Influence of vancomycin minimum inhibitory concentration on the outcome of methicillin-susceptible Staphylococcus aureus left-sided infective endocarditis treated with antistaphylococcal β-lactam antibiotics: a prospective cohort study by the International Collaboration on Endocarditis

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Abstract

Objectives: A recent study showed a poorer prognosis in left-sided MSSA endocarditis treated with cloxacillin when the vancomycin MIC was ≥1.5 mg/L. We aimed to validate these results using the International Collaboration on Endocarditis cohort and to analyze whether specific genetic characteristics were associated with a high vancomycin MIC (≥1.5 mg/L) phenotype.

Methods: All patients with left-sided MSSA IE treated with anti-staphylococcal beta-lactam antibiotics between 2000 and 2006 with available isolates were included. Vancomycin MIC (VAN MIC) was determined by E-test as either *high* (≥1.5 mg/L) or *low* (<1.5 mg/L). Isolates underwent *spa* typing to infer clonal complexes (CC) and multiplex polymerase chain reaction for identifying virulence genes. Univariate analysis was performed to evaluate the association between in-hospital and one-year mortality and vancomycin MIC phenotype.

Results: 62 cases met the inclusion criteria. VAN MIC was *low* in 28 cases (45%) and *high* in 34 cases (55%). No significant differences in patient demographic data or characteristics of infection were observed between patients with IE due to *high* and *low* VAN MIC isolates. Isolates with *high* and *low* VAN MIC had similar distributions of virulence genes and clonal lineages. In-hospital and one-year mortality did not differ significantly between the two groups (32% (9/28) vs. 27% (9/34), P=0.780; and 43% (12/28) vs. 29% (10/34), P=0.298, for *low* and *high* VAN MIC, respectively).

Conclusion: In this international cohort of patients with left-sided MSSA endocarditis treated with anti-staphylococcal beta-lactams, vancomycin MIC phenotype was not associated with patient demographics, clinical outcome, or virulence gene repertoire.

BACKGROUND

Methicillin-susceptible *Staphylococcus aureus* (MSSA) is the leading cause of infective endocarditis (IE) worldwide [1]. Despite the advances on diagnosis and management, the in-hospital mortality of MSSA IE has stabilized at 20-25% over the last three decades [2,3].

The relationship between vancomycin minimum inhibitory concentration (MIC) of bloodstream *S. aureus* isolates and patient outcome is unresolved. Although several studies have now shown an association between *high* MIC (≥1.5 mg/L) in methicillin-resistant *S. aureus* (MRSA) bloodstream isolates and a worse clinical outcome in patients with MRSA bacteremia [4], other reports have failed to confirm this observation [5]. However, no clinical studies have addressed the potential association between *high* vancomycin MIC and worse patient outcome in patients with MRSA IE. Furthermore, studies in the experimental model of IE have failed to demonstrate such associations [6].

The impact of a *high* vancomycin MIC phenotype (HMV; ≥1.5 mg/L) in MSSA bacteremia and IE and the relationship with *agr* dysfunction is also poorly defined. To date, several studies reported higher rates of complications and mortality in patients with MSSA bacteremia caused by strains with HVM [1,2] as well as a correlation with *agr* dysfunction and *agr* type II polymorphism [3]. Nonetheless, a more recent study did not find significant differences on *agr* subgroup and function according to the vancomycin MIC [4]. The association between HVM and significantly higher mortality was also demonstrated in a Spanish a cohort of 93 patients with MSSA IE treated with cloxacillin [5]. These clinical findings await international validation.

Objectives

We aimed to explore the association between higher vancomycin MIC and clinical outcome among patients with left-sided MSSA IE in the International Collaboration Endocarditis (ICE) Cohort and whether HMV was identifiable by a genetic "signature" of specific polymorphisms and virulence factors. The aims of this study were to validate the findings of our previous study in the and to analyze whether specific genetic characteristics were associated with the presence of *high* vancomycin MIC in a large, well-characterized cohort of geographically diverse MSSA isolates causing IE.

METHODS

Database

ICE-Prospective Cohort Study has been described previously [6]. Briefly, participating members from 64 sites in 28 countries reported patients prospectively on a standard case report form from 2000 through 2006. The case report form included 275 variables and was developed by ICE collaborators according to standard definitions [1]. All patients were included from sites that met performance criteria for participation. These criteria include the following: minimum enrolment of 12 patients per year in a centre with access to cardiac surgery; the presence of patient identification measures to ensure consecutive enrolment and to minimize ascertainment bias [1]; high quality data with query resolution. and the strains obtained from IE episodes were available in the ICE-Microbiology Repository [7], which included >1300 bloodstream isolates from prospectively identified patients with definite IE from 16 countries, obtained between June 2000 and September 2006. All participating sites had institutional review board or ethical committee approval or a waiver and informed consent (verbal or written) from all patients based on local standards as required by the Coordinating centre (Duke University Medical Center).

Study Sample

Patients and Settings

IE isolates were obtained from the Microbiological Repository of ICE-PCS [7]. Subjects in the ICE-Microbiology Repository cohort with available frozen bloodstream isolates with definite MSSA IE treated only with beta-lactams were eligible for inclusion in this study. Seventeen of these strains were included in the Spanish cohort [5]. Variables collected included demographics, characteristics of the episodes of IE, complications, treatment,

and outcome. All patients who survived had, at least, one year of follow-up. Antibiotic therapy was decided by the treating physician at the individual study site.

Definitions

IE was defined according to the modified Duke Criteria [8] and was considered to be left-sided if no right-sided (tricuspid or pulmonary valve) vegetations were present on echocardiographic examination, surgery, or autopsy. A strain was considered to have HVM when it was ≥1.5 mg/L by E-test, and a *low* vancomycin MIC (LVM) when it was <1.5 µg/mL. The rest of definitions have been previously provided in detail [9].

Geographic regions

Geographic regions included the following 25 participating sites (see **Appendix**): United States (4 sites), South America (2 sites from Brazil and Chile), Europe (12 sites from Croatia, France, Greece, Italy, Slovenia and Spain), Australia and New Zealand (6 sites) and Middle East (1 site from Lebanon).

Microbiological methods

ICE methodology for microbiological procedures has been defined elsewhere [6,10]. Detailed microbiological methods can be found in the **Supplemental Appendix**. MSSA strains were stored at -80º C in a central laboratory (Duke University) and tested for nafcillin, cloxacillin, vancomycin and daptomycin MIC by E-test, following the manufacturer's recommendations (bioMérieux, Marcy l'Etoile, France).

Multiplex Polymerase Chain Reaction. Genomic DNA was prepared as previously described [12]. Bacterial determinants including adhesins, toxins, *agr* group I–IV, and

other genes were screened by multiplex polymerase chain reaction (PCR) as previously described [12]. All negative calls on the multiplex PCR were confirmed by uniplex PCR.

Spa Typing. Spa typing was performed as previously described [12, 15]. PCR oligonucleotide primers for *spa* were described previously [15]. Samples were sequenced at the Duke University sequencing laboratory. For spa typing, eGenomics software (http://tools.egenomics.com/) was used to scan the primary sequence to help identify the orders and names of each repeat. The spa type number is representative of the repeat organization. Clonal complexes (CC) for the isolates were identified via repeats pattern recognition from existing spa type and CC database previously confirmed via multilocus sequence typing (MLST). Isolates whose spa type did not map to a known CC underwent MLST typing. For MLST, the sequence chromatograms for unique alleles were deposited in the MLST database (http://www.mlst.net). Alleles at the 7 loci (*arcC*, *aroE*, *glpF*, *gmk*, *pta*, *tpi,* and *yqiL*) were used to identify a unique sequence type (ST). MLST allele names and STs were derived from http://www.mlst.net. CCs were assigned to groups of isolates sharing 6 of 7 alleles by using the eBURST algorithm (http://eburst.mlst.net) [16].

Statistical analysis

Categorical variables were expressed as percentages and compared with Fisher's exact test. Continuous variables were expressed as means or medians (depending on the variable's homogeneity) and compared by non-parametric Wilcoxon's score/Mann Whitney's U tests. Survival analysis was performed by Kaplan-Meier analysis, and curves were compared by the log-rank test. Given the limited sample size, logistic regression analysis was not performed. A two-sided p-value <0.05 was considered to be statistically significant. To determine the rate of type I errors in null hypothesis testing when

conducting multiple comparisons among sociodemographic variables, the false discovery rate (FDR) was calculated after adjustment at P<0.10.

