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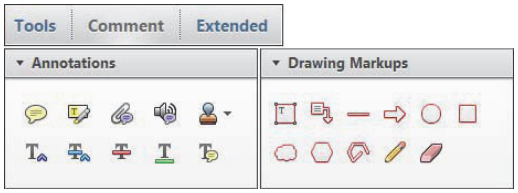

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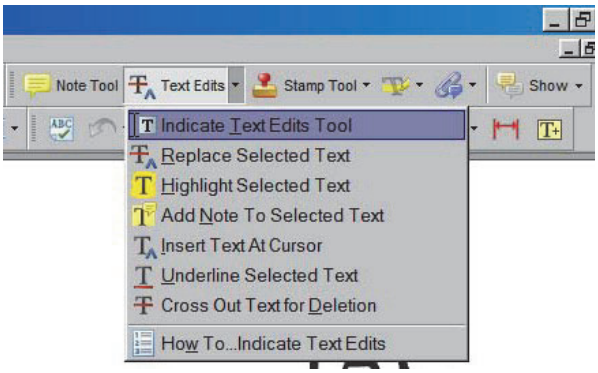

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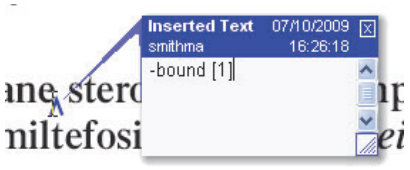

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Journal of Antimicrobial Chemotherapy

Is reduced vancomycin susceptibility a factor associated with poor prognosis in MSSA bacteraemia?

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Objectives: The known data about the influence of vancomycin MIC on *Staphylococcus aureus* bacteraemia are contradictory. Our objective was to study the possible impact of vancomycin MIC ≥1.5 mg/L on short- and medium-term mortality.

Methods: A prospective cohort study was carried out from March 2008 to January 2011 on adult patients with MSSA bacteraemia admitted to a tertiary hospital located in Seville (Spain). We studied the relationship between vancomycin MIC, accessory gene regulator (*agr*) type and absence of δ-haemolysin and poor prognosis. All isolates were genotyped by PFGE. Multivariate analysis, including a propensity score for having a vancomycin MIC of ≥1.5 mg/L, was performed by Cox regression.

Results: One-hundred and thirty-five episodes of bacteraemia due to MSSA were included in the analysis. Twenty-nine (21.5%) isolates had a vancomycin MIC of ≥1.5 mg/L by Etest. There were no differences in *agr* distribution or absence of δ-haemolysin between isolates with reduced vancomycin susceptibility (RVS) and those without. RVS was not more frequent in specific clones; RVS was not associated with higher 14 or 30 day crude mortality (RR=0.44, 95% CI=0.14–1.35; and RR=1.01, 95% CI=0.52–1.96) rates, and it did not show higher rates of complicated bacteraemia (14.2% versus 13.8%, *P*=0.61). Cox regression analysis did not significantly modify the results for 14 day mortality (HR=0.39, 95% CI=0.11–1.34) or 30 day mortality (HR=0.89, 95% CI=0.39–2.04).

Conclusions: Contrary to previously published data, we did not find a relationship between RVS and higher mortality in patients with MSSA bacteraemia and we did not find a link with higher complicated bacteraemia rates.

Keywords: *S. aureus*, MICs, virulence factors

Introduction

The prognosis of *Staphylococcus aureus* bacteraemia (SAB) is determined by several factors, not all of them well known. While the severity of underlying diseases and variables related to clinical management have been clearly associated with prognosis, the influence of microbiological factors is more controversial. In some studies and a meta-analysis, higher vancomycin MICs (even in the susceptible range) have been associated with a worse outcome.[1–3] It was hypothesized that the probability of achieving appropriate pharmacokinetic–pharmacodynamic targets in patients with MRSA bacteraemia treated with vancomycin was much lower when the vancomycin MIC was ≥1.5 mg/L.[4] However, other studies also found this association in patients with MSSA bacteraemia not treated with vancomycin,[5,6] which suggests that reduced vancomycin susceptibility (RVS) could itself be a marker for some other intrinsic property of these isolates associated with a poorer outcome. Significantly, a recent well-conducted meta-analysis did not find vancomycin MIC to be associated with mortality among patients with SAB,[7] although mortality associated with MSSA bacteraemia and vancomycin MIC could not be evaluated in that analysis because the individual studies did not consider that outcome. Other microbiological factors, such as accessory gene regulator (*agr*) type or *agr* dysfunction, have been associated with RVS, although their involvement in the outcome is not clear. The objective of our study was to analyse the relationship between specific microbiological determinants, including vancomycin MIC, and mortality in patients with MSSA bacteraemia.

