



HAL
open science

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

J.M. Pericàs, A. Messina, C. Garcia-De-La-Mària, L. Park, B. Sharma-Kuinkel, F. Marco, D. Wray, A. Kanafani, M. Carugati, E. Durante-Mangoni, et al.

► **To cite this version:**

J.M. Pericàs, A. Messina, C. Garcia-De-La-Mària, L. Park, B. Sharma-Kuinkel, et al.. 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. *Clinical Microbiology and Infection*, 2017, 23 (8), pp.544 - 549. 10.1016/j.cmi.2017.01.017 . hal-01621675

HAL Id: hal-01621675

<https://univ-rennes.hal.science/hal-01621675>

Submitted on 23 Oct 2017

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

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.

Authors: Juan M. Pericàs¹, Julia A. Messina^{2,3}, Cristina Garcia-de-la-Maria¹, Lawrence Park^{2,4}, Batu.K. Sharma-Kuinkel², Francesc Marco⁵, Dannah Wray⁶, Zeina Kanafani⁷, Manuela Carugati², Emanuele Durante-Mangoni⁸, Pierre Tattevin⁹, Vivian H. Chu^{2,3}, Asuncion Moreno¹, Vance G. Fowler Jr.^{2,3}, Jose M. Miró¹ and the International Collaboration on Endocarditis Microbiology- Investigators¹⁰.

Affiliations:

1. Infectious Diseases Service, Hospital Clínic of Barcelona, Institut d'Investigacions Biomèdiques Pi i Sunyer (IDIBAPS), University of Barcelona, Spain.

2. Duke University Medical Center. Division of Infectious Diseases and International Health, Durham, North Carolina, US.

3. Duke Clinical Research Institute, Durham, North Carolina, US.

4. Duke Global Health Institute, Durham, North Carolina, US.

5. Department of Microbiology, Institute for Global Health, Hospital Clínic of Barcelona, University of Barcelona, Barcelona, Spain.

6. Infectious Disease Division, Medical University of South Carolina, Charleston, South Carolina, US.

7. Division of Infectious Diseases, American University of Beirut, Beirut, Lebanon.

8. Internal Medicine, Department of Clinical and Experimental Medicine, University of Campania 'Luigi Vanvitelli'; Unit of Infectious & Transplant Medicine, 'V. Monaldi' Hospital, AORN dei Colli, Naples, Italy

9. Infectious Diseases Service, CHU Rennes, Rennes, France.

10. See **Annex I**.

Corresponding author: Dr. Jose M. Miró, Infectious Diseases Service, Hospital Clínic, Villarroel 170, 08036-Barcelona, Spain. (jmmiro@ub.edu).

1
2
3 30 **Alternative corresponding author:** Dr. Vance G Fowler Jr., Duke University Medical
4
5 31 Center. Division of Infectious Diseases and International Health, Durham, North Carolina,
6
7 32 US. (vance.fowler@duke.edu)
8

9
10 33 **Keywords:** *Staphylococcus aureus*, vancomycin MIC, endocarditis, phenotype,
11
12 34 genotype.
13

14 35 **Keypoints:** In this international cohort of patients with left-sided MSSA endocarditis
15
16 36 treated with anti-staphylococcal beta-lactam antibiotics, vancomycin MIC phenotype was
17
18 37 not associated with patient demographics, clinical outcome, or virulence gene repertoire.
19

20 38 **Running title:** “Vancomycin MIC and MSSA endocarditis”.

21 39 **Word count:** 1,398

22
23 40 **Abstract word count:** 249

24
25 41 **References:** 15

26
27 42 **Tables:** 3

28
29 43 **Figures:** 2

30
31 44 **Supplementary Appendix:** 1 table, 1 figure and microbiological methods.

32
33
34 45 **Funding.** Supported in part by R01-AI068804 and K24-AI093969 (US), as well as
35
36 46 20152610 project by “La Marató de TV3”, Barcelona (Spain), FIS PI14/00603 from
37
38 47 Instituto de Salud Carlos III and the Ministerio de Economía and Competitividad, Madrid
39
40 48 (Spain) and the 2016 ESCMID Research Grant. JMP received a “Rio Hortega” Research
41
42 49 Grant (CM14/00135; 2015-16) from Instituto de Salud Carlos III and the Ministerio de
43
44 50 Economía and Competitividad, Madrid (Spain) and the ESCMID/FEMS Joint Research
45
46 51 Fellowship Award 2016. Instituto de Salud Carlos III, Ministerio de Economía y
47
48 52 Competitividad, Madrid (Spain) provided to JMM a personal intensification research grant
49
50 53 #INT15/00168 during 2016.
51
52
53
54
55
56
57
58
59
60

1
2
3 54 These data were partially presented at the 55th ICAAC, September 18-21, 2015. San
4
5 55 Diego, CA (abstract number 2423).
6
7
8 56
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For Peer Review

57 **Abstract**

58 **Objectives:** A recent study showed a poorer prognosis in left-sided MSSA endocarditis
59 treated with cloxacillin when the vancomycin MIC was ≥ 1.5 mg/L. We aimed to validate
60 these results using the International Collaboration on Endocarditis cohort and to analyze
61 whether specific genetic characteristics were associated with a high vancomycin MIC
62 (≥ 1.5 mg/L) phenotype.

63 **Methods:** All patients with left-sided MSSA IE treated with anti-staphylococcal beta-
64 lactam antibiotics between 2000 and 2006 with available isolates were included.
65 Vancomycin MIC (VAN MIC) was determined by E-test as either *high* (≥ 1.5 mg/L) or *low*
66 (< 1.5 mg/L). Isolates underwent *spa* typing to infer clonal complexes (CC) and multiplex
67 polymerase chain reaction for identifying virulence genes. Univariate analysis was
68 performed to evaluate the association between in-hospital and one-year mortality and
69 vancomycin MIC phenotype.

70 **Results:** 62 cases met the inclusion criteria. VAN MIC was *low* in 28 cases (45%) and
71 *high* in 34 cases (55%). No significant differences in patient demographic data or
72 characteristics of infection were observed between patients with IE due to *high* and *low*
73 VAN MIC isolates. Isolates with *high* and *low* VAN MIC had similar distributions of
74 virulence genes and clonal lineages. In-hospital and one-year mortality did not differ
75 significantly between the two groups (32% (9/28) vs. 27% (9/34), $P=0.780$; and 43%
76 (12/28) vs. 29% (10/34), $P=0.298$, for *low* and *high* VAN MIC, respectively).

