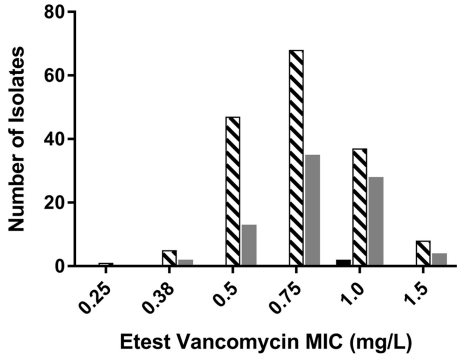

FIG 1 Comparison of Etest and Microscan MICs. Microscan vancomycin MIC: ■, 0.5 mg/liter; ▧, 1.0 mg/liter; ▨, 2.0 mg/liter.

had a CCI ≥ 3 (73% versus 88%; $P = 0.0002$). There was a similar frequency of sources of infection across the groups, but significantly more patients in the RVS phenotype group developed endovascular infection (26% versus 12%; $P = 0.004$). Furthermore, a greater proportion of patients in the RVS group had metastatic seeding or embolic spread of (36% versus 17%; $P = 0.001$). Significantly more isolates from the Spa-CC002 or Spa-CC008 lineages were found in the RVS phenotype group (44% versus 29%; $P = 0.01$) compared to isolates from the non-CC002/CC008 clonal backgrounds. There were no significant differences in antibiotic susceptibility patterns between RVS and non-RVS groups.

Complications of MSSA bacteremia and RVS phenotype. In unadjusted analyses of 30-day mortality, we found no difference in frequency of the RVS phenotype between groups (Table 2). Significant predictors of 30-day mortality included age ≥ 65 years (56% versus 32%; $P = 0.003$), hospital admission within the past 1 month (49% versus 31%; $P = 0.02$), a Pitt bacteremia score ≥ 4 (57% versus 15%; $P < 0.0001$), and pneumonia as a primary source of bacteremia (27% versus 14%; $P = 0.03$; Table 2). Hemodialysis was protective in unadjusted analysis (2% versus 17%; $P = 0.03$). In a multivariable analysis of 30-day mortality predictors, only hospital admission within the past 1 month remained a significant predictor of mortality (adjusted odds ratio [aOR], 2.12 [1.06 to 4.24]; $P = 0.03$).

Complicated bacteremia, as defined by metastatic or embolic seeding, endovascular infection, or duration >3 days, was evaluated in univariable and multivariable models. In unadjusted analysis, there were significantly more isolates with vancomycin MIC of ≥2 mg/liter in the complicated bacteremia group (43% versus 29%; $P = 0.04$). The multivariable model included the variables of age, gender, and any variables from the univariable analysis with a P value ≤ 0.2 (Table 3). A vancomycin MIC of ≥2 mg/liter remained a significant predictor of complicated infection in the multivariable model (aOR, 2.35 [1.26 to 4.37]; $P = 0.007$). A Charlson score of ≥3 was also a significant predictor of complicated infection (aOR, 2.63 [1.09 to 6.37]; $P = 0.03$) in this model. There were no significant differences in genotype (as dichotomized into Spa-CC002/CC008 versus others) between groups in either univariable or adjusted analyses (Table 3).

DISCUSSION

In this retrospective observational study of MSSA bloodstream infections we found increased rates of complicated bacteremia, as defined by metastatic spread of disease, endovascular infection or duration >3 days, in the MSSA BSIs caused by isolates exhibiting the RVS phenotype. However, we did not observe a significant effect of a high vancomycin MIC on all-cause 30-day mortality.

Our data show high overall mortality rates in MSSA BSI, consistent with those seen in prior studies (5, 6, 25). The persistence of these high mortality rates and other