Methods

Study design and patients

We carried out a prospective cohort study that included all episodes of bacteraemia due to MSSA diagnosed in patients aged ≥18 years, admitted to a 950 bed tertiary hospital located in Seville (Spain) from March 2008 to January 2011. The analysis was reported following the STROBE recommendations (please see the Supplementary data available at *JAC* Online). Aspects of the methodology employed have been previously reported in detail.[8] In summary, patients with SAB were detected through daily review of microbiology reports. An experienced team of clinical microbiologists and infectious diseases specialists followed all included patients from admission to up to 30 days after discharge. For the prognosis analysis, patients with limitation of therapeutic effort were excluded because of their short expected survival. The study was approved by the Ethics Committee of the Hospital Universitario Virgen Macarena, Seville, which waived the need to obtain written informed consent from patients because of the observational nature of the study.

Variables and definitions

The main outcome variable was 14 and 30 day all-cause mortality after the first positive MSSA isolate from blood culture. Mortality among patients who were discharged before day 14 or day 30 was assessed by outpatient clinic visits and/or phone calls. As secondary endpoints, the presence of severe sepsis or septic shock at presentation and complicated and persistent bacteraemia was also assessed.

Explanatory variables included vancomycin MIC by Etest, demographics, type and severity of underlying conditions, acquisition of SAB, severity of systemic inflammatory response syndrome at presentation,[9] antimicrobial therapy, support therapy and outcome. We used the Charlson comorbidity index to measure the severity of chronic underlying conditions,[10] which has been validated as predictive of mortality among patients with SAB.[11] Acute severity of illness was assessed using the Pitt bacteraemia score measured retrospectively on the day before SAB was diagnosed, which is also validated as a predictor of mortality in SAB.[12,13] The patients whose estimated expectancy of life was <72 h the first day when *S. aureus* was isolated as specified by the physician in charge were classified as 'limitation of therapeutic effort'. Type of acquisition was classified following Friedman's criteria.[14] Primary sources of SAB were defined according to the CDC[15] and evaluated in agreement with two investigators. Sources of SAB associated with high mortality in previous studies were classified as high-risk sources; these included endocarditis, endovascular infections other than catheter-related, CNS infections, intra-abdominal infections and respiratory tract infections.[4,16] For evaluation of clinical management, we considered appropriate empirical treatment, early source control, echocardiography in patients with complicated bacteraemia, and early use of intravenous cloxacillin as definitive therapy in accordance with previous definitions and published results.[17,18] Early use of with intravenous cloxacillin was defined as the administration of this antibiotic within the first 24 h after susceptibility results were available, at a dose of at least 2 g every 6 h (or adjusted based on renal function). Early source control was defined as removal of non-permanent vascular catheters whenever the catheter was suspected or confirmed as the source of SAB, or drainage of an abscess within 72 h after *S. aureus* isolation. Duration of antimicrobial therapy was considered adequate if it was at least 14 days for uncomplicated bacteraemia and 28 days for complicated bacteraemia. We defined inadequate management as any of the following: inadequate empirical treatment; no early source control; patients with complicated bacteraemia who did not undergo echocardiography; and absence of early use of intravenous cloxacillin as definitive therapy. Empirical therapy was considered appropriate if an *in vitro* active drug was administered during the first 24 h after obtaining the blood culture, and inappropriate otherwise. Persistent SAB was defined as the isolation of *S. aureus* in blood cultures obtained from peripheral veins for ≥3 days, in spite of active antimicrobial therapy according to susceptibility testing. To facilitate the comparison of results, we used the same definition of complicated bacteraemia as Aguado *et al*.,[5] who defined it as bacteraemia that fulfilled one of the following events occurring after the first bacteraemia episode: (i) the development of endocarditis, septic thrombophlebitis (defined as MSSA bacteraemia persisting at least 72 h after initiating active antimicrobial drug therapy+documented thrombi), arthritis or spondylitis, as well as end-organ haematogenous spread of infection to other locations; or (ii) infection involving vascular or osteoarticular prostheses (excluding intravascular catheter) not removed within 4 days. The clinical situation on the day when the definitive blood culture results were reported (48–72 h later) was compared with that prevailing on the day when the blood cultures were taken and classified as unfavourable if there was a worsening or an evident lack of improvement in the signs of sepsis.[9] Recurrence was defined as *S. aureus* isolated from a blood culture or deep-seated focus of infection with the same PFGE pattern within 3 months of reaching clinical cure.