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

80

81

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 (HVM; ≥ 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.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

107 **Objectives**

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

For Peer Review

115
116
117
118
119
120
121
122
123
124
125
126
127
128
129
130
131

METHODS

Database

ICE-Pro 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,

1
2
3 156 and outcome. All patients who survived had, at least, one year of follow-up. Antibiotic
4
5 157 therapy was decided by the treating physician at the individual study site.
6
7
8 158

9 159 **Definitions**

10
11 160 IE was defined according to the modified Duke Criteria [8] and was considered to be left-
12
13 161 sided if no right-sided (tricuspid or pulmonary valve) vegetations were present on
14
15 162 echocardiographic examination, surgery, or autopsy. A strain was considered to have
16
17 163 HVM when it was ≥ 1.5 mg/L by E-test, and a low vancomycin MIC (LVM) when it was
18
19 164 < 1.5 $\mu\text{g/mL}$. The rest of definitions have been previously provided in detail [9].
20
21
22
23 165

24 166 **Geographic regions**

25
26 167 Geographic regions included the following 25 participating sites (see Appendix): United
27
28 168 States (4 sites), South America (2 sites from Brazil and Chile), Europe (12 sites from
29
30 169 Croatia, France, Greece, Italy, Slovenia and Spain), Australia and New Zealand (6 sites)
31
32 170 and Middle East (1 site from Lebanon).
33
34
35
36 171

37 172 **Microbiological methods**

38
39 173 ICE methodology for microbiological procedures has been defined elsewhere [6,10].
40
41 174 Detailed microbiological methods can be found in the Supplemental Appendix. MSSA
42
43 175 strains were stored at -80°C in a central laboratory (Duke University) and tested for
44
45 176 nafcillin, cloxacillin, vancomycin and daptomycin MIC by E-test, following the
46
47 177 manufacturer's recommendations (bioMérieux, Marcy l'Etoile, France).
48
49 178 **Multiplex Polymerase Chain Reaction.** Genomic DNA was prepared as previously
50
51 179 described [12]. Bacterial determinants including adhesins, toxins, *agr* group I–IV, and
52
53
54
55
56
57
58
59
60

1
2
3 180 other genes were screened by multiplex polymerase chain reaction (PCR) as previously
4
5 181 described [12]. All negative calls on the multiplex PCR were confirmed by uniplex PCR.
6
7
8
9 182

10 183 **Spa Typing.** Spa typing was performed as previously described [12, 15]. PCR
11
12 184 oligonucleotide primers for *spa* were described previously [15]. Samples were sequenced
13
14 185 at the Duke University sequencing laboratory. For *spa* typing, eGenomics software
15
16 186 (<http://tools.egenomics.com/>) was used to scan the primary sequence to help identify the
17
18 187 orders and names of each repeat. The *spa* type number is representative of the repeat
19
20 188 organization. Clonal complexes (CC) for the isolates were identified via repeats pattern
21
22 189 recognition from existing *spa* type and CC database previously confirmed via multilocus
23
24 190 sequence typing (MLST). Isolates whose *spa* type did not map to a known CC underwent
25
26 191 MLST typing. For MLST, the sequence chromatograms for unique alleles were deposited
27
28 192 in the MLST database (<http://www.mlst.net>). Alleles at the 7 loci (*arcC*, *aroE*, *glpF*, *gmk*,
29
30 193 *pta*, *tpi*, and *yqiL*) were used to identify a unique sequence type (ST). MLST allele names
31
32 194 and STs were derived from <http://www.mlst.net>. CCs were assigned to groups of isolates
33
34 195 sharing 6 of 7 alleles by using the eBURST algorithm (<http://eburst.mlst.net>) [16].
35
36
37
38
39
40
41
42

197 **Statistical analysis**

43 198 Categorical variables were expressed as percentages and compared with Fisher's exact
44
45 199 test. Continuous variables were expressed as means or medians (depending on the
46
47 200 variable's homogeneity) and compared by non-parametric Wilcoxon's score/Mann
48
49 201 Whitney's U tests. Survival analysis was performed by Kaplan-Meier analysis, and curves
50
51 202 were compared by the log-rank test. Given the limited sample size, logistic regression
52
53 203 analysis was not performed. A two-sided p-value <0.05 was considered to be statistically
54
55 204 significant. To determine the rate of type I errors in null hypothesis testing when
56
57
58
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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

For Peer Review

214 RESULTS

215 Cases included

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

222 Vancomycin MIC distribution in the cohort

223 The distribution of vancomycin MIC among the 62 cases included in the study is
224 displayed in **Figure 2** along with the respective rates of mortality for 0.75, 1, 1.5 and ≥ 2
225 $\mu\text{g/mL}$ vancomycin MIC determinations.

227 Demographics and clinical characteristics

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

234 Outcomes

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

1
2
3 23 32% (9/28) and 43% (12/28), respectively, while in-hospital mortality was 27% (9/34) and
4
5 239 one-year mortality 29% (10/34) for HVM.

240 Genotypic characteristics

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

249 Risk factors for mortality

250 The univariate analysis of risk factors for one-year mortality is shown in **Table 2**. HVM,
251 type of *agr* and CC were not significantly associated with mortality. The analysis for in-
252 hospital mortality using the same variables did not differ from that of one-year mortality
253 (data not shown). Among clinical markers, paravalvular complications [OR 4.0 (95%CI
254 1.1-14.6); $P=0.021$] and stroke [OR 4.72 (95%CI 1.2-19.1); $P=0.015$] were associated
255 with a poorer prognosis at one-year. With regard to virulence factors, see [OR 3.33 (0.9-
256 12.2); $P=0.044$] and *sei* [OR 2.94 (1.1-13.7); $P=0.044$] were identified as risk factors for
257 mortality whereas *chp* was found to be a protective factor [OR 0.07 (0-0.7); $P=0.006$].

258
259 The Kaplan-Meier survival plot at one-year according to vancomycin MIC group is
260 displayed in the **Supplemental Appendix**.

261

262

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.