RESULTS

Cases included

There were 1,072 cases of MSSA IE cases included in the ICE-PCS, of which 114 isolates were available in the ICE-Microbiology Repository substudy. Of these, 62 (28 LVM, 34 HVM) were cases of left-sided MSSA treated with anti-staphylococcal beta-lactam antibiotics with enough clinical data for the final analysis. The study flowchart is shown in **Figure 1**.

Vancomycin MIC distribution in the cohort

The distribution of vancomycin MIC among the 62 cases included in the study is displayed in **Figure 2** along with the respective rates of mortality for 0.75, 1, 1.5 and ≥2 µg/mL vancomycin MIC determinations.

Demographics and clinical characteristics

Demographics and clinical characteristics of patients with LVM and HVM are shown in **Table 1**. There were no significant differences found in age, sex, place of acquisition of the infection, or geographical area between the two groups. Most episodes were native valve IE (77.4%) acquired in the community (64.5%). After adjustment, no variables were significant at FDR <0.10.

Outcomes

Complications, surgical rates and mortality according to vancomycin MIC groups are shown in **Table 1**. Differences between the two groups did not reach statistical significance in any of the variables. For LVM, in-hospital and one-year mortality were

32% (9/28) and 43% (12/28), respe tivel , while in-h spital rtalit was 27% (9/34) and one-year mortality 29% (10/34) for HVM.

Genotypic characteristics

Differences in genotypic characteristics between the two groups according to MIC phenotypes are shown in the **Supplemental Appendix, Table 2**. No differences were detected regarding adhesins, toxins or other putative virulence factors. Thirty-eight percent of patients with HVM presented *agr*-II compared to 19% of patients with LVM. However, this difference was not statistically significant (*P*=0.157). With regard to CC, CC30 was the most frequent CC in the whole cohort. Differences in the prevalence of the CCs between the HVM and LVM groups were not statistically significant.

Risk factors for mortality

The univariate analysis of risk factors for one-year mortality is shown in **Table 2**. HVM, type of *agr* and CC were not significantly associated with mortality. The analysis for in-hospital mortality using the same variables did not differ from that of one-year mortality (data not shown). Among clinical markers, paravalvular complications [OR 4.0 (95%CI 1.1-14.6); *P*=0.021] and stroke [OR 4.72 (95%CI 1.2-19.1); *P*=0.015) were associated with a poorer prognosis at one-year. With regard to virulence factors, *see* [OR 3.33 (0.9-12.2); *P*=0.044] and *sei* [OR 2.94 (1.1-13-7); *P*=0.044] were identified as risk factors for mortality whereas *chp* was found to be a protective factor [OR 0.07 (0-0.7); *P*=0.006].

The Kaplan-Meier survival plot at one-year according to vancomycin MIC group is displayed in the **Supplemental Appendix**.

Discussion

Vancomycin MIC was not associated with complications or mortality among patients with MSSA left-sided IE treated with beta-lactams. Thus, this study was unable to validate findings from the Cervera et al study [5], Holmes et al. described significantly higher 30-day mortality in patients with MSSA bacteremia caused by strains with HVM, regardless of the treatment received (either cloxacillin or vancomycin) [7]. Another study found that HVM was significantly associated with a higher likelihood of developing a complicated bacteremia in a cohort of 99 patients with MSSA catheter-associated bacteremia [8]. In an 84-patient MSSA bacteremia cohort study, HVM was significantly related with *agr* dysfunction and *agr* type II polymorphism [9]. In the Cervera et al study we found MSSA endocarditis caused by strains with a HVM had a 3-fold higher in-hospital mortality rate and that major embolic events, especially stroke, were more frequent within this subgroup [11], from which two main hypotheses were raised: 1) HVM in MSSA isolates was associated with higher mortality in patients with left-sided IE due to an increased rate of major embolic events; and 2) a genomic signature identified MSSA isolates with HVM. However, in the present study systemic embolic events were higher in the LVM group and stroke was almost equally common in the two groups. Moreover, both in-hospital and one-year mortality were higher in the LVM group although these differences were not statistically significant probably because of the small sample size.

Main findings from studies investigating the relationship between VAN MIC and prognosis of MSSA and IE, as well as its relationship with *agr* dysfunction are shown in **Table 3.** Higher mortality among patients with LVM than in patients with HVM was found in the present study, as well as higher rates of systemic embolic events (36% vs. 23%) and persistent bacteremia (14% vs.12%), neither of them reaching statistical significance.

Given that no significant differences were found in virulence factors between the two groups, we might speculate that in this dataset, due to the high proportion of patients with MSSA strains harbouring LVM and CC30, a high rate of detected and not detected hematogenous complications in the LVM may explain the higher mortality in this group.

With regard to genotypic features, the analysis of *agr* subgroup did not reveal a significant association between a specific *agr* polymorphism and HVM or CC. We expected to find an association between a HVM and *agrII* polymorphism relying on previous studies performed in MSSA bacteremia [3,11]. Holmes et al. found an overall statistically significant association between a HVM and *agrII* in a cohort of 242 patients with *S. aureus* bacteremia (104 MRSA and only 38 MSSA) [17]. However, a subanalysis of the MSSA subset was not performed but would have probably been limited by small sample size. A more recent study that included 135 episodes of MSSA bacteremia of various origins did not find significant differences on *agr* subgroup and function according to the vancomycin MIC with a threshold of ≥1.5 µg/mL [10]. In our study, *agrII* was two times more frequent in the HVM than in the LVM (38% vs. 19%), but did not reach statistical significance. In our cohort, CC5 was only slightly more common in patients with HMV and CC30 was two times more common in the LVM group. CC30 was the most common CC within the whole cohort as previously described in a cohort of 228 patients with MSSA IE and skin and STI [6]. As in our study, Viedma et al. did not find specific associations between CC subtypes and HVM although in their cohort there was a trend toward a higher proportion of CC5 in the HVM group compared to the LVM group (21.8% vs. 6.9%; *P*=0.070). Fowler and colleagues found a significantly higher proportion of CC5 (and CC30) among patients with *S. aureus* bacteremia with hematogenous complications but not in the subgroup of MSSA patients [15]. In the study by Holmes et al [17], CC8

was significantly associated with HVM, and in the case of CC5, the association approached statistical significance (6.5% in the LVM group and 14.2% in the HVM group; *P*=0.055). Thus, the hypothesis of a higher embolic potential among HVM strains could not be demonstrated. However, Further studies are needed before firm conclusions can be drawn regarding the association between CC and hematogenous complications in left-sided IE caused by MSSA strains.

We did not identify a specific repertoire of virulence factors in the HVM group, as other previous studies did [6,7,10]. The virulence factors we found to be associated with worse prognosis have not been previously described as risk factors for mortality in the clinical field. In the Nienaber et al study, MSSA IE strains were shown to more likely contain 3 adhesins (*clfB, cna, map/eap*) and 5 enterotoxins (*tst, sea, sed, see, and sei*) than STI strains with no significant geographical variations [12]. Holmes et al found a significantly higher proportion of *sea* and *clfA* among *S. aureus* strains with HVM causing bacteremia [17]. In a recently published Indian study, the most frequently identified toxin genes among patients with MSSA bacteremia were *sei* and *seg* [18]. In an epidemiologic study analyzing CC and diversity of exotoxin gene profiles performed in 81 strains of *S. aureus* isolated in a Spanish hospital (68% of which were MSSA), *agrII* was the most common gene and was linked to CC5, *sea* and *sec* [19]. In the study by Nienaber et al., no significant *agrII* variations among geographical areas where found, with 68% of isolates being from Europe and Middle East, 23% from North America and 9% from Oceania [12]. Although geographical regions are almost equally represented, changes in the genetic expression and phenotypic pattern in MSSA might be common over the time and geographical variations are also likely to occur as years pass. In the present cohort, the geographical regions are almost equally represented. However, since changes in the

genetic expression and phenotypic pattern in MSSA might be common over the time and geographical variations are also likely to occur as years pass, we wonder if some of the different findings between the Cervera et al. study [5] and the present study could be due to different periods of time (1995-2011 in the Spanish cohort and 2000-2006 in the ICE) and geographic regions (local vs. intercontinental samples). Actually, the proportion of MSSA strains with HMV in Cervera et al. study significantly decreased along the period of the study [5]. Another factor that could also have influence in the percentage of MSSA strains identified as HVM is the effect of freezer storage. Ludwig et al. demonstrated a progressive decline of VAN MIC determination by Etest in MRSA bloodstream samples from the moment they were frozen [14]. Either due to locoregional or global variations of MSSA clones, further studies on the occurrence of variations of MSSA genotypic features are needed to correlate our results with more precise data.