Microbiological data

Two or three sets of two blood samples (corresponding to four or six bottles, respectively) containing 15 mL of blood each were drawn at 20–30 min intervals from patients presenting with fever of >38°C or when bacteraemia was suspected. Positive blood cultures were processed following standard protocols.[19] Isolates were identified and tested for antimicrobial susceptibility using the semiautomatic WIDER I system (Soria Melguizo, Madrid, Spain) during daily microbiology work, and stored at −80°C. Vancomycin susceptibility was studied in stored isolates by broth microdilution and Etest (AB Biodisk, Solana, Sweden), which were performed in parallel on January 2011, following EUCAST guidelines.[20] Vancomycin MIC was read by two independent observers. Etest was repeated for confirmation in all isolates with MIC=1.5 mg/L. Any isolate showing a vancomycin MIC of ≥1.5 mg/L by Etest was considered to have RVS. All isolates were genotyped by PFGE after SmaI digestion of chromosomal DNA. A dendrogram was constructed with Fingerprinting II (Bio-Rad) software, using the Dice coefficient and the unweighted pair group method with arithmetic averages, position tolerance of 1% and optimization setting of 0.5%. PFGE types were defined using similarity cut-off points of 80%. Gels were also analysed by visual inspection, in accordance with the criteria of Tenover *et al*.[21] Amplification of the polymorphic X region of the protein A gene (*spa*) from all *S. aureus* isolates was carried out using spa-1113f 5′-TAAAGACGATCCTTCGGTGAGC-3′ and spa-1514r 5′-CAGCAGTAGTGCCGTTTGCTT-3′ primers, recommended in http://www.ridom.de/staphtype/spa_sequencing.shtml, then sequenced. Nucleotide sequences were analysed with Ridom StaphTypeTM software version 1.5 (Ridom GmbH, Würzburg, Germany). The BURP algorithm was used to calculate *spa* clonal complexes (CCs), with the following default parameters for group/cluster definition: 'exclude *spa* types that are shorter than 5 repeats' and '*spa* types are clustered if the cost is ≤4'. The PCR-based method described by Gilot *et al*.[22] was used to determine specific *agr* groups. δ-Haemolysin activity was determined by cross-streaking perpendicularly to RN4220, which produces only β-haemolysin, on a sheep blood agar plate.

Statistical analysis

Univariate analyses were performed using the χ^2 or Fisher's test for qualitative variables, and the Student's *t*-test for continuous variables, as appropriate. Multivariate analyses were performed by Cox regression. Variables were selected using a backward stepwise procedure; *P* values <0.2 and <0.1 were used as the respective cut-offs for inclusion of variables in models or for their deletion. Effect modifications between exposures of interest and other variables were investigated. A propensity

score for being infected with an isolate showing RVS was calculated using a non-parsimonious multivariate logistic regression model and was included in the multivariate analysis. Explanatory variables considered as potentially influencing the presence of a vancomycin MIC ≥1.5 mg/L by Etest included age, sex, diabetes, COPD, chronic renal insufficiency, cardiac insufficiency, prior antibiotic therapy, type of acquisition, source, type of *agr* and presence or absence of δ-haemolysin. This model showed a *P* value of 0.35 with the Hosmer–Lemeshow goodness-of-fit test and an area under the receiver operating characteristic curve of 0.71, so showing an acceptable ability to predict the presence of an isolate with RVS. The SPSS v17.0 package was used for statistical analysis.

Results

During the study period there were 141 episodes of bacteraemia due to MSSA. Six were excluded from the outcome analyses because of the decision of limitation of therapeutic effort, so that 135 episodes were analysed for outcome. By microdilution and Etest, the MIC_{50} values for vancomycin were 0.5 and 1 mg/L, the MIC_{90} values were 1 and 1.5 mg/L, and the MIC ranges were 0.5–1 and 0.75–1.5 mg/L, respectively. Twenty-nine (21.5%) isolates had a vancomycin MIC of ≥1.5 mg/L by Etest. None of the isolates had a vancomycin MIC of ≥2 mg/L by this method.