1
2
3 288 Given that no significant differences were found in virulence factors between the two
4
5 289 groups, we might speculate that in this dataset, due to the high proportion of patients with
6
7 290 MSSA strains harbouring LVM and CC30, a high rate of detected and not detected
8
9 291 hematogenous complications in the LVM may explain the higher mortality in this group.
10
11
12 292

13
14 293 With regard to genotypic features, the analysis of *agr* subgroup did not reveal a
15
16 294 significant association between a specific *agr* polymorphism and HVM or CC. We
17
18 295 expected to find an association between a HVM and *agrII* polymorphism relying on
19
20 296 previous studies performed in MSSA bacteremia [3,11]. Holmes et al. found an overall
21
22 297 statistically significant association between a HVM and *agrII* in a cohort of 242 patients
23
24 298 with *S. aureus* bacteremia (104 MRSA and only 38 MSSA) [17]. However, a subanalysis
25
26 299 of the MSSA subset was not performed but would have probably been limited by small
27
28 300 sample size. A more recent study that included 135 episodes of MSSA bacteremia of
29
30 301 various origins did not find significant differences on *agr* subgroup and function according
31
32 302 to the vancomycin MIC with a threshold of ≥ 1.5 $\mu\text{g/mL}$ [10]. In our study, *agrII* was two
33
34 303 times more frequent in the HVM than in the LVM (38% vs. 19%), but did not reach
35
36 304 statistical significance. In our cohort, CC5 was only slightly more common in patients with
37
38 305 HVM and CC30 was two times more common in the LVM group. CC30 was the most
39
40 306 common CC within the whole cohort as previously described in a cohort of 228 patients
41
42 307 with MSSA IE and skin and STI [6]. As in our study, Viedma et al. did not find specific
43
44 308 associations between CC subtypes and HVM although in their cohort there was a trend
45
46 309 toward a higher proportion of CC5 in the HVM group compared to the LVM group (21.8%
47
48 310 vs. 6.9%; $P=0.070$). Fowler and colleagues found a significantly higher proportion of CC5
49
50 311 (and CC30) among patients with *S. aureus* bacteremia with hematogenous complications
51
52 312 but not in the subgroup of MSSA patients [15]. In the study by Holmes et al [17], CC8
53
54
55
56
57
58
59
60

1
2
3 313 was significantly associated with HVM, and in the case of CC5, the association
4
5 314 approached statistical significance (6.5% in the LVM group and 14.2% in the HVM group;
6
7 315 $P=0.055$). Thus, the hypothesis of a higher embolic potential among HVM strains could
8
9 316 not be demonstrated. However, Further studies are needed before firm conclusions can
10
11 317 be drawn regarding the association between CC and hematogenous complications in left-
12
13
14 318 sided IE caused by MSSA strains.
15
16 319

17
18 320 We did not identify a specific repertoire of virulence factors in the HVM group, as other
19
20 321 previous studies did [6,7,10]. The virulence factors we found to be associated with worse
21
22 322 prognosis have not been previously described as risk factors for mortality in the clinical
23
24 323 field. In the Nienaber et al study, MSSA IE strains were shown to more likely contain 3
25
26 324 adhesins (*clfB*, *cna*, *map/eap*) and 5 enterotoxins (*tst*, *sea*, *sed*, *see*, and *sei*) than STI
27
28 325 strains with no significant geographical variations [12]. Holmes et al found a significantly
29
30 326 higher proportion of *sea* and *clfA* among *S. aureus* strains with HVM causing bacteremia
31
32 327 [17]. In a recently published Indian study, the most frequently identified toxin genes
33
34 328 among patients with MSSA bacteremia were *sei* and *seg* [18]. In an epidemiologic study
35
36 329 analyzing CC and diversity of exotoxin gene profiles performed in 81 strains of *S. aureus*
37
38 330 isolated in a Spanish hospital (68% of which were MSSA), *agrII* was the most common
39
40 331 gene and was linked to CC5, *sea* and *sec* [19]. In the study by Nienaber et al., no
41
42 332 significant *agrII* variations among geographical areas where found, with 68% of isolates
43
44 333 being from Europe and Middle East, 23% from North America and 9% from Oceania [12].
45
46 334 Although geographical regions are almost equally represented, changes in the genetic
47
48 335 expression and phenotypic pattern in MSSA might be common over the time and
49
50 336 geographical variations are also likely to occur as years pass. In the present cohort, the
51
52 337 geographical regions are almost equally represented. However, since changes in the
53
54
55
56
57
58
59
60

1
2
3 338 genetic expression and phenotypic pattern in MSSA might be common over the time and
4
5 339 geographical variations are also likely to occur as years pass, we wonder if some of the
6
7 340 different findings between the Cervera et al. study [5] and the present study could be due
8
9 341 to different periods of time (1995-2011 in the Spanish cohort and 2000-2006 in the ICE)
10
11 342 and geographic regions (local vs. intercontinental samples). Actually, the proportion of
12
13 343 MSSA strains with HMV in Cervera et al. study significantly decreased along the period of
14
15 344 the study [5]. Another factor that could also have influence in the percentage of MSSA
16
17 345 strains identified as HVM is the effect of freezer storage. Ludwig et al. demonstrated a
18
19 346 progressive decline of VAN MIC determination by Etest in MRSA bloodstream samples
20
21 347 from the moment they were frozen [14]. Either due to locoregional or global variations of
22
23 348 MSSA clones, further studies on the occurrence of variations of MSSA genotypic features
24
25 349 are needed to correlate our results with more precise data.
26
27
28
29
30

31
32 351 The superantigen genes *see* and *sei*, encoded by the enterotoxin gene cluster, conferred
33
34 352 worse prognosis at one year, with 3-fold higher mortality. There are some *in vivo* data
35
36 353 pointing to an important role of these virulence genes in the severity of invasive *S. aureus*
37
38 354 infections [14]. However, the pathway in which these genes are involved, as other
39
40 355 superantigens, is T-cell activation by binding to major histocompatibility complex class II
41
42 356 molecules and to certain V β varieties on the T-cell receptor, leading to septic shock and
43
44 357 not embolic events, including stroke. Chemotaxis inhibitory protein (*chp*), a protective
45
46 358 factor in this cohort, is a major staphylococcal defense evasion mechanism against the
47
48 359 human innate immune system [21]. Potentially, the presence of *chp* involves a loss of
49
50 360 fitness and thus lowers virulence in patients with MSSA strains harboring this gene in this
51
52 361 cohort. Yet, it is important to stress that outcomes of IE patients may not be easily
53
54 362 attributable to simply virulence factors. The role played by the host is crucial and definite
55
56
57
58
59
60