The superantigen genes *see* and *sei*, encoded by the enterotoxin gene cluster, conferred worse prognosis at one year, with 3-fold higher mortality. There are some *in vivo* data pointing to an important role of these virulence genes in the severity of invasive *S. aureus* infections [14]. However, the pathway in which these genes are involved, as other superantigens, is T-cell activation by binding to major histocompatibility complex class II molecules and to certain Vβ varieties on the T-cell receptor, leading to septic shock and not embolic events, including stroke. Chemotaxis inhibitory protein (*chp*), a protective factor in this cohort, is a major staphylococcal defense evasion mechanism against the human innate immune system [21]. Potentially, the presence of *chp* involves a loss of fitness and thus lowers virulence in patients with MSSA strains harboring this gene in this cohort. Yet, it is important to stress that outcomes of IE patients may not be easily attributable to simply virulence factors. The role played by the host is crucial and definite

conclusions should be drawn from studies analyzing both sides of the interplay, namely the host and the pathogen. For example, outcomes of patients with IE due to relatively low-virulence microorganisms, such as coagulase-negative staphylococci, are associated with heart failure, the presence of prosthetic devices, comorbidities of the patient and the need of surgery [15-17] albeit coagulase-negative staphylococci causing IE have unique genetic signatures which are found across vast geographic distances [18], and HMV has also been identified as a marker of bad prognosis [19].

This study has several limitations. First, the low sample size is a major shortcoming, severely limiting the statistical power of the study and leading to the potential for type II error in the analysis. Second, the number of isolates tested and available is not representative of the overall ICE dataset. However, we conducted a subanalysis comparing ICE left-sided MSSA frozen strains and those that were not frozen, and we did not detect significant differences regarding in-hospital and one-year mortality and surgery rates (data not shown). Third, *agrII* dysfunction was not measured, so correlations with *agr* subgroup, vancomycin MIC, and outcomes were not performed. Fourth, only cases occurring in the 2000-2006 period were included, which precluded observations regarding the temporal trends on vancomycin MIC, CC, or virulence factors according to each geographical region. Given that the data presented are older than 10 years, the clinical relevance of current MSSA circulating strains may have changed. Fifth, the potential variations of MSSA clones between geographical regions were not investigated. And finally, all patients were treated with anti-staphylococcal beta-lactam antibiotics (cloxacillin, nafcillin and first generation cephalosporins), but the specific type of beta-lactam used was not available. Despite these limitations, this is the first study on this subject including genetic studies of the

bacterial isolates in order to find an association with the vancomycin phenotype and clinical outcomes.

In conclusion, in this international cohort of left-sided MSSA IE treated with beta-lactams, vancomycin MIC ≥1.5 µg/mL was not found to be an independent risk factor for complications of IE or for mortality. Stroke, paravalvular complications, and some *S. aureus* genes were associated with a worse outcome. Differences in clonality and virulence factors were not found between strains with LVM and HVM. Further studies in this field are warranted to expand upon these findings and elucidate if the contradictory results obtained in this field [15] are due to methodological limitations or rather to a difficult-to-interpret phenomenon.

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VGF reports the following potential conflicts of interest: Chair of the Scientific Advisory Board for Merck V710; paid consultant for Pfizer, Novartis, Galderma, Novadigm, Durata, Debiopharm, Genentech, Achaogen, Affinium, Medicines Co., Cerexa, Tetraphase, Trius, MedImmune, Bayer, Theravance, Cubist, Basilea, Affinergy; Grants pending from MedImmune, Actavis/Forest/Cerexa, Pfizer, Merck/Cubist, Advanced Liquid Logics, Theravance, Novartis, Medical Surfaces, and Locus Biotechnology; royalties from UpToDate, personal fees for development or presentation of educational presentations (Green Cross, Cubist, Cerexa, Durata, Theravance), outside the submitted work; and patent pending related to sepsis diagnostics. JMM has received consulting honoraria and/or research grants from AbbVie, Bristol-Myers Squibb, Cubist, Novartis, Gilead Sciences, and ViiV. All other authors: no conflicts.

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FIGURE LEGENDS

Figure 1. Flowchart of the patients included in the study. Abbreviation: ICE, International Collaboration on Endocarditis; IE, infective endocarditis; MIC, Minimum Inhibitory Concentration; MSSA, methicillin-susceptible *Staphylococcus aureus*; VAN, vancomycin.

Figure 2. Distribution of the vancomycin MIC within the cohort of 62 left-sided MSSA Infective endocarditis and overall one-year mortality rates according to vancomycin MIC determination.

Figure 3. Kaplan–Meier survival graph of mortality at one-year days of follow-up according to vancomycin minimum inhibitory concentration. Abbreviation: MIC, minimum inhibitory concentration.

Table 1. Demographics, Clinical Characteristics and Outcomes of 62 Episodes of Left-sided Methicillin-Susceptible *Staphylococcus aureus* Infective Endocarditis Treated with Beta-lactams According to "High" or "Low" Vancomycin Minimum Inhibitory Concentration (MIC).

	Vancomycin MIC <1.5 µg/mL n=28	**Vancomycin MIC ≥1.5 µg/mL n=34**	***P***
Mean age (SD)	61.1 (18.5)	60.1 (14.3)	0.396
Male gender (%)	19 (68%)	27 (79%)	0.386
Type of endocarditis:			0.548
▪ Native valve	23 (82%)	25 (74%)	
▪ Prosthetic valve	5 (19%)	9 (27%)	
Origin of acquisition:			0.916
▪ Community-acquired	17 (61%)	23 (68%)	
▪ Nosocomial	8 (29%)	9 (26%)	
▪ Healthcare-associated	2 (7%)	2 (6%)	
▪ Unknown	1 (3%)	0	
Geographical area:			0.333
▪ North America	4 (14%)	10 (29%)	
▪ Europe/Mid East	20 (71%)	22 (65%)	
▪ South America	1 (4%)	0	
▪ Australia/New Zealand	3 (11%)	2 (6%)	
Complications:			
▪ Heart failure	13 (46%)	10 (29%)	0.195
▪ Systemic emboli	10 (36%)	8 (23%)	0.400
▪ Stroke	7 (25%)	9 (27%)	1.000
▪ Paravalvular complications	8 (29%)	11 (32%)	0.788
▪ New conduction abnormality	0 (-)	3 (9%)	0.245
▪ Persistent bacteremia	4 (14%)	4 (12%)	1.000
Surgical treatment	9 (32%)	14 (41%)	0.599
Relapses*	0	0	1.000

Mortality			
▪ In-hospital mortality	9 (32%)	9 (27%)	0.780
▪ One-year mortality	12 (43%)	10 (29%)	0.298

* Defined as a new episode of endocarditis due to the same microorganism that caused the first IE within the following 12 months.

Table 2. Genotypic Characteristics and Distribution of Clonal Complexes of 62 Episodes of Left-sided Methicillin-Susceptible *Staphylococcus aureus* Infective Endocarditis Treated with Beta-lactams According to *High* or *Low* Vancomycin Minimum Inhibitory Concentration (MIC).

	Vancomycin MIC <1.5 µg/mL n=28	Vancomycin MIC ≥1.5 µg/mL n=34	*P*
Adhesins			
- *fnbA*	28 (100%)	34 (100%)	1.000
- *fnbB*	5 (19%)	7 (21%)	1.000
- *clfA*	28 (100%)	34 (100%)	1.000
- *clfB*	28 (100%)	34 (100%)	1.000
- *cna*	25 (90%)	29 (85%)	0.720
- *spa*	27 (96%)	33 (97%)	1.000
- *scrC*	12 (43%)	16 (47%)	0.801
- *sdrD*	18 (64%)	19 (56%)	0.606
- *sdrE*	12 (43%)	16 (47%)	0.801
- *bbp*	25 (89%)	28 (82%)	0.495
- *ebpS*	28 (100%)	34 (100%)	1.000
- *map/eap*	17 (61%)	25 (74%)	0.413
Toxins			
- *eta*	4 (14%)	10 (29%)	0.225
- *etb*	1 (4%)	1 (3%)	1.000
- *tst*	27 (96%)	31 (91%)	0.620
- *sea*	21 (75%)	19 (56%)	0.182
- *seb*	0	2 (6%)	0.497
- *sec*	6 (21%)	10 (29%)	0.566
- *sed*	5 (18%)	9 (27%)	0.546
- *see*	9 (32%)	9 (27%)	0.780
- *seg*	22 (79%)	24 (71%)	0.566
- *seh*	1 (4%)	4 (12%)	0.366