The main genetic features of the isolates with and without RVS are summarized in Table 1. Sixty-five (48.1%) of the isolates were non-producers of δ-haemolysin and the proportion of non-producers was much higher among isolates harbouring *agr* type III (*n*=38, 58.5%) compared with *agr* type II (*n*=14, 21.4%) or *agr* type I (*n*=13, 20%), with *P*<0.001. There were no differences in *agr* distribution or absence of δ-haemolysin between isolates that had RVS and those that did not. PFGE profiles are shown in Figure 1; 101 of the isolates (74.8%) were clustered into seven major pulse types (marked with asterisks). The remaining presented either sporadic profiles or belonged to minor pulse types and one isolate could not be typed. Twenty out of 29 isolates

Table 1. Genetic features of the isolates and features of included patients

Variable	All patients (*n*=135)	Vancomycin MIC <1.5 mg/L (*n*=106)	Vancomycin MIC ≥1.5 mg/L (*n*=29)	*P*
agr subgroup				
I	47 (34.8)	35 (33)	12 (41.2)	0.35
II	39 (28.9)	28 (26.4)	11 (37.9)	0.23
III	41 (30.4)	36 (34)	5 (17.2)	0.08
IV	8 (5.9)	7 (6.6)	1 (3.4)	0.45
Absence of δ-haemolysin	65 (48.1)	53 (50)	12 (41.4)	0.41
Age (years), median (SD)	67 (13.7)	67 (13.3)	66 (15.1)	0.55
Female	56 (41.5)	41 (38.7)	15 (51.7)	0.21
Comorbidity				
diabetes mellitus	45 (33.3)	36 (34)	9 (31)	0.77
chronic pulmonary disease	23 (17)	16 (15.1)	7 (24.1)	0.19
chronic heart failure	15 (11.1)	14 (13.2)	1 (3.4)	0.12
malignancy	28 (20.7)	24 (22.6)	4 (13.8)	0.30
chronic renal disease	25 (18.5)	20 (18.9)	5 (17.2)	0.84
Immunosuppression	14 (10.4)	13 (12)	1 (3.4)	0.17
Condition predisposing to endocarditis	30 (22.2)	25 (23.6)	5 (17.2)	0.47
Charlson index, median (SD)	2 (2)	2 (1.9)	1 (1.9)	0.32
Pitt score, median (SD)	2 (1.8)	2 (1.8)	1 (1.8)	0.79
Acquisition				
hospital	84 (62.2)	68 (64.2)	16 (55.2)	0.38
healthcare	26 (19.3)	18 (17)	8 (27.6)	0.20
Source of bacteraemia				
vascular catheter	61 (45.2)	47 (44.3)	14 (48.3)	0.71
unknown source	24 (17.8)	19 (17.9)	5 (17.2)	0.93
skin and/or soft tissue	18 (13.3)	14 (13.9)	4 (13.8)	0.57
respiratory tract	10 (7.4)	8 (7.5)	2 (6.9)	0.91
high-risk source[a]	43 (31.9)	34 (32.1)	9 (31)	0.92

Data are expressed as number of patients (%), except where specified.
[a]High-risk source: endocarditis, endovascular infections other than catheter-related, CNS infections, intra-abdominal infections and respiratory tract infections.