1
2
3 363 conclusions should be drawn from studies analyzing both sides of the interplay, namely
4
5 364 the host and the pathogen. For example, outcomes of patients with IE due to relatively
6
7 365 low-virulence microorganisms, such as coagulase-negative staphylococci, are associated
8
9 366 with heart failure, the presence of prosthetic devices, comorbidities of the patient and the
10
11 367 need of surgery [15-17] albeit coagulase-negative staphylococci causing IE have unique
12
13 368 genetic signatures which are found across vast geographic distances [18], and HMV has
14
15 369 also been identified as a marker of bad prognosis [19].
16
17
18
19
20

21 371 This study has several limitations. First, the low sample size is a major shortcoming,
22
23 372 severely limiting the statistical power of the study and leading to the potential for type II
24
25 373 error in the analysis. Second, the number of isolates tested and available is not
26
27 374 representative of the overall ICE dataset. However, we conducted a subanalysis
28
29 375 comparing ICE left-sided MSSA frozen strains and those that were not frozen, and we did
30
31 376 not detect significant differences regarding in-hospital and one-year mortality and surgery
32
33 377 rates (data not shown). Third, *agrII* dysfunction was not measured, so correlations with
34
35 378 *agr* subgroup, vancomycin MIC, and outcomes were not performed. Fourth, only cases
36
37 379 occurring in the 2000-2006 period were included, which precluded observations
38
39 380 regarding the temporal trends on vancomycin MIC, CC, or virulence factors according to
40
41 381 each geographical region. Given that the data presented are older than 10 years, the
42
43 382 clinical relevance of current MSSA
44
45 383 circulating strains may have changed. Fifth, the potential variations of MSSA clones
46
47 384 between geographical regions were not investigated. And finally, all patients were treated
48
49 385 with anti-staphylococcal beta-lactam antibiotics (cloxacillin, nafcillin and first generation
50
51 386 cephalosporins), but the specific type of beta-lactam used was not available. Despite
52
53 387 these limitations, this is the first study on this subject including genetic studies of the
54
55
56
57
58
59
60

1
2
3 388 bacterial isolates in order to find an association with the vancomycin phenotype and
4
5 389 clinical outcomes.
6

7
8 390

9
10 391 In conclusion, in this international cohort of left-sided MSSA IE treated with beta-lactams,
11 392 vancomycin MIC ≥ 1.5 $\mu\text{g/mL}$ was not found to be an independent risk factor for
12 393 complications of IE or for mortality. Stroke, paravalvular complications, and some *S.*
13
14 394 *aureus* genes were associated with a worse outcome. Differences in clonality and
15
16 395 virulence factors were not found between strains with LVM and HVM. Further studies in
17
18 396 this field are warranted to expand upon these findings and elucidate if the contradictory
19
20 397 results obtained in this field [15] are due to methodological limitations or rather to a
21
22
23 398 difficult-to-interpret phenomenon.
24
25
26
27
28 399

1
2
3 400 **Financial disclosures**

4 401 VGF reports the following potential conflicts of interest: Chair of the Scientific Advisory
5
6 402 Board for Merck V710; paid consultant for Pfizer, Novartis, Galderma, Novadigm, Durata,
7
8 403 Debiopharm, Genentech, Achaogen, Affinium, Medicines Co., Cerexa, Tetrphase, Trius,
9
10 404 MedImmune, Bayer, Theravance, Cubist, Basilea, Affinergy; Grants pending from
11
12 405 MedImmune, Actavis/Forest/Cerexa, Pfizer, Merck/Cubist, Advanced Liquid Logics,
13
14 406 Theravance, Novartis, Medical Surfaces, and Locus Biotechnology; royalties from
15
16 407 UpToDate, personal fees for development or presentation of educational presentations
17
18 408 (Green Cross, Cubist, Cerexa, Durata, Theravance), outside the submitted work; and
19
20 409 patent pending related to sepsis diagnostics. JMM has received consulting honoraria
21
22 410 and/or research grants from AbbVie, Bristol-Myers Squibb, Cubist, Novartis, Gilead
23
24 411 Sciences, and ViiV. All other authors: no conflicts.
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

References

[1] Murdoch DR, Corey GR, Hoen B, Miró JM, Fowler VG Jr, Bayer AS, et al. Clinical presentation, etiology, and outcome of infective endocarditis in the 21st century: the International Collaboration on Endocarditis-Pro prospective Cohort Study. *Arch Intern Med.* 2009;169:463-73.

[2] Miro JM, Anguera I, Cabell CH, Chen AY, Stafford JA, Corey GR, et al. *Staphylococcus aureus* native valve infective endocarditis: report of 566 episodes from the International Collaboration on Endocarditis Merged Database. *Clin Infect Dis* 2005;41:507 -14.

[3] Fowler VG Jr, Miro JM, Hoen B, Cabell CH, Abrutyn E, Rubinstein E, et al. *Staphylococcus aureus* endocarditis: a consequence of medical progress. *JAMA* 2005;293:3012 -21.

[4] van Hal SJ, Lodise TP, Paterson DL. The clinical significance of vancomycin minimum inhibitory concentration in *Staphylococcus aureus* infections: a systematic review and meta-analysis. *Clin Infect Dis.* 2012;54:755-71.

[5] Kalil AC, Van Schooneveld TC, Fey PD, Rupp ME. Association between vancomycin minimum inhibitory concentration and mortality among patients with *Staphylococcus aureus* bloodstream infections: a systematic review and meta-analysis. *JAMA.* 2014;312:1552-64.

[6] Seidl K, Chen L, Bayer AS, Hady WA, Kreiswirth BN, Xiong YQ. Relationship of agr expression and function with virulence and vancomycin treatment outcomes in experimental endocarditis due to methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother.* 2011;55:5631-9.