- *sei*	24 (86%)	31 (91%)	0.691
- *sej*	1 (4%)	2 (6%)	1.000
- *pvl*	6 (21%)	3 (9%)	0.277
- *hlg*	28 (100%)	34 (100%)	1.000
Other putative virulence genes			
- *efb*	28 (100%)	34 (100%)	1.000
- *icaA*	24 (86%)	31 (91%)	0.691
- *chp*	25 (89%)	30 (88%)	1.000
- V8	9 (32%)	19 (56%)	0.077
***Agr* subgroup**			
- I	10 (37%)	12 (35%)	1.000
- II	5 (19%)	13 (38%)	0.157
- III	12 (44%)	8 (24%)	0.105
- IV	0	1 (3%)	1.000
Clonal Complex			
– CC1	2 (7%)	1 (3%)	0.579
– CC5	4 (15%)	6 (18%)	1.000
– CC8	2 (7%)	2 (6%)	1.000
– CC15	1 (4%)	4 (12%)	0.371
– CC30	8 (30%)	5 (15%)	0.212
– CC45	5 (19%)	6 (18%)	1.000
– Other	5 (19%)	10 (29%)	0.382

Table 2. Bi-variable Analysis of Risk Factors for One-year Mortality

Variable	Alive n=39	Dead n=22	OR (95%CI)	*P*
Demographics				
Age >65years	16 (40%)	11 (50%)	1.5 (0.5-4.9)	0.593
Male sex	31 (78%)	15 (68%)	0.62 (0.2-2.4)	0.546
Prosthetic valve IE	11 (29%)	3 (14%)	0.41 (0.1-1.9)	0.338
Diabetes	8 (20%)	4 (18%)	0.89 (0.2-3.9)	1.000
Acquisition in the community	26 (65%)	14 (64%)	1.76 (0.0-141.9)	0.889
North America	8 (20%)	6 (27%)	1.77 (0.1-143.1)	0.444
Clinical features				
Paravalvular complications	8 (20%)	11 (50%)	4.0 (1.1-14.6)	0.021
Stroke	6 (15%)	10 (46%)	4.72 (1.2-19.1)	0.015
Heart failure	10 (46%)	10 (46%)	1.73 (0.5-5.7)	0.411
Persistent bacteremia	5 (13%)	3 (14%)	1.11 (0.2-6.4)	1.000
Surgery	14 (35%)	9 (41%)	1.29 (0.4-4.2)	0.785
Microbiological features				
Vancomycin MIC ≥ 1.5 mg/L	24 (60%)	10 (45%)	0.56 (0.2-1.8)	0.298
CC30	7 (18%)	6 (27%)	1.77 (0.4-7.3)	0.516
CC8	1 (3%)	3 (14%)	6.16 (0.4-331.3)	0.546
CC15	6 (15%)	0	0.0 (0-1.1)	0.081
AGR subgroup I	15 (38%)	7 (32%)	0.78 (0.2-2.6)	0.784
AGR subgroup II	13 (33%)	6 (27%)	0.78 (0.2-2.7)	0.777
AGR subgroup III	11 (28%)	9 (41%)	1.83 (0.5-6.2)	0.394
See	8 (20%)	10 (46%)	3.33 (0.9-12.2)	0.044
Sei	33 (83%)	22 (100%)	2.94 (1.1-13.7)	0.044
Chp	39 (98)	16 (73%)	0.07 (0.0-0.7)	0.006
Eta	8 (20%)	6 (27%)	1.5 (0.4-5.9)	0.539
Pvl	4 (10%)	5 (23%)	2.65 (0.5-14.9)	0.259

The analysis for in-hospital mortality using the same variables did not differ from that of one-year mortality (data not shown).

Table 3. Summary of Main Findings from Studies Assessing the Relationship between Vancomycin MIC and Prognosis in MSSA Bacteremia and Left-sided IE.

First author, year	Design	SAB/IE (n)	Overall mortality *Low* VAN MIC < 1.5 µg/mL	Overall mortality *High* VAN MIC ≥ 1.5 µg/mL	Genetic factors	Main outcomes analysis
Kalil, 2014 [15]	Systematic review and metaanalysis	SAB (8291, both MRSA and MSSA)	25.8% (1430/5551)	26.8% (734/2740)	NA	RD 1.6% [95%CI, −2.3%to 5.6%]; P = 0.43 (for absolute risk of mortality, combining 30-day mortality and in-hospital mortality)
Holmes, 2011 [1]	Prospective multicenter cohort study	SAB (532; 266 of which MSSA treated with betalactams only)	12.2% (24/193)	26.8% (18/68)	NA	P= 0.011 (for 30-day mortality)
Holmes, 2014 [11]	Analysis of a subset of strains from [1]	SAB (252 MSSA isolates)	NA	NA	Associated with HVM: CC8, *agr* dysfunction, *agr* genotype II, *blaZ, sea, clfA, splA* and ACME locus. Associated to LVM: CC22, CC88 and CC188	Associated with HVM: CC8 P < 0.001), *agr* dysfunction (P= 0.014), *agr* genotype II (P=0.043), *blaZ* (P=0.002), *sea* (P < 0.001), *clfA* (P < 0.001), *splA* (P 0.001) and ACME locus (P=0.02). Associated with LVM: CC22 (P < 0.001), CC88 ([P < 0.001) and CC188 (P=0.002)
Aguado, 2011 [2]	Retrospective, single-center Cohort	Catheter-related SAB (99, all MSSA)	10.5% (8/76)	26.1% (6/23)	NA	P=0.13 (for 30-day mortality) OR=22.9, [95%CI 6.7–78.1] for complicated SAB.

López-Cortes, 2015 [4]	Prospective, single-center cohort	SAB (135, all MSSA)	23.6% (25/106)	10.3% (3/29)	There were no differences in *agr* distribution or absence of δ-haemolysin between isolates with HVM and those with LVM. HVM was not more frequent in specific clones.	RR=0.44 [95%CI , 0.14-1.35] for 14-day mortality
Viedma, 2014 [3]	Retrospective, single-center cohort	SAB (84, all MSSA)	24.1%, (7/29) *agr II* polymorphism: 17.2%; average levels of RNAIII gene expression: ΔCt 1.5 ± 2.11	45.5% (25/55) *agr II* polymorphism: 41.8%; average levels of RNAIII gene expression: ΔCt 4.05 ± 3.29	HVM: *agr II* polymorphism: 17.2%; average levels of RNAIII gene expression: ΔCt 1.5 ± 2.11 LVM: *agr II* polymorphism: 41.8%; average levels of RNAIII gene expression: ΔCt 4.05 ± 3.29	In-hospital mortality: P=0.057 *agr* dysfunction: P= 0.023. RNAIII expression: P<.01
Cervera, 2014 [5]	Prospective, single-center cohort	MSSA IE (93)	31% (16/53)	53% (21/40)	NA	In-hospital mortality: P=0.035; Patients with HVM presented significantly more severe embolic events
Current study	Prospective, multicenter cohort	MSSA IE (62)	32% (9/28)	27% (9/34)	HVM: *agrII* polymorphism: 19% LVM: *agrII* polymorphism: 38%	In-hospital mortality: P=0.780. *agrII* polymorphism: P=0.157

CI: Confidence interval; IE: Infective endocarditis; MRSA: methicillin-resistant *Staphylococcus aureus*; NA: not addressed; OR: Odds ratio; RD: relative difference; RR: relative risk; SAB: *S. aureus* bacteremia.

Supplemen l Appen x

1. Microbiological Methods

E-test for Vancomycin MIC

MSSA strains were stored at -80º C in a central laboratory (Duke University) and tested for vancomycin MIC by E-test after the freezing of the samples, following the manufacturer's recommendations (bioMérieux, Marcy l'Etoile, France). E-test were done by duplicate and interpreted by consensus with a second researcher.

Multiplex Polymerase Chain Reaction. Genomic DNA was prepared as previously described [1]. Bacterial determinants including adhesins, toxins, *agr* group I–IV, and other genes were screened by multiplex polymerase chain reaction (PCR) as described before [1]. All negative calls on the multiplex PCR were confirmed by uniplex PCR.