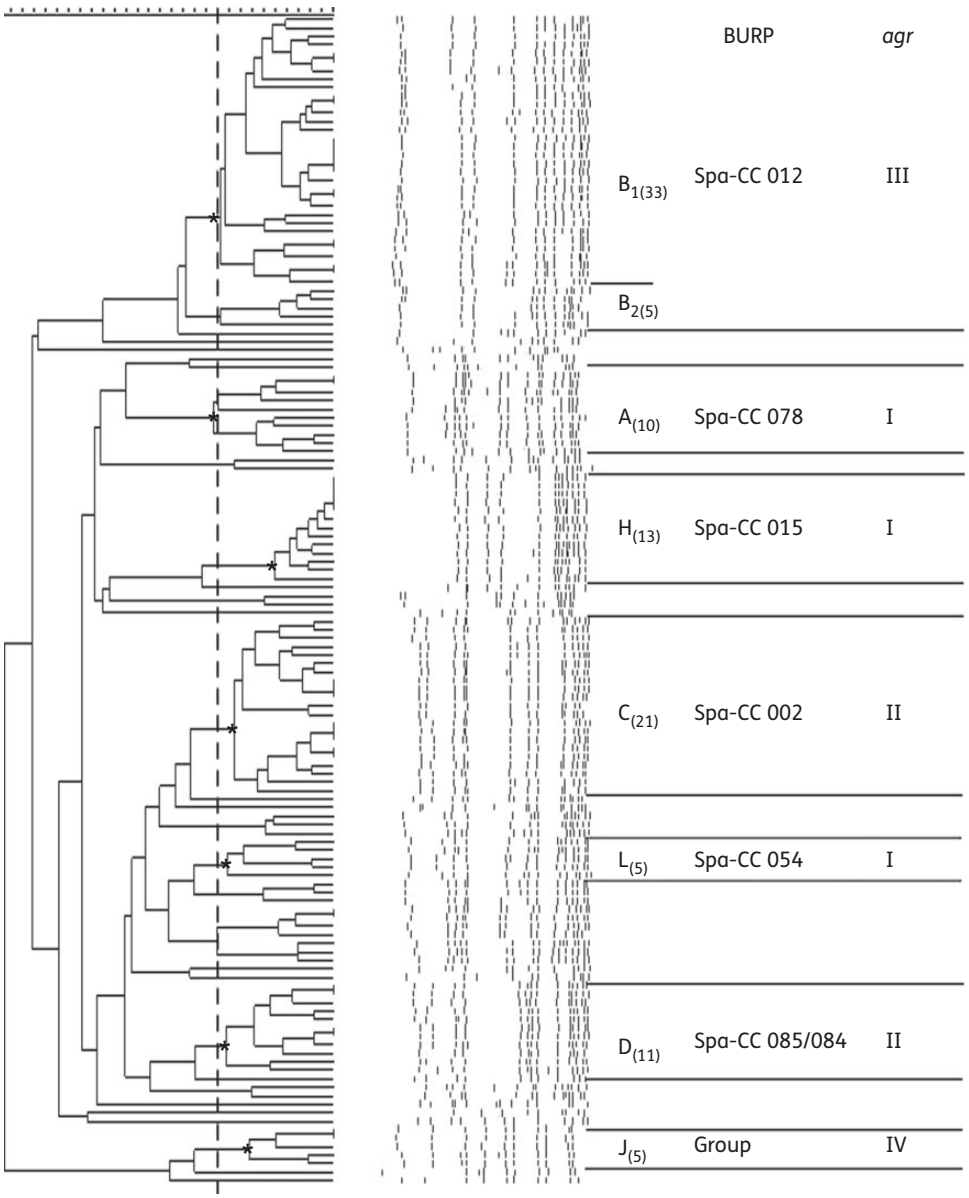

Figure 1. Dendrogram showing the genetic relationships between the 135 MSSA isolates and correlations between the different typing methods. The broken line indicates the similarity cut-off of 80%. Asterisks indicate the major clusters found.

with RSV were distributed among the major pulse types [A (4, 20%), B (2, 10%), C (8, 40%), D (2, 10%), H (2, 10%), L (1, 5%) and J (1, 5%)], the rest being found in minor or sporadic pulse types. RVS was not more frequent in any specific pulse type in our collection. All but two isolates underwent successful *spa* typing. Seventy-four *spa* types were obtained and further clustering rendered six *spa* CCs and one group showing a good correspondence with the major clusters identified by PFGE. When analysing *spa* types or *spa* CCs within the RVS group, no significant conclusion could be obtained. Fifteen isolates with RVS had *spa* types that were either shorter than five repeats or not included in the main *spa* CCs. The characteristics of patients with and without RVS were similar (Table 1). Targeted therapy and clinical management are summarized in Table 2. The frequency of inappropriate empirical treatment was higher in the non-RVS group. The remaining clinical management indicators showed no differences between the compared groups. Cases with RVS did not present a worse clinical evolution at 48 h or worse rates of ICU admission or mortality at 14 and 30 days after the first positive blood culture. Cases with RVS presented higher rates of persistent bacteraemia in the bivariate analysis, although without statistically significant differences ($P=0.25$). Multivariate analysis including appropriate empirical therapy, early source control, *agr* type and δ-haemolysin activity did not show different results (OR=1.53, 95% CI=0.56–4.17, $P=0.41$). We found no association between *agr* type or δ-haemolysin activity and higher frequencies of severe sepsis or shock at SAB presentation, persistent bacteraemia or death at days 14 and 30 (Table 3).