TABLE 2 Predictors of 30-day mortality

Variable	30-day mortality		
	No. (%) of patients[a]		
	No (n = 207)	Yes (n = 45)	P
Demographics			
Female sex	80 (39)	24 (53)	0.07
Age >65 yrs	66 (32)	25 (56)	0.003[b]
Health care risk factors			
Admission from NH/LTCF	22 (11)	8 (18)	0.18
Hemodialysis, <1 yr	35 (17)	1 (2)	0.01[b]
Hospital admission, <1 mo	64 (31)	22 (49)	0.02[b]
Hospital admission, <6 mo	108 (52)	23 (51)	0.90
Hospital associated[c] (n = 250)	166 (81)	38 (85)	
Health care onset (HA/HO)	117 (57)	21 (47)	0.16
Community onset (HA/CO)	49 (24)	17 (38)	
Community associated (CA)	39 (19)	7 (16)	
Surgery, <72 h	12 (6)	3 (7)	0.73
Surgery, <1 year	61 (29)	10 (22)	0.33
Central venous catheter (temporary)	25 (12)	9 (20)	0.16
Central venous catheter (permanent)	38 (18)	5 (11)	0.07[b]
Percutaneous device	20 (10)	4 (9)	1.0
Primary site of infection (categorized)			0.061
Bacteremia only	41 (20)	12 (27)	
Catheter associated	48 (23)	5 (11)	
Pneumonia	28 (14)	12 (27)	
SSTI	27 (13)	7 (16)	
Other[d]	63 (30)	9 (19)	
Secondary site of infection			0.34
Metastatic or embolic	46 (22)	13 (29)	
No secondary infection	161 (78)	32 (71)	
Mean comorbidity and disease severity scores			
Mean Charlson comorbidity index (IQR)	5.2 (3–7)	6.0 (5–7)	0.10
Score ≥3	167 (81)	42 (93)	0.04[b]
Mean Pitt bacteremia score (n = 246) (IQR)	1.6 (0–2)	4.5 (2–9)	<0.001[b]
Score ≥4	31 (15)	25 (57)	<0.001[b]
Treatment and clinical course			
Initial treatment (n = 245)			
Vancomycin only	61 (31)	11 (24)	0.42
Vancomycin with other agent	70 (35)	21 (47)	0.14
Beta-lactam with or without nonvancomycin agent	56 (28)	11 (24)	0.62
Other antistaphylococcal agent	8 (4)	2 (4)	1.0
Ineffective or absent therapy	5 (3)	0 (0)	0.59
Treated with ≥1 vancomycin dose (n = 245)	140 (70)	37 (82)	0.10
Treatment alteration (n = 245)			
Altered within 2 days of initiation	46 (23)	8 (18)	0.44
Altered within 7 days of initiation	74 (37)	14 (31)	0.46
ID consult	120 (60)	24 (53)	0.41
Mean bacteremia duration (IQR)			
>1 day	77 (37)	18 (40)	0.73
>3 days	39 (19)	9 (20)	0.86
>5 days	14 (7)	6 (13)	0.14
Strain characteristics			
SpaCC, dichotomized			0.27
Spa-CC002 or spa-CC008	73 (35)	12 (27)	
Other	134 (65)	33 (73)	
agr deficient	18 (9)	2 (4)	0.54
Vancomycin MIC, 2 mg/liter	70 (34)	14 (31)	0.72

[a]Except as indicated otherwise in column 1.
[b]Statistically significant ($P < 0.05$) as determined by chi-square, Fisher exact, two sample t test, or Wilcoxon rank sum tests, where appropriate.
[c]Comparison of HA/CO versus HA/HO versus CA infection categories.
[d]"Other" includes UTI, device, surgical site, endovascular, CNS, and osteoarticular primary sites.

TABLE 3 Predictors of complicated bacteremia[a]

Variable	No. (%) of patients[b]		Univariable *P* value	aOR (95% CI)[c]	Multivariable *P* value
	No (n = 172)	Yes (n = 80)			
Vancomycin MIC (2 mg/liter)	50 (29)	34 (43)	0.04	2.35 (1.26–4.37)	0.007
Spa-CC (dichotomized)			0.15		0.08
Spa-CC002 or Spa-CC008	63 (37)	22 (28)		0.56 (0.30–1.06)	
Other Spa-CC	109 (63)	58 (73)		REF	
Race			0.04		0.03
White	43 (25)	29 (36)		1.07 (0.48–2.37)	0.87
African-American	40 (23)	9 (11)		0.29 (0.11–0.78)	0.01
Hispanic	57 (33)	23 (29)		0.61 (0.27–1.36)	0.22
Other	29 (17)	19 (24)		REF	
Hemodialysis, <1 yr	20 (12)	16 (20)	0.08	2.02 (0.95–4.43)	0.08
Hospital admission, <1 yr	98 (57)	53 (66)	0.16	1.49 (0.82–2.70)	0.19
Percutaneous device	20 (12)	4 (5)	0.10	0.29 (0.09–0.94)	0.04
Charlson score ≥3	138 (80)	71 (89)	0.09	2.63 (1.09–6.37)	0.03

[a]Except as indicated otherwise in column 1.
[b]That is, metastatic, embolic, or endovascular infection for >3 days.
[c]aOR, adjusted odds ratio; REF, reference category; CI, confidence interval.

adverse outcomes, even in the presence of effective antimicrobial therapy, highlights the urgent need to better understand prognostic and modifiable risk factors for poor outcome in MSSA BSI. We controlled for comorbidity burden and severity of disease in our analysis. While our study was retrospective in nature, our clinical data were rich, and we additionally evaluated the role of genotypic background in clinical outcomes. There have been few studies on the relationship between vancomycin MIC and mortality in MSSA BSI that incorporate both genotype and *agr* dysfunction into the assessment of MIC effect.