Spa Typing. Spa typing was performed as previously described [1, 2]. PCR oligonucleotide primers for *spa* were described previously [2]. Samples were sequenced at the Duke University sequencing laboratory. For spa typing, eGenomics software (http://tools.egenomics.com/) was used to scan the primary sequence to help identify the orders and names of each repeat. The spa type number is representative of the repeat organization. Clonal complexes (CC) for the isolates were identified via repeats pattern recognition from existing spa type and CC database previously confirmed via multilocus sequence typing (MLST). Isolates whose spa type did not map to a known CC underwent MLST typing. For MLST, the sequence chromatograms for unique alleles were deposited in the MLST database (http://www.mlst.net). Alleles at the 7 loci

(*arcC*, *aroE*, *glpF*, *gmk*, *pta*, *tpi,* and *yqiL*) were used to identify a unique sequence type (ST). MLST allele names and STs were derived from http://www.mlst.net. CCs were assigned to groups of isolates sharing 6 of 7 alleles by using the eBURST algorithm (http://eburst.mlst.net) [3].

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2. Genotypic characteristics of MSSA strains according to VAN MIC.

Differences in genotypic characteristics between the two groups according to MIC phenotypes are displayed in the **Table 1**. No differences were detected regarding adhesins, toxins or other putative virulence factors. Thirty-eight percent of patients with HVM presented *agr*-II compared to 19% of patients with LVM. However, this difference was not statistically significant (*P*=0.157). With regard to CC, CC30 was the most frequent CC in the whole cohort. Differences

in the prevalence of the CCs between the HVM and LVM groups were not statistically significant.

Table 1. Genotypic Characteristics and Distribution of Clonal Complexes of 62 Episodes of Left-sided Methicillin-susceptible *Staphylococcus aureus* Infective Endocarditis Treated with Beta-lactams According to *High* or *Low* Vancomycin Minimum Inhibitory Concentration (MIC).

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- *fnbB*	5 (19%)	7 (21%)	1.000
- *clfA*	28 (100%)	34 (100%)	1.000
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- *cna*	25 (90%)	29 (85%)	0.720
- *spa*	27 (96%)	33 (97%)	1.000
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- *sei*	24 (86%)	31 (91%)	0.691
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- *efb*	28 (100%)	34 (100%)	1.000
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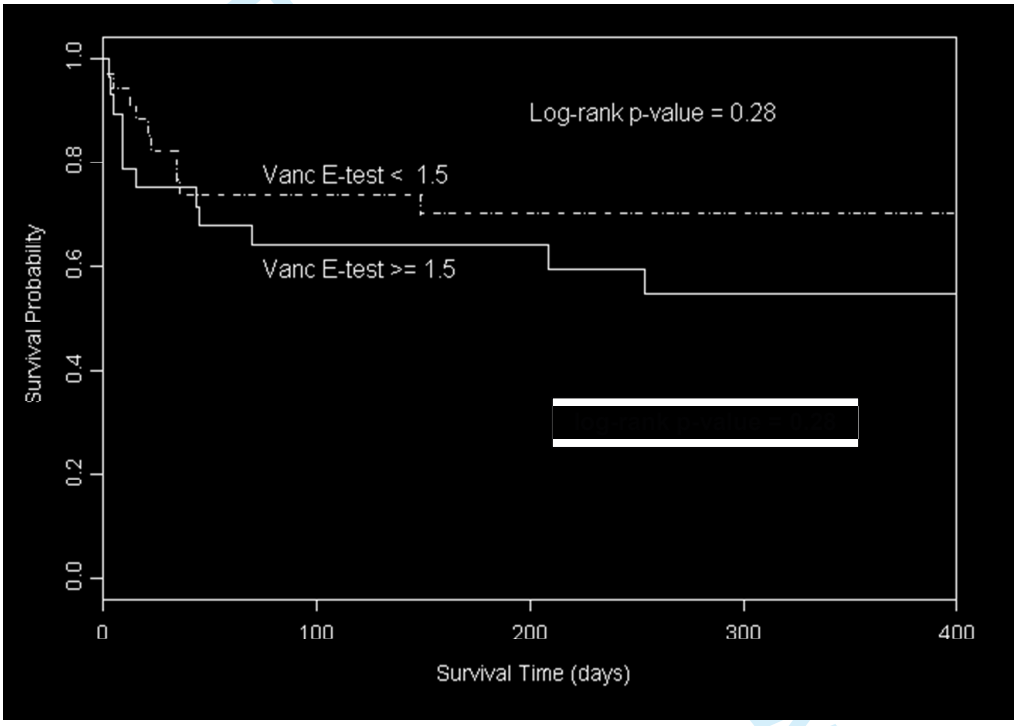
- V8	9 (32%)	19 (56%)	0.077
***Agr* subgroup**			
- I	10 (37%)	12 (35%)	1.000
- II	5 (19%)	13 (38%)	0.157
- III	12 (44%)	8 (24%)	0.105
- IV	0	1 (3%)	1.000
Clonal Complex			
– CC1	2 (7%)	1 (3%)	0.579
– CC5	4 (15%)	6 (18%)	1.000
– CC8	2 (7%)	2 (6%)	1.000
– CC15	1 (4%)	4 (12%)	0.371
– CC30	8 (30%)	5 (15%)	0.212
– CC45	5 (19%)	6 (18%)	1.000
– Other*	5 (19%)	10 (29%)	0.382

* Other CC analysed c pr sed CC9, CC22, CC25, CC34, CC51, CC59, CC72, and 125.

Figure 1. Kaplan–Meier survival graph of mortality at one-year days of follow-up according to vancomycin minimum inhibitory concentration. Abbreviation: MIC, minimum inhibitory concentration.



Title: Influence of Vancomycin Minimum Inhibitory Concentration on the Outcome of Methicillin-Susceptible *Staphylococcus aureus* Left-Sided Infective Endocarditis Treated with Anti-staphylococcal Beta-Lactam Antibiotics; a Prospective Cohort Study by the International Collaboration on Endocarditis.

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Keypoints: In this international cohort of patients with left-sided MSSA endocarditis treated with anti-staphylococcal beta-lactam antibiotics, vancomycin MIC phenotype was not associated with patient demographics, clinical outcome, or virulence gene repertoire.

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Abstract

Objectives: A recent study showed a poorer prognosis in left-sided MSSA endocarditis treated with cloxacillin when the vancomycin MIC was ≥1.5 mg/L. We aimed to validate these results using the International Collaboration on Endocarditis cohort and to analyze whether specific genetic characteristics were associated with a high vancomycin MIC (≥1.5 mg/L) phenotype.

Methods: All patients with left-sided MSSA IE treated with anti-staphylococcal beta-lactam antibiotics between 2000 and 2006 with available isolates were included. Vancomycin MIC (VAN MIC) was determined by E-test as either *high* (≥1.5 mg/L) or *low* (<1.5 mg/L). Isolates underwent *spa* typing to infer clonal complexes (CC) and multiplex polymerase chain reaction for identifying virulence genes. Univariate analysis was performed to evaluate the association between in-hospital and one-year mortality and vancomycin MIC phenotype.

Results: 62 cases met the inclusion criteria. VAN MIC was *low* in 28 cases (45%) and *high* in 34 cases (55%). No significant differences in patient demographic data or characteristics of infection were observed between patients with IE due to *high* and *low* VAN MIC isolates. Isolates with *high* and *low* VAN MIC had similar distributions of virulence genes and clonal lineages. In-hospital and one-year mortality did not differ significantly between the two groups (32% (9/28) vs. 27% (9/34), P=0.780; and 43% (12/28) vs. 29% (10/34), P=0.298, for *low* and *high* VAN MIC, respectively).

Conclusion: In this international cohort of patients with left-sided MSSA endocarditis treated with anti-staphylococcal beta-lactams, vancomycin MIC phenotype was not associated with patient demographics, clinical outcome, or virulence gene repertoire.

BACKGROUND

The impact of a *high* vancomycin MIC phenotype (HMV; ≥1.5 mg/L) in MSSA bacteremia and IE is poorly known. To date, several studies reported higher rates of complications and mortality in patients with MSSA bacteremia caused by strains with HVM [1,2], as well as a correlation with *agr* dysfunction and *agr* type II polymorphism [3]. Nonetheless, a more recent study did not find significant differences on *agr* subgroup and function according to the vancomycin MIC [4]. The association between HVM and significantly higher mortality was also demonstrated in a Spanish a cohort of 93 patients with MSSA IE treated with cloxacillin [5].

Objectives

We aimed to explore the association between higher vancomycin MIC and clinical outcome among patients with left-sided MSSA IE in the International Collaboration Endocarditis (ICE) Cohort and whether HMV was identifiable by a genetic “signature” of specific polymorphisms and virulence factors.