Table 2. Treatment and outcomes, according to vancomycin MIC

Variable	All patients ($n=135$)	Vancomycin MIC <1 mg/L ($n=106$)	Vancomycin MIC ≥1.5 mg/L ($n=29$)	*P*
Clinical management				
appropriate empirical therapy	122 (90.4)	93 (87.7)	29 (100)	
early source control	125 (92.6)	97 (91.5)	28 (96.6)	0.04
echocardiography for complicated bacteraemia	17/19 (89.5)	14/15 (93.3)	3/4 (75)	0.32
early use of intravenous cloxacillin as definitive therapy[a]	104/125 (83.9)	84/98 (85.7)	20/26 (76.9)	0.37
treatment duration according to complexity of infection[b]	79/108 (73.1)	60/84 (71.4)	19/24 (79.2)	0.21
Targeted therapy				
cloxacillin	104/124 (83.9)	84/124 (85.7)	20 (76.9)	0.21
glycopeptides	8/124 (6.5)	8/98 (8.2)	0/26 (0)	0.14
antistaphylococcal β-lactams other than cloxacillin[c]	9/124 (7.3)	5/98 (5.1)	4/26 (15.4)	0.09
non-β-lactam antistaphylococcal agents[d]	8/124 (6.5)	5/98 (5.1)	3/26 (11.5)	0.22
Complicated bacteraemia	19 (14.1)	15 (14.2)	4 (13.8)	0.61
endocarditis[e]	11 (8.1)	9 (8.5)	2 (6.9)	0.57
septic thrombophlebitis	2 (1.5)	0 (0)	2 (6.9)	0.05
arthritis, spondylitis and haematogenous secondary foci of infection	3 (2.2)	3 (2.8)	0 (0)	0.48
infection involving indwelling devices that were not removed	3 (2.2)	3 (2.8)	0 (0)	0.48
Severe sepsis or septic shock during treatment	38 (28.1)	31 (29.2)	7 (24.1)	0.59
Adequate support treatment	33/38 (86.8)	27/31 (87.1)	6/7 (85.7)	0.66
Persistent bacteraemia[a]	26/125 (20.8)	18/98 (18.4)	8/26 (30.8)	0.25
Unfavourable course at 48 h[f]	57 (45.2)	43 (44.3)	14 (48.3)	0.71
ICU admission after MSSA bacteraemia diagnosed	17 (12.6)	11 (10.4)	6 (20.6)	0.12
Mortality				
crude 14	28 (20.7)	25 (23.6)	3 (10.3)	0.12
crude 30	37 (27.4)	29 (27.4)	8 (27.6)	0.98

Data are expressed as number of patients (%).
[a]In patients surviving at day 3.
[b]In patients surviving at day 10 for non-complicated bacteraemia and at day 28 for complicated bacteraemia.
[c]Third-generation cephalosporins, carbapenems, amoxicillin/clavulanic acid or piperacillin/tazobactam.
[d]Clindamycin, quinolones, linezolid or daptomycin.
[e]Only considered in patients who received echocardiography.

Table 3. Genetic features of isolates

	Persistent bacteraemia[a]	*P*	Severe sepsis or septic shock	*P*	14 Day mortality	*P*	30 Day mortality	*P*
agr subgroup		0.58		0.83		0.21		0.23
I	8/41 (19.5)		12/47 (25.5%)		11/47 (23.4)		15/47 (31.9)	
II	10/38 (26.3)		10/39 (26.6)		4/39 (10.3)		7/39 (17.9)	
III	8/40 (20)		13/41 (31.7)		10/41 (24.4)		11/41 (26.8)	
IV	0/6 (0)		3/8 (35.7)		3/8 (37.5)		4/8 (50)	
δ-Haemolysin		0.18		0.79		0.84		0.14
positive	19/70 (20.1)		14/62 (22.6)		15/70 (21.4)		23/70 (22.9)	
negative	19/65 (29.2)		12/63 (19)		13/65 (20)		14/65 (21.5)	

Data are expressed as number of patients (%).
[a]Only patients who survived long enough to investigate persistent bacteraemia were included.

Table 4. Univariate analysis of variables associated with 14 day mortality

Variable	Dead/ exposed (%)	RR (95% CI)	*P*
Age (years)			
<60	4 (11.8)	reference	
≥60	24 (23.8)	2.01 (0.75–5.41)	0.14
Acquisition			
hospital	22 (20)	reference	
community	6 (24)	1.20 (0.54–2.65)	0.66
Charlson index			
<3	18 (21.2)	1.06 (0.53–2.11)	0.87
≥3	10 (20)	reference	
Source			
skin and/or soft tissue	1/18 (5.6)	reference	
unknown	9/61 (14.8)	2.66 (0.36–19.58)	0.28
respiratory	6/24 (25)	4.50 (0.59–34.16)	0.10
catheter	6/10 (60)	10.80 (1.50–77.51)	0.003
High-risk source[a]			
no	13 (14.1)	reference	
yes	15 (34.9)	2.47 (1.29–4.72)	0.006
Pitt score			
<3	13 (13.5)	reference	
≥3	15 (38.5)	2.84 (1.49–5.40)	0.002
Complicated bacteraemia			
no	24 (20.7)	reference	
yes	4 (21.1)	1.02 (0.39–2.61)	0.97
Inadequate clinical management			
no	17 (17.3)	reference	
yes	11 (29.7)	1.71 (0.89–3.31)	0.11
Vancomycin Etest MIC (mg/L)			
<1.5	25 (23.6)	reference	
≥1.5	3 (10.3)	0.44 (0.14–1.35)	0.12

[a]High-risk source: endocarditis, endovascular infections other than catheter-related, CNS infections, intra-abdominal infections and respiratory tract infections.