[1] Holmes NE, Turnidge JD, Munckhof WJ, Robinson JO, Korman TM, O'Sullivan MV. Antibiotic choice may not explain poorer outcomes in patients with *Staphylococcus*

1
2
3 441 *aureus* bacteremia and high vancomycin minimum inhibitory concentrations. J Infect Dis.
4
5 442 2011;204:340-7.
6

7 443 [2] Aguado JM, San-Juan R, Lalueza A, Sanz F, Rodríguez-Otero J, Gómez-Gonzalez C,
8
9 444 et al. High vancomycin MIC and complicated methicillin-susceptible *Staphylococcus*
10
11 445 *aureus* bacteremia. Emerg Infect Dis. 2011;17:1099-102.
12

13
14 446 [3] Viedma E, Sanz F, Orellana MA, San Juan R, Aguado JM, Otero JR, et al.
15
16 447 Relationship between *agr* dysfunction and reduced vancomycin susceptibility in
17
18 448 methicillin-susceptible *Staphylococcus aureus* causing bacteraemia. J Antimicrob
19
20 449 Chemother. 2014;69:51-8.
21

22
23 450 [4] López-Cortés LE, Velasco C, Retamar P, del Toro MD, Gálvez-Acebal J, de Cueto M,
24
25 451 et al. Is reduced vancomycin susceptibility a factor associated with poor prognosis in
26
27 452 MSSA bacteraemia? J Antimicrob Chemother. 2015;70:2652-60.
28

29
30 453 [5] Cervera C, Castañeda X, de la Maria CG, del Rio A, Moreno A, Soy D, et al. Effect of
31
32 454 vancomycin minimal inhibitory concentration on the outcome of methicillin-susceptible
33
34 455 *Staphylococcus aureus* endocarditis. Clin Infect Dis. 2014;58:1668-75.
35

36 456 [6] Nienaber JJ, Sharma Kuinkel BK, Clarke-Pearson M, Lamlertthon S, Park L, Rude
37
38 457 TH, et al. Methicillin-susceptible *Staphylococcus aureus* endocarditis isolates are
39
40 458 associated with clonal complex 30 genotype and a distinct repertoire of enterotoxins and
41
42 459 adhesins. J Infect Dis. 2011;204:704-13.
43
44

45 460 [7] Bae IG, Federspiel JJ, Miro JM, Woods CW, Park L, Rybak MJ, et al. Heterogeneous
46
47 461 vancomycin intermediate susceptibility phenotype in bloodstream methicillin-resistant
48
49 462 *Staphylococcus aureus* isolates from an international cohort of patients with infective
50
51 463 endocarditis: prevalence, genotype, and clinical significance. J Infect Dis. 2009;
52
53 464 200:1355–66
54
55
56
57
58
59
60

- 1
2
3 465 [8] Li JS, Sexton DJ, Mick N, Nettles R, Fowler VG Jr, Ryan T, et al. Proposed
4
5 466 modifications to the Duke criteria for the diagnosis of infective endocarditis. Clin Infect
6
7 467 Dis. 2000;30:633-8.
8
9
10 468 [9] Fowler VG Jr, Miro JM, Hoen B, Cabell CH, Abrutyn E, Rubinstein E, et al.
11
12 469 *Staphylococcus aureus* endocarditis: a consequence of medical progress. JAMA
13
14 470 2005;293:3012-21.
15
16 471 [10] Fowler VG Jr, Nelson CL, McIntyre LM, Kreiswirth BN, Monk A, Archer GL, et al.
17
18 472 Potential associations between hematogenous complications and bacterial genotype in
19
20 473 *Staphylococcus aureus* infection. J Infect Dis. 2007;196:738-47.
21
22
23 474 [16] Feil EJ, Li BC, Aanensen DM, Hanage WP, Spratt BG. eBURST: inferring patterns of
24
25 475 evolutionary descent among clusters of related bacterial genotypes from multilocus
26
27 476 sequence typing data. J Bacteriol. 2004;186:1518-30.
28
29
30 477 [11] Holmes NE, Turnidge JD, Munckhof WJ, Robinson JO, Korman TM, O'Sullivan MV,
31
32 478 et al. Genetic and molecular predictors of high vancomycin MIC in *Staphylococcus*
33
34 479 *aureus* bacteremia isolates. J Clin Microbiol. 2014;52:3384-93.
35
36 480 [12] Deodhar D, Varghese G, Balaji V, John J, Rebekah G, Janardhanan J, et al.
37
38 481 Prevalence of Toxin Genes among the Clinical Isolates of *Staphylococcus aureus* and its
39
40 482 Clinical Impact. J Glob Infect Dis. 2015;7:97-102.
41
42
43 483 [13] Argudín MA, Mendoza MC, Méndez FJ, Martín MC, Guerra B, Rodicio MR. Clonal
44
45 484 complexes and diversity of exotoxin gene profiles in methicillin-resistant and methicillin-
46
47 485 susceptible *Staphylococcus aureus* isolates from patients in a Spanish hospital. J Clin
48
49 486 Microbiol. 2009;47:2097-105.
50
51
52 487 [14] Ludwig F, Edwards B, Lawes T, Gould IM. Effects of storage on vancomycin and
53
54 488 daptomycin MIC in susceptible blood isolates of methicillin-resistant *Staphylococcus*
55
56 489 *aureus*. J Clin Microbiol. 2012;50:3383-7.
57
58
59
60

1
2
3 490 [15] Kalil AC, Van Schooneveld TC, Fey PD, Rupp ME. Association between vancomycin
4
5 491 minimum inhibitory concentration and mortality among patients with *Staphylococcus*
6
7 492 *aureus* bloodstream infections: a systematic review and meta-analysis. JAMA.
8
9 493 2014;312:1552-64.

11 494 [14] Nowrouzian FL, Ali A, Badiou C, Dauwalder O, Lina G, Josefsson E. Impacts of
12
13 495 enterotoxin gene cluster-encoded superantigens on local and systemic experimental
14
15 496 *Staphylococcus aureus* infections. Eur J Clin Microbiol Infect Dis. 2015;34:1443-9.

17 497 [21] van Wamel WJ, Rooijackers SH, Ruyken M, van Kessel KP, van Strijp JA. The
18
19 498 innate immune modulators staphylococcal complement inhibitor and chemotaxis
20
21 499 inhibitory protein of *Staphylococcus aureus* are located on betahemolysin-converting
22
23 500 bacteriophages. J Bacteriol 2006;188:1310–1315.

25 501 [15] Chu VH, Woods CW, Miro JM, Hoen B, Cabell CH, Pappas PA, et al. Emergence of
26
27 502 coagulase-negative staphylococci as a cause of native valve endocarditis. Clin Infect Dis.
28
29 503 2008;46:232-42.

31 504 [16] Chu VH, Miro JM, Hoen B, Cabell CH, Pappas PA, Jones P, et al. Coagulase-
32
33 505 negative staphylococcal prosthetic valve endocarditis--a contemporary update based on
34
35 506 the International Collaboration on Endocarditis: prospective cohort study. Heart.
36
37 507 2009;95:570-6.

39 508 [17] Chu VH, Cabell CH, Abrutyn E, Corey GR, Hoen B, Miro JM, et al. Native valve
40
41 509 endocarditis due to coagulase-negative staphylococci: report of 99 episodes from the
42
43 510 International Collaboration on Endocarditis Merged Database. Clin Infect Dis.
44
45 511 2004;39:1527-30.