METHODS

Database

ICE-Prospective Cohort Study has been described previously [6]. All patients were included from sites that met performance criteria for participation and the strains obtained from IE episodes were available in the ICE-Microbiology Repository [7]. All participating sites had institutional review board or ethical committee approval or a waiver and informed consent from all patients based on local standards as required by the Coordinating centre (Duke University Medical Center).

Study Sample

Patients and Settings

IE isolates were obtained from the Microbiological Repository of ICE-PCS [7]. Subjects in the ICE-Microbiology Repository cohort with available frozen bloodstream isolates with definite MSSA IE treated with beta-lactams were eligible for inclusion in this study. Seventeen of these strains were included in the Spanish cohort [5]. All patients who survived had, at least, one year of follow-up.

Definitions

IE was defined according to the modified Duke Criteria [8] and was considered to be left-sided if no right-sided (tricuspid or pulmonary valve) vegetations were present on echocardiographic examination, surgery, or autopsy. The rest of definitions have been previously provided in detail [9].

Geographic regions

Twenty-five sites from all Geographic regions participated in the study (see **Appendix**)

Microbiological methods

ICE methodology for microbiological procedures has been defined elsewhere [6,10]. Detailed microbiological methods can be found in the **Supplemental Appendix**.

Statistical analysis

Categorical variables were expressed as percentage and compared with Fisher's exact test. Continuous variables were expressed as means or medians and compared by non-parametric tests. Survival analysis was performed by Kaplan-Meier analysis, and curves were compared by the log-rank test. A two-sided p-value <0.05 was considered to be statistically significant.

RESULTS

Cases included

The study flowchart is shown in **Figure 1**.

Vancomycin MIC distribution in the cohort

The distribution of vancomycin MIC among the 62 cases included in the study is displayed in **Figure 2** along with the respective rates of mortality.

Demographics and clinical characteristics

Demographics and clinical characteristics of patients with LVM and HVM are shown in **Table 1**.

Outcomes

Complications, surgical rates and mortality according to vancomycin MIC groups are shown in **Table 1**. Differences between the two groups did not reach statistical significance in any of the variables. For LVM, in-hospital and one-year mortality were 32% (9/28) and 43% (12/28), respectively, while in-hospital mortality was 27% (9/34) and one-year mortality 29% (10/34) for HVM.

Genotypic characteristics

Differences in genotypic characteristics between the two groups according to MIC phenotypes are shown in the **Supplemental Appendix**. No differences were detected regarding adhesins, toxins or other putative virulence factors.

Risk factors for mortality

The univariate analysis of risk factors for one-year mortality is shown in **Table 2**. HVM, type of *agr* and CC were not significantly associated with mortality. The analysis for in-hospital mortality using the same variables did not differ from that of one-year mortality (data not shown).

The Kaplan-Meier survival plot at one-year according to vancomycin MIC group is displayed in the **Supplemental Appendix**.

Discussion

Vancomycin MIC was not associated with complications or mortality among patients with MSSA left-sided IE treated with beta-lactams. Thus, this study was unable to validate findings from the Cervera et al study [5], from which two main hypotheses were raised: 1) HVM in MSSA isolates was associated with higher mortality in patients with left-sided IE due to an increased rate of major embolic events; and 2) a genomic signature identified MSSA isolates with HVM.

Main findings from studies investigating the relationship between VAN MIC and prognosis of MSSA and IE, as well as its relationship with *agr* dysfunction are shown in **Table 3.**

Higher mortality among patients with LVM than in patients with HVM was found in the present study, as well as higher rates of systemic embolic events (36% vs. 23%) and persistent bacteremia (14% vs.12%), neither of them reaching statistical significance. Given that no significant differences were found in virulence factors between the two groups, we might speculate that in this dataset, due to the high proportion of patients with MSSA strains harbouring LVM and CC30, a high rate of detected and not detected hematogenous complications in the LVM may explain the higher mortality in this group.

With regard to genotypic features, the analysis of *agr* subgroup did not reveal a significant association between a specific *agr* polymorphism and HVM or CC. We expected to find an association between a HVM and *agrII* polymorphism relying on previous studies performed in MSSA bacteremia [3,11]. In our study, *agrII* was two times more frequent in the HVM than in the LVM (38% vs. 19%), but did not reach statistical significance.

We did not identify a specific repertoire of virulence factors in the HVM group, as other previous studies did [6,7,10]. Although geographical regions are almost equally represented, changes in the genetic expression and phenotypic pattern in MSSA might be common over the time and geographical variations are also likely to occur as years pass. Another factor that could also have influence in the percentage of MSSA strains identified as HVM is the effect of freezer storage. Ludwig et al demonstrated a progressive decline of VAN MIC determination by Etest in MRSA bloodstream samples from the moment they were frozen [14].

This study has several limitations. First, the low sample size is a major shortcoming, severely limiting the statistical power of the study and leading to the potential for type II error in the analysis. Second, the number of isolates tested and available is not representative of the overall ICE dataset. However, we conducted a subanalysis comparing ICE left-sided MSSA frozen strains and those that were not frozen, and we did not detect significant differences regarding in-hospital and one-year mortality and surgery rates (data not shown). Third, *agrII* dysfunction was not measured, so correlations with *agr* subgroup, vancomycin MIC, and outcomes were not performed. Fourth, only cases occurring in the 2000-2006 period were included, which precluded observations regarding the temporal trends Fifth, the potential variations of MSSA clones between geographical regions were not investigated. And finally, the specific type of beta-lactam used was not available.

In conclusion, in this international cohort of left-sided MSSA IE treated with beta-lactams, vancomycin MIC ≥1.5 µg/mL was not found to be an independent risk factor for complications of IE or for mortality. Stroke, paravalvular complications, and some *S.*

aureus genes were associated with a worse outcome. Differences in clonality and virulence factors were not found between strains with LVM and HVM. Further studies in this field are warranted to expand upon these findings and elucidate if the contradictory results obtained in this field [15] are due to methodological limitations or rather to a difficult-to-interpret phenomenon.

Financial di cl res

VGF reports the following potential conflicts of interest: Chair of the Scientific Advisory Board for Merck V710; paid consultant for Pfizer, Novartis, Galderma, Novadigm, Durata, Debiopharm, Genentech, Achaogen, Affinium, Medicines Co., Cerexa, Tetraphase, Trius, MedImmune, Bayer, Theravance, Cubist, Basilea, Affinergy; Grants pending from MedImmune, Actavis/Forest/Cerexa, Pfizer, Merck/Cubist, Advanced Liquid Logics, Theravance, Novartis, Medical Surfaces, and Locus Biotechnology; royalties from UpToDate, personal fees for development or presentation of educational presentations (Green Cross, Cubist, Cerexa, Durata, Theravance), outside the submitted work; and patent pending related to sepsis diagnostics. JMM has received consulting honoraria and/or research grants from AbbVie, Bristol-Myers Squibb, Cubist, Novartis, Gilead Sciences, and ViiV. All other authors: no conflicts.

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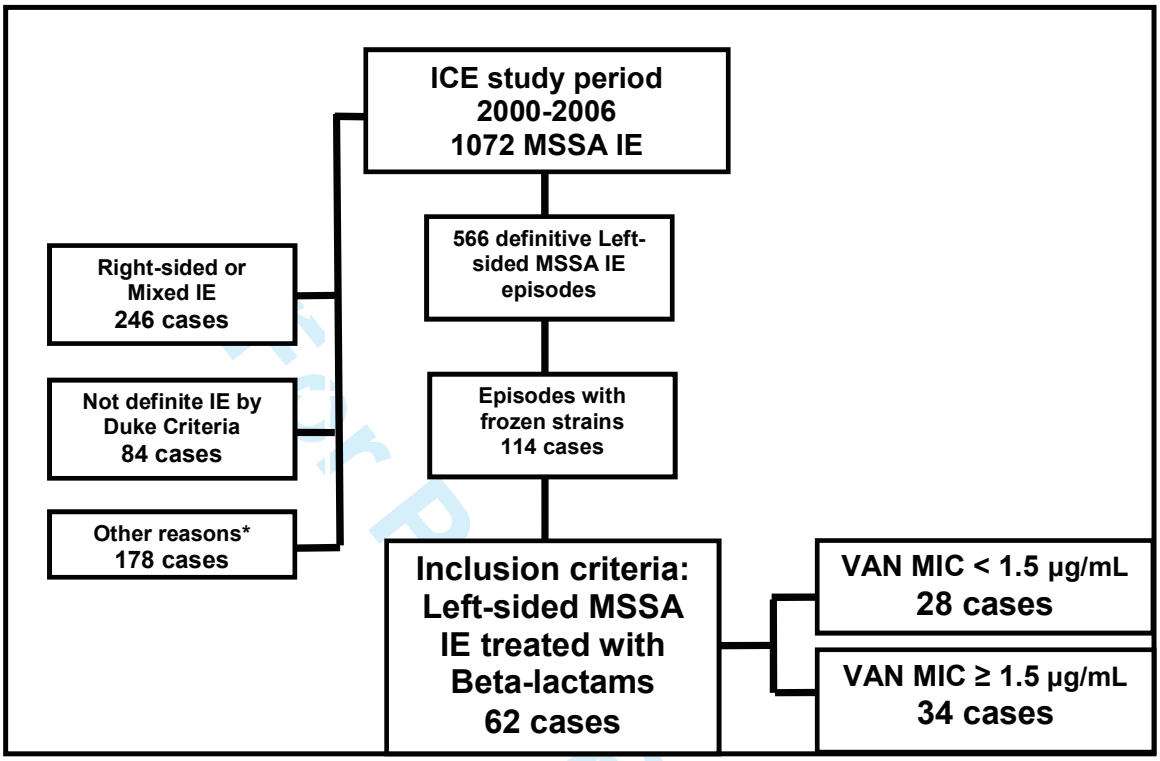
FIGURE LEGENDS