Table 5. Univariate analysis of variables associated with 30 day mortality

Variable	Dead/ exposed (%)	RR (95% CI)	*P*
Age (years)			
<60	5 (14.7)	reference	
≥60	32 (31.7)	2.15 (0.91–5.08)	0.06
Acquisition			
hospital	30 (27.3)	reference	
community	7 (28)	1.03 (0.51–2.06)	0.94
Charlson index			
<3	23 (27.1)	reference	
≥3	14 (28)	1.23 (0.74–2.06)	0.91
Source			
skin and/or soft tissue	2/18 (11.1)	reference	
unknown	11/61 (18)	1.62 (0.40–6.66)	0.39
respiratory	12/24 (50)	4.50 (1.14–17.64)	0.008
catheter	5/10 (50)	4.50 (1.06–19.11)	0.04
High-risk source[a]			
no	16 (17.4)	reference	
yes	24 (48.8)	2.80 (1.64–4.82)	<0.001
Pitt score			
<3	20 (20.8)	reference	
≥3	17 (43.6)	2.09 (1.23–3.55)	0.007
Complicated bacteraemia			
no	32 (27.6)	1.04 (0.47–2.35)	0.91
yes	5 (26.3)	reference	
Inadequate clinical management			
no	23 (23.5)	reference	
yes	14 (37.8)	1.61 (0.93–2.78)	0.09
Vancomycin Etest MIC (mg/L)			
<1.5	29 (27.4)	reference	
≥1.5	8 (27.6)	1.01 (0.52–1.96)	0.98

[a]High-risk source: endocarditis, endovascular infections other than catheter-related, CNS infections, intra-abdominal infections and respiratory tract infections.

The variables associated with 14 and 30 day mortality are summarized in Tables 4 and 5, respectively. Cox regression models for mortality including the propensity score are shown in Table 6. We also repeated the analyses by removing the variable Pitt score with no significant changes to the model (14 day mortality: HR=0.44, 95% CI=0.13–1.54, P=0.20; 30 day mortality: HR=0.96, 95% CI=0.41–2.23, P=0.92).

Discussion

Our data did not show a relation between RVS, *agr* type or δ-haemolysin activity and mortality among patients with SAB due to MSSA. Whether or not RVS is associated with a poorer outcome in SAB is a matter of debate. Two of three previous meta-analyses concluded that there was an association between them.[2,3] In our opinion, the significance of vancomycin MIC and its consequences for prognosis in MRSA bacteraemia cannot be applied to MSSA. The article published by Van Hal *et al.*[1] only found this association when the isolate had a vancomycin MIC ≥2 mg/L by Etest. In our study, it was not possible to evaluate whether a higher vancomycin MIC cut-off point could be associated with poor prognosis because none of the isolates had this property. The conclusion reached by the previous meta-analyses may be biased because they included different types of infection (with too many different prognoses) and very heterogeneous patient populations, and they did not analyse the influence of notable variables like comorbidity, severity of illness at presentation or clinical management. However, one recent meta-analysis that only included SAB failed to find this association even after a stratified analysis according to different MIC cut-off points, current or previous vancomycin treatment, year of publication and quality of study design, among others.[7]

Table 6. Cox regression for 14 and 30 day mortality; the propensity score for RVS was included in the models

Variable	HR (95% CI)	*P*
14 day mortality		
vancomycin MIC ≥1.5 mg/L	0.39 (0.11–1.34)	0.13
age >60 years	2.12 (0.70–6.43)	0.18
Pitt score ≥3	3.44 (1.58–7.48)	0.002
high-risk source	1.99 (0.91–4.40)	0.09
inadequate clinical management	1.67 (0.75–3.71)	0.21
30 day mortality		
vancomycin MIC ≥1.5 mg/L	0.89 (0.39–2.04)	0.78
age >60 years	2.19 (0.83–5.77)	0.12
Pitt score ≥3	2.52 (1.29–4.92)	0.007
high-risk source	2.64 (1.34–5.18)	0.005
inadequate clinical management	1.61 (0.81–3.22)	0.18