47 512 [18] Petti CA, Simmon KE, Miro JM, Hoen B, Marco F, Chu VH, et al. Genotypic diversity
48
49 513 of coagulase-negative staphylococci causing endocarditis: a global perspective. J Clin
50
51 514 Microbiol. 2008;46:1780-4.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

515 [19] García de la Mària C, Cervera C, Pericàs JM, Castañeda X, Armero Y, Soy D, et al.
516 Epidemiology and prognosis of coagulase-negative staphylococcal endocarditis: impact
517 of vancomycin minimum inhibitory concentration. PLoS One. 2015;10:e0125818.
518
519

For Peer Review

1
2
3 **Annex I: ICE Micro Investigators**
4
5

6
7 **Australia:** Eugene Athan, MD, Owen Harris, MBBS (*Barwon Health*); Tony M. Korman, MD
8 (*Monash Medical Centre*), Despina Kotsanas, BS (*Southern Health*); Phillip Jones, MD, Porl
9 Reinbott, Suzanne Ryan, MHS (*The University of New South Wales*); **Brazil:** Claudio
10 Querido Fortes, MD (*Hospital Universitario Clementino Fraga Filho/UFRJ*); **Chile:** Patricia
11 Garcia, MD, Sandra Braun Jones, MD (*Hosp. Clínico Pont. Universidad Católica de Chile*);
12 **Croatia:** Bruno Barsic, MD, PhD, Suzana Bukovski, MD (*Univ. Hospital for Infectious*
13 *Diseases*); **France:** Christine Selton-Suty, MD, Neijla Aissa, MD, Thanh Doco-Lecompte,
14 MD (CHU Nancy-Brabois); Francois Delahaye, MD, PhD, Francois Vandenesch, MD
15 (*Hopital Louis Pradel*); Pierre Tattevin, MD, PhD (*Pontchaillou University*); Bruno Hoen,
16 MD, PhD, Patrick Plesiat, MD (*University Medical Center of Besançon*); **Greece:** Helen
17 Giamarellou, MD, PhD, Efthymia Giannitsioti, MD, Ekaterini Tarpatzi, MD (*Attikon University*
18 *General Hospital*); **Italy:** Emanuele Durante-Mangoni MD, PhD, Domenico Iossa PhD,
19 Serena Orlando MD, Maria Paola Ursi MD, Pia Clara Pafundi PhD, Fabiana D'Amico BSc,
20 Mariano Bernardo MSc, Susanna Cuccurullo MSc, Giovanni Dialetto MD, Franco Enrico
21 Covino MD, Sabrina Manduca MD, Alessandro Della Corte MD, PhD, Marisa De Feo MD,
22 PhD (*Università della Campania*); Marie Françoise Tripodi MD (*University of Salerno*);
23 **Lebanon:** Tania Baban, MD, Zeina Kanafani, MD, MS, Souha S.Kanj, MD, Jad Sfeir, MD ,
24 Mohamad Yasmine, MD (*American University of Beirut Medical Center*); **New Zealand:**
25 Arthur Morris, MD (*Diagnostic Medlab*), David R. Murdoch MD, MSc, DTM&H (*University of*
26 *Otago*); **Slovenia:** Manica Mueller Premru MD PhD, Tatjana Lejko-Zupanc MD PhD
27 (*Medical Center Ljubljana*); **Spain:** Manel Almela, MD, Juan Ambrosioni, MD, PhD, Manuel
28 Azqueta, MD, Merce Brunet, MD, PhD, Carlos Cervera, MD, PhD, Elisa De Lazzari, MS,
29 Carlos Falces, MD, David Fuster, MD, PhD, Cristina Garcia- de- la- Maria, PhD, Javier
30 Garcia-Gonzalez, MS, Jose M. Gatell, MD, PhD, Francesc Marco, MD, PhD, , José M. Miró,
31 MD, PhD, Asuncion Moreno, MD, PhD, José Ortiz, MD, PhD, Salvador Ninot, MD, J. Carlos
32 Paré, MD, PhD, Juan M Pericas, MD, Eduard Quintana, MD, PhD, Jose Ramirez, MD, PhD,
33 Elena Sandoval, MD, Marta Sitges, MD, PhD, José M. Tolosana, MD, PhD, Barbara Vidal,
34 MD, PhD, Jordi Vila, MD, PhD (*Hospital Clinic – IDIBAPS. University of Barcelona*) Emilio
35 Bouza MD, PhD, Marta Rodríguez-Créixems, MD, PhD, Victor Ramallo, MD, (*Hospital*
36 *General Universitario Gregorio Marañón*); **USA:** Suzanne Bradley, MD (*Ann Arbor VA*
37 *Medical Center*); Dannah Wray, MD, Lisa Steed, PhD, MHS, Robert Cantey, MD, (*Medical*
38 *University of South Carolina*); Gail Peterson, MD, Amy Stancoven, MD (*UT-Southwestern*
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 554 *Medical Center*); Christopher Woods, MD, MPH, G. Ralph Corey, MD, L. Barth Reller, MD,
4 555 Vance G. Fowler Jr., MD, MHS, Vivian H. Chu, MD, MHS, (*Duke University Medical Center*).

556

557 **ICE Coordinating Center:** Khaula Baloch, MPH, Vivian H. Chu, MD, MHS, G. Ralph Corey,
558 MD, Christy C. Dixon, Vance G. Fowler, Jr., MD, MHS, Tina Harding, RN, BSN, Marian
559 Jones-Richmond, Paul Pappas, MS, Lawrence P. Park, PhD, Thomas Redick, MPH, Judy
560 Stafford, MS

561

562 **ICE Publications Committee:** Kevin Anstrom, PhD, Eugene Athan, MD, Arnold S. Bayer,
563 MD, Christopher H. Cabell, MD, MHS, Vivian H. Chu, MD, MHS, G. Ralph Corey, MD,
564 Vance G. Fowler, Jr., MD, MHS, Bruno Hoen, MD, PhD, A W Karchmer MD, José M. Miró,
565 MD, PhD, David R. Murdoch, MD, MSc, DTM&H, Daniel J. Sexton, MD, Andrew Wang, MD

566

567 **ICE Steering Committee:** Arnold S. Bayer, MD, Christopher H Cabell, MD, MHS, Vivian
568 Chu, MD, MHS, G. Ralph Corey MD, David T. Durack, MD, D Phil, Susannah Eykyn, MD,
569 Vance G. Fowler, Jr., MD, MHS, Bruno Hoen, MD, PhD, José M. Miró, MD, PhD, Phillipe
570 Moreillon, MD, PhD, Lars Olaison, MD, PhD, Didier Raoult, MD, PhD, Ethan Rubinstein MD,
571 LLB, Daniel J. Sexton, MD

572

573

574 **FIGURE LEGENDS**

575

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

579

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

583

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

587

588

589

590

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

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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.