Figure 1. Flowchart of the patients included in the study. Abbreviation: ICE, International Collaboration on Endocarditis; IE, infective endocarditis; MIC, Minimum Inhibitory Concentration; MSSA, methicillin-susceptible *Staphylococcus aureus*; VAN, vancomycin.

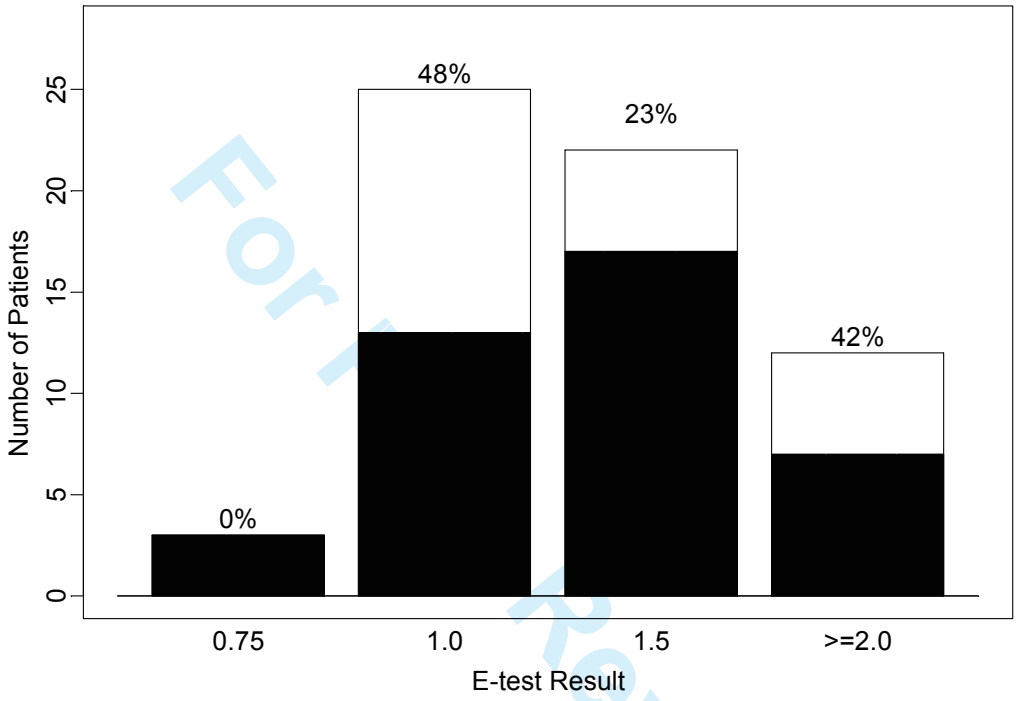
Figure 2. Distribution of the vancomycin MIC within the cohort of 62 left-sided MSSA Infective endocarditis and overall one-year mortality rates according to vancomycin MIC determination.

Figure 1.



*Other causes of exclusion were: lack of vegetations in the echocardiographic, surgical or post-mortem examination (123 patients), age below 18 years (7 patients) and relevant data missing in the CRD (48 patients).

Figure 2.



VAN Group	0.75	1.0	1.5	≥ 2.0
N	3	25	22	12
Deaths	0	12	5	5

Percentages and white areas of every column indicate rates of one-year mortality per VAN MIC group. Black areas represent the percentage of patients alive at one-year.

Table 1. Demographics, Clinical Characteristics and Outcomes of 62 Episodes of Left-sided Methicillin-susceptible *Staphylococcus aureus* Infective Endocarditis Treated with Beta-lactams According to "High" or "Low" Vancomycin Minimum Inhibitory Concentration (MIC).

	Vancomycin MIC < 1.5 µg/mL n=28	**Vancomycin MIC ≥ 1.5 µg/mL n=34**	***P***
Mean age (SD)	61.1 (18.5)	60.1 (14.3)	0.396
Male gender (%)	19 (68%)	27 (79%)	0.386
Type of endocarditis:			0.548
▪ Native valve	23 (82%)	25 (74%)	
▪ Prosthetic valve	5 (19%)	9 (27%)	
Origin of acquisition:			0.916
▪ Community-acquired	17 (61%)	23 (68%)	
▪ Nosocomial	8 (29%)	9 (26%)	
▪ Health-care related	2 (7%)	2 (6%)	
▪ Unknown	1 (3%)	0	
Geographical area:			0.333
▪ North America	4 (14%)	10 (29%)	
▪ Europe/Mid East	20 (71%)	22 (65%)	
▪ South America	1 (4%)	0	
▪ Australia/New Zealand	3 (11%)	2 (6%)	
Complications:			
▪ Heart failure	13 (46%)	10 (29%)	0.195
▪ Systemic emboli	10 (36%)	8 (23%)	0.400
▪ Stroke	7 (25%)	9 (27%)	1.000
▪ Paravalvular complications	8 (29%)	11 (32%)	0.788
▪ New conduction abnormality	0 (-)	3 (9%)	0.245
▪ Persistent bacteremia	4 (14%)	4 (12%)	1.000
Surgical treatment	9 (32%)	14 (41%)	0.599
Relapses*	0	0	1.000

Mortality			
▪ In-hospital mortality	9 (32%)	9 (27%)	0.780
▪ One-year mortality	12 (43%)	10 (29%)	0.298

* Defined as a new episode of endocarditis due to the same microorganism that caused the first IE within the following 12 months.

Table 2. Bi-variable Analysis of Risk Factors for One-year Mortality

Variable	Alive n=39	Dead n=22	OR (95%CI)	*P*
Demographics				
Age >65years	16 (40%)	11 (50%)	1.5 (0.5-4.9)	0.593
Male sex	31 (78%)	15 (68%)	0.62 (0.2-2.4)	0.546
Prosthetic valve IE	11 (29%)	3 (14%)	0.41 (0.1-1.9)	0.338
Diabetes	8 (20%)	4 (18%)	0.89 (0.2-3.9)	1.000
Acquisition in the community	26 (65%)	14 (64%)	1.76 (0.0-141.9)	0.889
North America	8 (20%)	6 (27%)	1.77 (0.1-143.1)	0.444
Clinical features				
Paravalvular complications	8 (20%)	11 (50%)	4.0 (1.1-14.6)	0.021
Stroke	6 (15%)	10 (46%)	4.72 (1.2-19.1)	0.015
Heart failure	10 (46%)	10 (46%)	1.73 (0.5-5.7)	0.411
Persistent bacteremia	5 (13%)	3 (14%)	1.11 (0.2-6.4)	1.000
Surgery	14 (35%)	9 (41%)	1.29 (0.4-4.2)	0.785
Microbiological features				
Vancomycin MIC ≥ 1.5 mg/L	24 (60%)	10 (45%)	0.56 (0.2-1.8)	0.298
CC30	7 (18%)	6 (27%)	1.77 (0.4-7.3)	0.516
CC8	1 (3%)	3 (14%)	6.16 (0.4-331.3)	0.546
CC15	6 (15%)	0	0.0 (0-1.1)	0.081
AGR subgroup I	15 (38%)	7 (32%)	0.78 (0.2-2.6)	0.784
AGR subgroup II	13 (33%)	6 (27%)	0.78 (0.2-2.7)	0.777
AGR subgroup III	11 (28%)	9 (41%)	1.83 (0.5-6.2)	0.394
see	8 (20%)	10 (46%)	3.33 (0.9-12.2)	0.044
sei	33 (83%)	22 (100%)	2.94 (1.1-13.7)	0.044
chp	39 (98)	16 (73%)	0.07 (0.0-0.7)	0.006
eta	8 (20%)	6 (27%)	1.5 (0.4-5.9)	0.539
pvl	4 (10%)	5 (23%)	2.65 (0.5-14.9)	0.259

The analysis for in-hospital mortality using the same variables did not differ from that of one-year mortality (data not shown).