Regarding mortality effect, some prior studies on the relationship between vancomycin MIC and outcomes in MSSA BSI have found an increased mortality risk (19, 20) associated with MICs ≥ 2 mg/liter, however other recent studies have failed to demonstrate a statistically significant difference in death rates (2, 23, 26). A meta-analysis looking at effect of vancomycin MIC on mortality in *S. aureus* bacteremia failed to show a significant difference in 30-day mortality rates in SAB across a range of vancomycin MIC cutoffs (21). Most of the studies in this meta-analysis focused on MRSA, as only a limited number of prior studies have examined the predictive value of vancomycin MIC in MSSA SAB. The variation in results may be due to lack of power, differences in clinical features of the study populations, failure to control for confounding factors such as comorbidity burden, and varying MIC measurement methodologies (automated versus nonautomated) that may alter the MIC groupings. It also may be that the effect of MIC on short-term mortality in MSSA BSI is minimal but the reduced susceptibility phenotype is a marker of virulence that drives morbidity and complications such as endocarditis.

Our findings in unadjusted analyses found that the RVS phenotype was associated with younger age, lower comorbidity burden, metastatic or embolic spread of infection, and endovascular infection and suggest that a vancomycin MIC ≥ 2 mg/liter may be a marker for heightened virulence. The association between MIC ≥ 2 mg/liter and complicated infection remained significant in adjusted analyses. Similarly, a different study of catheter-related MSSA bloodstream infections found that an MIC ≥ 1.5 mg/liter by Etest was associated with complicated MSSA BSI, defined by the presence of either endocarditis, septic thrombophlebitis, metastatic or hematogenous spread, persistent bacteremia, or persistent fever (2). Another study of MSSA BSI found increased rates of septic thrombophlebitis with vancomycin MIC ≥ 1.5 mg/liter (Etest) but failed to show differences in risk for mortality, endocarditis or hematogenous seeding (26). This study, however, was limited by small cohort size and small number

of isolates with MIC $\geq$ 1.5 mg/liter that were included in the analysis. If pathogenicity is attributable to heightened MIC, it is still unclear what exact mechanism is responsible for this relationship. Different explanations ranging from impaired therapeutic efficacy in the setting of a thickened cell wall to metabolic changes or changes in virulence factor expression have been proposed (17, 27).

It is also possible that the increased MIC is a marker for other mediating factors, such as clonal background or broader antibiotic resistance. We did not observe any significant differences in susceptibilities to other antibiotic classes for RVS versus non-RVS isolates. However, we found differences in frequency of the RVS phenotype among clonal backgrounds. Despite the high genetic diversity among MSSA isolates in our study, the RVS phenotype was significantly overrepresented in Spa-CC002 and Spa-CC008 strains versus non-CC002/CC008 strains. Most CC002 and CC008 strains belong to the ST5 and ST8 lineages, respectively (28). Along similar lines, in a prior examination of candidate gene loci of VISA and hVISA MRSA isolates, we found a greater number of polymorphisms in genes that contribute to mechanisms of intermediate vancomycin susceptibility in ST5 and ST8 strains (29), suggesting a link between susceptibility phenotype and clonal lineage. The exact nature of this association cannot be fully elucidated based on sequencing of the *spa* locus alone. Both bacterial genome-wide association studies (GWAS) and whole-genome sequencing (WGS) approaches would help to identify contributions of clone-specific polymorphisms to the RVS phenotype.

We chose to use vancomycin MICs derived from automated methods (Microscan) in our study, since such methods are the ones more often used in clinical practice and therefore inform real-time clinical decision-making. Particularly in the case of MSSA infection, nonautomated methods such as Etest or broth microdilution (BMD) are rarely used in the clinical setting. The use of MICs generated by automated methods limits full comparison with prior studies that have used nonautomated methods such as Etest or BMD (2, 19, 20). Studies comparing methodologies show that, depending on the standard to which compared, automated methods such as Microscan may overestimate, approximate, or underestimate the true MIC values (30–33). Therefore, use of an automated method may produce a different categorization with regard to this exposure variable, in part because of differences in the concentrations assayed. When we compared Etests to Microscan, we found the Etest and Microscan results were in essential agreement in a majority of cases, with MIcroScan overall yielding higher MIC values. While automated methods such as Microscan do not perfectly replicate results from clinical standards, due to their widespread use they are of important clinical relevance and thus were the focus of our analysis.

Several other limitations in our study deserve consideration. First, it occurred at a single institution, potentially limiting the generalizability of our findings (2, 18–20). Second, the available clinical data did not include granular information on treatment (e.g., duration or therapy adjustments), limiting our ability to control for this important determinant of patient outcomes. Furthermore, while we had access to information on hospital courses within our medical system, we did not have access to information regarding whether or not patients had outside hospital admissions that may have captured further morbidity and mortality. Third, the high genetic diversity of our MSSA collection decreased our power to detect meaningful differences in clinical outcomes based on clonal lineage. We were also underpowered to assess the impact of *agr* deficiency on morbidity and mortality.