For Peer Review

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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

- <i>sei</i>	24 (86%)	31 (91%)	0.691
- <i>sej</i>	1 (4%)	2 (6%)	1.000
- <i>pvi</i>	6 (21%)	3 (9%)	0.277
- <i>hlg</i>	28 (100%)	34 (100%)	1.000
Other putative virulence genes			
- <i>efb</i>	28 (100%)	34 (100%)	1.000
- <i>icaA</i>	24 (86%)	31 (91%)	0.691
- <i>chp</i>	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

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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

Variable	Alive n=39	Dead n=22	OR (95%CI)	<i>P</i>
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 \geq 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
<i>See</i>	8 (20%)	10 (46%)	3.33 (0.9-12.2)	0.044
<i>Sei</i>	33 (83%)	22 (100%)	2.94 (1.1-13.7)	0.044
<i>Chp</i>	39 (98)	16 (73%)	0.07 (0.0-0.7)	0.006
<i>Eta</i>	8 (20%)	6 (27%)	1.5 (0.4-5.9)	0.539
<i>Pvl</i>	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, <i>agr</i> dysfunction, <i>agr</i> genotype II, <i>blaZ</i> , <i>sea</i> , <i>clfA</i> , <i>spIA</i> and ACME locus. Associated to LVM: CC22, CC88 and CC188	Associated with HVM: CC8 P < 0.001), <i>agr</i> dysfunction (P= 0.014), <i>agr</i> genotype II (P=0.043), <i>blaZ</i> (P=0.002), <i>sea</i> (P < 0.001), <i>clfA</i> (P < 0.001), <i>spIA</i> (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 <i>agr</i> 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) <i>agr II</i> polymorphism: 17.2%; average levels of RNAIII gene expression: Δ Ct 1.5 \pm 2.11	45.5% (25/55) <i>agr II</i> polymorphism: 41.8%; average levels of RNAIII gene expression: Δ Ct 4.05 \pm 3.29	HVM: <i>agr II</i> polymorphism: 17.2%; average levels of RNAIII gene expression: Δ Ct 1.5 \pm 2.11 LVM: <i>agr II</i> polymorphism: 41.8%; average levels of RNAIII gene expression: Δ Ct 4.05 \pm 3.29	In-hospital mortality: P=0.057 <i>agr</i> 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: <i>agrII</i> polymorphism: 19% LVM: <i>agrII</i> polymorphism: 38%	In-hospital mortality: P=0.780. <i>agrII</i> 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.

Supplement Appendix

1. Microbiological Methods

E-test for Vancomycin MIC

MSSA strains were stored at -80° 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

1
2
3
4 26 (*arcC*, *aroE*, *glpF*, *gmk*, *pta*, *tpi*, and *yqiL*) were used to identify a unique
5
6 27 sequence type (ST). MLST allele names and STs were derived from
7
8 28 <http://www.mlst.net>. CCs were assigned to groups of isolates sharing 6 of 7
9
10 29 alleles by using the eBURST algorithm (<http://eburst.mlst.net>) [3].
11
12
13

14 31 References

15 32
16 33 [1] Campbell SJ, Deshmukh HS, Nelson CL, Bae IG, Stryjewski ME, Federspiel
17
18 34 JJ, et al. Genotypic characteristics of *Staphylococcus aureus* isolates from a
19
20 35 multinational trial of complicated skin and skin structure infections. *J Clin*
21
22 36 *Microbiol.* 2008;46:678-84.

23
24
25 37 [2] Fowler VG Jr, Nelson CL, McIntyre LM, Kreiswirth BN, Monk A, Archer GL,
26
27 38 et al. Potential associations between hematogenous complications and bacterial
28
29 39 genotype in *Staphylococcus aureus* infection. *J Infect Dis.* 2007;196:738-47.

30
31 40 [3] Feil EJ, Li BC, Aanensen DM, Hanage WP, Spratt BG. eBURST: inferring
32
33 41 patterns of evolutionary descent among clusters of related bacterial genotypes
34
35 42 from multilocus sequence typing data. *J Bacteriol.* 2004;186:1518-30.
36
37
38
39
40
41
42
43
44

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

45 45 Differences in genotypic characteristics between the two groups according to
46
47 46 MIC phenotypes are displayed in the **Table 1**. No differences were detected
48
49 47 regarding adhesins, toxins or other putative virulence factors. Thirty-eight
50
51 48 percent of patients with HVM presented *agr*-II compared to 19% of patients with
52
53 49 LVM. However, this difference was not statistically significant ($P=0.157$). With
54
55 50 regard to CC, CC30 was the most frequent CC in the whole cohort. Differences
56
57
58
59
60