Table 3. Summary of Main Findings from Studies Assessing the Relationship between Vancomycin MIC and Prognosis in MSSA Bacteremia and Left-sided IE.

First author, year	Design	SAB/IE (n)	Overall mortality *Low* VAN MIC < 1.5 µg/mL	Overall mortality *High* VAN MIC ≥ 1.5 µg/mL	Genetic factors	Main outcomes analysis
Kalil, 2014 [15]	Systematic review and metaanalysis	SAB (8291, both MRSA and MSSA)	25.8% (1430/5551)	26.8% (734/2740)	NA	RD 1.6% [95%CI, −2.3%to 5.6%]; P = 0.43 (for absolute risk of mortality, combining 30-day mortality and in-hospital mortality)
Holmes, 2011 [1]	Prospective multicenter cohort study	SAB (532; 266 of which MSSA treated with betalactams only)	12.2% (24/193)	26.8% (18/68)	NA	P= 0.011 (for 30-day mortality)
Holmes, 2014 [11]	Analysis of a subset of strains from [1]	SAB (252 MSSA isolates)	NA	NA	Associated to HVM: CC8, *agr* dysfunction, *agr* genotype II, *blaZ, sea, clfA, splA* and ACME locus. Associated to LVM: CC22, CC88 and CC188	Associated to HVM: CC8 P < 0.001), *agr* dysfunction (P= 0.014), *agr* genotype II (P=0.043), *blaZ* (P=0.002), *sea* (P < 0.001), *clfA* (P < 0.001), *splA* (P 0.001) and ACME locus (P=0.02). Associated to LVM: CC22 (P < 0.001), CC88 ([P < 0.001) and CC188 (P=0.002)
Aguado, 2011 [2]	Retrospective, single-center Cohort	Catheter-related SAB (99, all MSSA)	10.5% (8/76)	26.1% (6/23)	NA	P=0.13 (for 30-day mortality) OR=22.9, [95%CI 6.7–78.1] for complicated SAB.

López-Cortes, 2015 [4]	Prospective, single-center cohort	SAB (135, all MSSA)	23.6% (25/106)	10.3% (3/29)	There were no differences in *agr* distribution or absence of δ-haemolysin between isolates with HVM and those with LVM. HVM was not more frequent in specific clones.	RR=0.44 [95%CI , 0.14-1.35] for 14-day mortality
Viedma, 2014 [3]	Retrospective, single-center cohort	SAB (84, all MSSA)	24.1%, (7/29)	45.5% (25/55)	HVM: *agr II* polymorphism: 17.2%; average levels of RNAIII gene expression: ΔCt 1.5 ± 2.11 LVM: *agr II* polymorphism: 41.8%; average levels of RNAIII gene expression: ΔCt 4.05 ± 3.29	In-hospital mortality: P=0.057 *agr* dysfunction: P= 0.023. RNAIII expression: P<.01
Cervera, 2014 [5]	Prospective, single-center cohort	MSSA IE (93)	31% (16/53)	53% (21/40)	NA	In-hospital mortality: P=0.035; Patients with HVM presented significantly more severe embolic events
Current study	Prospective, multicenter cohort	MSSA IE (62)	32% (9/28)	27% (9/34)	HVM: *agrII* polymorphism: 19% LVM: *agrII* polymorphism: 38%	In-hospital mortality: P=0.780. *agrII* polymorphism: P=0.157

CI: Confidence interval; IE: Infective endocarditis; MRSA: methicillin-resistant *Staphylococcus aureus*; NA: not addressed; OR: Odds ratio; RD: relative difference; RR: relative risk; SAB: *S. aureus* bacteremia.

Supplemen l Appen x

1. Microbiological Methods

E-test for Vancomycin MIC

MSSA strains were stored at -80º C in a central laboratory (Duke University) and tested for vancomycin MIC by E-test after the freezing of the samples, following the manufacturer's recommendations (bioMérieux, Marcy l'Etoile, France). E-test were done by duplicate and interpreted by consensus with a second researcher.

Multiplex Polymerase Chain Reaction. Genomic DNA was prepared as previously described [1]. Bacterial determinants including adhesins, toxins, *agr* group I–IV, and other genes were screened by multiplex polymerase chain reaction (PCR) as described before [1]. All negative calls on the multiplex PCR were confirmed by uniplex PCR.

Spa Typing. Spa typing was performed as previously described [1, 2]. PCR oligonucleotide primers for *spa* were described previously [2]. Samples were sequenced at the Duke University sequencing laboratory. For spa typing, eGenomics software (http://tools.egenomics.com/) was used to scan the primary sequence to help identify the orders and names of each repeat. The spa type number is representative of the repeat organization. Clonal complexes (CC) for the isolates were identified via repeats pattern recognition from existing spa type and CC database previously confirmed via multilocus sequence typing (MLST). Isolates whose spa type did not map to a known CC underwent MLST typing. For MLST, the sequence chromatograms for unique alleles were deposited in the MLST database (http://www.mlst.net). Alleles at the 7 loci

(*arcC*, *aroE*, *glpF*, *gmk*, *pta*, *tpi,* and *yqiL*) were used to identify a unique sequence type (ST). MLST allele names and STs were derived from http://www.mlst.net. CCs were assigned to groups of isolates sharing 6 of 7 alleles by using the eBURST algorithm (http://eburst.mlst.net) [3].

References

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[2] Fowler VG Jr, Nelson CL, McIntyre LM, Kreiswirth BN, Monk A, Archer GL, et al. Potential associations between hematogenous complications and bacterial genotype in *Staphylococcus aureus* infection. J Infect Dis. 2007;196:738-47.

[3] Feil EJ, Li BC, Aanensen DM, Hanage WP, Spratt BG. eBURST: inferring patterns of evolutionary descent among clusters of related bacterial genotypes from multilocus sequence typing data. J Bacteriol. 2004;186:1518-30.

2. Genotypic characteristics of MSSA strains according to VAN MIC.

Differences in genotypic characteristics between the two groups according to MIC phenotypes are displayed in the **Table 1**. No differences were detected regarding adhesins, toxins or other putative virulence factors. Thirty-eight percent of patients with HVM presented *agr*-II compared to 19% of patients with LVM. However, this difference was not statistically significant (*P*=0.157). With regard to CC, CC30 was the most frequent CC in the whole cohort. Differences

in the prevalence of the CCs between the HVM and LVM groups were not statistically significant.

Table 1. Genotypic Characteristics and Distribution of Clonal Complexes of 62 Episodes of Left-sided Methicillin-susceptible *Staphylococcus aureus* Infective Endocarditis Treated with Beta-lactams According to *High* or *Low* Vancomycin Minimum Inhibitory Concentration (MIC).

	Vancomycin MIC < 1.5 µg/mL n=28	**Vancomycin MIC ≥ 1.5 µg/mL n=34**	***P***
Adhesins			
- *fnbA*	28 (100%)	34 (100%)	1.000
- *fnbB*	5 (19%)	7 (21%)	1.000
- *clfA*	28 (100%)	34 (100%)	1.000
- *clfB*	28 (100%)	34 (100%)	1.000
- *cna*	25 (90%)	29 (85%)	0.720
- *spa*	27 (96%)	33 (97%)	1.000
- *scrC*	12 (43%)	16 (47%)	0.801
- *sdrD*	18 (64%)	19 (56%)	0.606
- *sdrE*	12 (43%)	16 (47%)	0.801
- *bbp*	25 (89%)	28 (82%)	0.495
- *ebpS*	28 (100%)	34 (100%)	1.000
- *map/eap*	17 (61%)	25 (74%)	0.413
Toxins			
- *eta*	4 (14%)	10 (29%)	0.225
- *etb*	1 (4%)	1 (3%)	1.000
- *tst*	27 (96%)	31 (91%)	0.620
- *sea*	21 (75%)	19 (56%)	0.182
- *seb*	0	2 (6%)	0.497
- *sec*	6 (21%)	10 (29%)	0.566
- *sed*	5 (18%)	9 (27%)	0.546