In the case of MSSA, two recent studies involving bacteraemia[5,6] and a third involving left-sided infective endocarditis[23] concluded that a higher vancomycin MIC (employing the same cut-off point as MRSA) was associated with poor prognosis, assessed in terms of crude mortality. It should be specified that the first article published by Holmes *et al.*[6] did not include comorbidity or clinical management variables in the analysis. Therefore, they repeated the analysis 2 years later, using a retrospective methodology to correct this bias, although they were not finally included in the multivariate analysis.[24] The authors explained that, for reasons of sample size, they excluded relevant prognostic variables with *P* values of between 0.05 and 0.2 in the univariate model (e.g. Charlson comorbidity or ICU admission), to achieve a multivariate model that was more predictive than explanatory. To avoid that bias, we included relevant clinical variables using a prospective methodology and a propensity score in our analysis. We were unable to find any link between RVS and higher mortality, either in the short term or the medium term. To facilitate comparison with the results published by Aguado *et al.*,[5] we also used the same definition of complicated bacteraemia. Despite this, we did not find a link between RVS and complicated bacteraemia in our cohort. The meta-analysis published in 2014 by Kalil *et al.*[7] did not help to resolve the question because none of the 38 included studies reported data for MSSA mortality independently.

Nobody to date has offered a pathophysiological explanation linking the potential association between vancomycin MIC and outcome. Some authors think that the concept of vancomycin-intermediate *S. aureus* and heterogeneous vancomycin-intermediate *S. aureus* could arise with MSSA,[25,26] whereby isolates present increased cell-wall thickness, reduced autolytic activity and metabolic changes that would explain its association with poor prognosis. A higher vancomycin MIC would be a marker of such special bacterial characteristics. We do not really know the clinical significance of this condition, or whether it could be a consequence of previous vancomycin therapy. In our cohort, none of the patients had received vancomycin in the previous year, and only 6.5% of cases received vancomycin as targeted therapy in the current episode (none of them in the RVS group). This contradicts the hypothesis of prior exposure to vancomycin and RVS.[27] There are several potential hypotheses, none of which have been confirmed.

agr is a quorum-sensing operon that up-regulates the production of secreted virulence factors and down-regulates the production of cell-associated virulence factors. *agr* dysfunction (evaluated using the absence of δ-haemolysin as its surrogate marker) has been associated with attenuated vancomycin bactericidal activity and higher mortality among critically ill patients.[28,29] *agr* dysfunction was detected in nearly half of the isolates, with high rates in *agr* type III and lower rates in *agr* types I and II. Like Viedma *et al.*,[30] we studied the possible relationship between *agr* group, *agr* dysfunction and RVS, but were unable to find one. The previous series had a smaller sample size than ours (84 versus 135 cases), which would not explain our different results. Compared with previous publications, our study analyses in greater depth whether other microbiological characteristics might explain differences in prognosis other than MIC. In our cohort, cases with RVS presented no differences related to PFGE distribution, *agr* type or δ-haemolysin activity.

The main limitation of our study is its single-centre methodology and its relatively limited sample size. Although our methodology tried to control for potential confounding factors by using a Cox regression model that included a propensity score, it is possible that other factors that we did not measure influenced the results. We carried out several multivariate models with similar results. In the final model, we removed the Pitt score variable, since it might have been a variable intermediate between RVS (or some other unknown characteristic of the isolate) and mortality. Although our univariate results did not show an association between these variables, we wanted to stop and explore this possibility.

In 2008, it was not common practice to assess vancomycin MIC by Etest on MSSA isolates, so we performed the Etest on previously stored isolates. This could be of relevance because some authors have described changes in MIC after defrosting the isolates.[31] This effect may be a bias, but, if it is real, it would affect most previous publications.

The strengths of our study are its prospective design, the molecular characterization of the isolates performed by PFGE, the use of clinical and management indicators and the attempt to control for the effect of confounders. To our knowledge, this is the first published series showing these results.

In conclusion, in contrast to previously published data, we did not find a relationship between RVS and higher mortality in patients with MSSA bacteraemia, and we did not find a link with higher complicated bacteraemia rates. Cases with RVS did not present higher rates of severe sepsis or septic shock at presentation of the bacteraemia.

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Transparency declarations

A. P. has been a consultant for Pfizer, has served as speaker for Wyeth and Pfizer, and has received research support from Pfizer, Wyeth and Novartis. J. R.-B. has served as scientific advisor and speaker for Merck, AstraZeneca and Pfizer. All other authors: none to declare.

Author contributions

J. R.-B. and L. E. L.-C. had full access to all the data in this study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: J. R.-B., J. G.-A., A. P. and L. E. L.-C.

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Critical revision of the manuscript for important intellectual content: P. R., J. G.-A., M. D. d. T., C. V. and A. P.

Statistical analysis: L. E. L.-C. and J. R.-B.

Study supervision: J. R.-B.

Supplementary data

Supplementary data are available at *JAC* Online (http://jac.oxfordjournals.org/).

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