In summary, while we found no association between the RVS phenotype and mortality in our study, we identified an important association with complicated infection. We also found an association between the phenotype and specific clonal background, younger age and lower burden of comorbidity. Taken together, these findings suggest the RVS phenotype as a potential marker of strain virulence. Further studies need to be conducted to explore this host-pathogen relationship.

MATERIALS AND METHODS

Study population. We carried out a retrospective, observational cohort study of all patients ≥ 18 years of age with a blood culture positive for MSSA from 1 January 2010 through 1 January 2012. The study was approved by the Institutional Review Board. Patients infected with MSSA BSIs were identified via blood culture logs maintained by the clinical microbiology laboratory. This academic tertiary care medical center is comprised of a large adult inpatient hospital, as well as a community hospital that is a referral center for local nursing facilities. Patients were excluded if medical charts were not available, the first identifiable culture was postmortem, or the culture was from a sterile body fluid other than blood.

An infectious diseases physician was responsible for reviewing all chart information. We extracted demographic and clinical information from the patient's charts, including prior hospital admissions, transfer from a nursing home/long-term-care facility, dialysis, invasive surgery, presence of a central venous catheter, and presence of a percutaneous device. The bloodstream infections were categorized as community onset (CO) if the initial culture was collected within 72 h of admission and hospital-onset (HO) if collected greater than 72 h after admission. Data on primary site of infection were collected (34). The primary site of infection was defined based on reported symptoms prior to bacteremia, coupled with diagnostic studies. Metastatic infections were defined as additional sites of infections that developed with a temporal delay after the onset of bacteremia or when the clinical evidence (i.e., multiple septic emboli on central nervous system [CNS] imaging) clearly supported metastatic or embolic disease. In cases where the site of infection was not clearly stated in the chart, the source was inferred from available clinical indicators or, if none were identifiable, it was categorized as an "unknown source." Data on patient comorbidities were collected to assign a Charlson comorbidity index (CCI) score to each patient (35). The Pitt bacteremia score (PBS) was also used to assess the severity of illness at time of the incident blood culture (36, 37). Both scores were dichotomized based on previously investigated cutoffs that are predictive of mortality (14). Only the first bacteremia episode during the study period was included in this analysis. The main outcome was all-cause 30-day mortality. The secondary outcome was complicated bacteremia, defined by metastatic or embolic seeding, endovascular infection, or a duration of >3 days.

Microbiological and molecular studies. Vancomycin and all other antibiotic MICs were determined by automated Microscan with Prompt (Beckman Coulter) inoculation according to Clinical and Laboratory Standards Institute (CLSI) standards (38). Vancomycin Etests (bioMérieux) were performed on 99% (250/252) of isolates according to the manufacturer's instructions. The vancomycin MIC was dichotomized, with the RVS phenotype defined as an MIC of ≥2 mg/liter (Microscan).

agr dysfunction was detected via the standard delta-hemolysin production assay (strains negative for delta-hemolysin production are considered *agr* deficient) (39). The repeat region of the staphylococcal protein A (*spa*) gene was sequenced, assigned a *spa* type, and clustered using the BURP (based upon repeat pattern) algorithm via Ridom StaphType software (v2.2.1) as previously described (40, 41).

Statistical analyses. All statistical analyses were completed using SAS v9.4 (SAS Institute, Inc.). Agreement between the Microscan and Etest measurements of vancomycin MIC was measured using the nonparametric Spearman correlation coefficient. Univariable relationships of strain and patient characteristics with the RVS phenotype were tested using chi-squared, Fisher exact, Student *t* test, or Wilcoxon rank sum tests where appropriate. Similar tests were used to investigate the relationship of clinical factors and strain characteristics to the primary and secondary outcomes, all-cause mortality and complicated bacteremia, respectively. These relationships were further assessed using multivariable logistic regression models. The RVS phenotype, gender, and age were included in the final model *a priori*. Other demographic and known risk factors were selected into the model if the univariable *P* value of <0.20. After assessing multicollinearity of variables, the most parsimonious model was selected.

Logistic regression models were used to test the hypothesized interaction between the RVS phenotype on mortality. To examine how clonal background modifies the effect of RVS phenotype on outcome, we included an interaction term. Similarly, interaction between either the RVS phenotype or clonal lineage and the infection category (i.e., health care associated/hospital onset, health care associated/community onset, and community associated) was tested using multivariable logistic regression models. The interaction terms were evaluated using the Wald chi-square test. We also hypothesized that PBS (as a proxy for disease severity) or embolic/metastatic spread would mediate the relationship between clonal lineage and outcome. Since there was no significant association between dichotomized PBS or embolic/metastatic spread and outcome, the requirements for mediation analysis were not met.

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