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

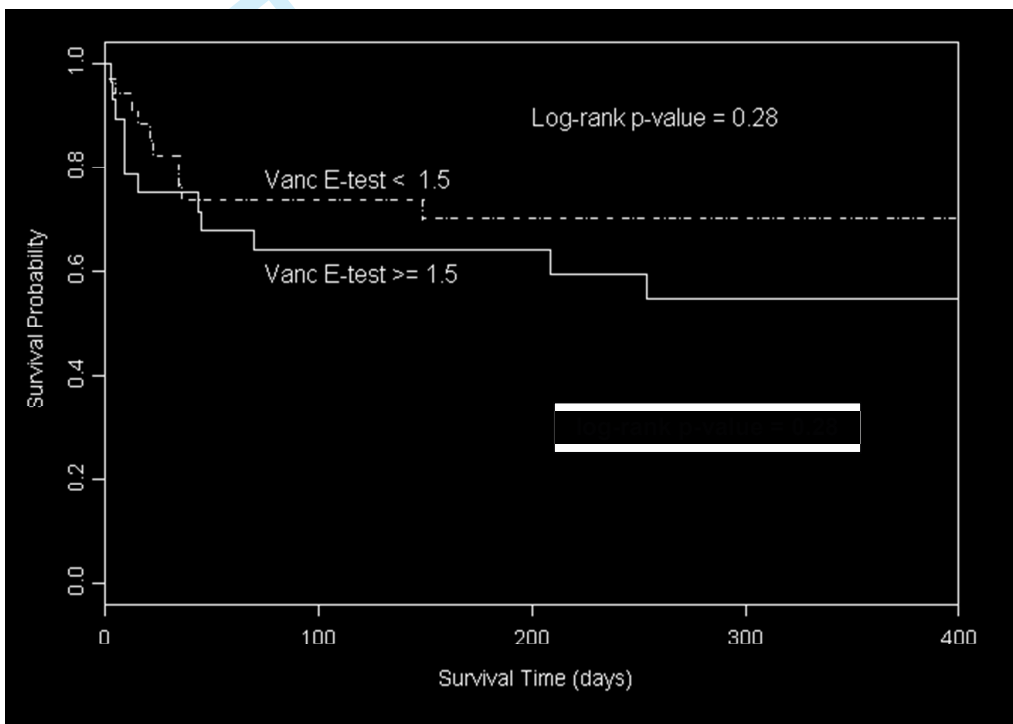
* Other CC analysed comprised CC9, CC22, CC25, CC34, CC51, CC59, CC72, and 125.

5
59
60
61
62
63

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

64
65
66
67
68
69
70
71
72
73

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.



74
75
76
77
78
79
80



1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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.

Authors: Juan M. Pericàs¹, Julia A. Messina^{2,3}, Cristina Garcia-de-la-Maria¹, Lawrence Park^{2,4}, Batu.K. Sharma-Kuinkel², Francesc Marco⁵, Dannah Wray⁶, Zeina Kanafani⁷, Manuela Carugati², Emanuele Durante-Mangoni⁸, Pierre Tattevin⁹, Vivian H. Chu^{2,3}, Asuncion Moreno¹, Vance G. Fowler Jr.^{2,3}, Jose M. Miró¹ and the International Collaboration on Endocarditis Microbiology- Investigators¹⁰.

Affiliations:

1. Infectious Diseases Service, Hospital Clínic of Barcelona, Institut d'Investigacions Biomèdiques Pi i Sunyer (IDIBAPS), University of Barcelona, Spain.

2. Duke University Medical Center. Division of Infectious Diseases and International Health, Durham, North Carolina, US.

3. Duke Clinical Research Institute, Durham, North Carolina, US.

4. Duke Global Health Institute, Durham, North Carolina, US.

5. Department of Microbiology, Institute for Global Health, Hospital Clínic of Barcelona, University of Barcelona, Barcelona, Spain.

6. Infectious Disease Division, Medical University of South Carolina, Charleston, South Carolina, US.

7. Division of Infectious Diseases, American University of Beirut, Beirut, Lebanon.

8. Internal Medicine, Department of Clinical and Experimental Medicine, University of Campania 'Luigi Vanvitelli'; Unit of Infectious & Transplant Medicine, 'V. Monaldi' Hospital, AORN dei Colli, Naples, Italy

9. Infectious Diseases Service, CHU Rennes, Rennes, France.

10. See **Annex I**.

Corresponding author: Dr. Jose M. Miró, Infectious Diseases Service, Hospital Clínic, Villarroel 170, 08036-Barcelona, Spain. (jmmiro@ub.edu).

1
2
3 30 **Alternative corresponding author:** Dr Vance G Fowler Jr., Duke University Medical
4
5 31 Center. Division of Infectious Diseases and International Health, Durham, North Carolina,
6
7 32 US. (vance.fowler@duke.edu)
8

9
10 33 **Keywords:** *Staphylococcus aureus*, vancomycin MIC, endocarditis, phenotype,
11
12 34 genotype.
13

14 35 **Keypoints:** In this international cohort of patients with left-sided MSSA endocarditis
15
16 36 treated with anti-staphylococcal beta-lactam antibiotics, vancomycin MIC phenotype was
17
18 37 not associated with patient demographics, clinical outcome, or virulence gene repertoire.
19

20 38 **Running title:** “Vancomycin MIC and MSSA endocarditis”.

21 39 **Word count:** 1,198

22 40 **Abstract word count:** 249

23 41 **References:** 15

24 42 **Tables:** 3

25 43 **Figures:** 2

26 44 **Supplementary Appendix:** 1 table, 1 figure and microbiological methods.

27 45 **Funding.** Supported in part by R01-AI068804 and K24-AI093969 (US), as well as
28
29 46 20152610 project by “La Marató de TV3”, Barcelona (Spain), FIS PI14/00603 from
30
31 47 Instituto de Salud Carlos III and the Ministerio de Economía and Competitividad, Madrid
32
33 48 (Spain) and the 2016 ESCMID Research Grant. JMP received a “Rio Hortega” Research
34
35 49 Grant (CM14/00135; 2015-16) from Instituto de Salud Carlos III and the Ministerio de
36
37 50 Economía and Competitividad, Madrid (Spain) and the ESCMID/FEMS Joint Research
38
39 51 Fellowship Award 2016. Instituto de Salud Carlos III, Ministerio de Economía y
40
41 52 Competitividad, Madrid (Spain) provided to JMM a personal intensification research grant
42
43 53 #INT15/00168 during 2016.
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

54 These data were partially presented at the 55th ICAAC, September 18-21, 2015. San
55 Diego, CA (abstract number 2423).

56

For Peer Review

1
2
3 57 **Abstract**
4

5 58 **Objectives:** A recent study showed a poorer prognosis in left-sided MSSA endocarditis
6
7 59 treated with cloxacillin when the vancomycin MIC was ≥ 1.5 mg/L. We aimed to validate
8
9 60 these results using the International Collaboration on Endocarditis cohort and to analyze
10
11 61 whether specific genetic characteristics were associated with a high vancomycin MIC
12
13 62 (≥ 1.5 mg/L) phenotype.
14

15
16 63 **Methods:** All patients with left-sided MSSA IE treated with anti-staphylococcal beta-
17
18 64 lactam antibiotics between 2000 and 2006 with available isolates were included.
19
20 65 Vancomycin MIC (VAN MIC) was determined by E-test as either *high* (≥ 1.5 mg/L) or *low*
21
22 66 (< 1.5 mg/L). Isolates underwent *spa* typing to infer clonal complexes (CC) and multiplex
23
24 67 polymerase chain reaction for identifying virulence genes. Univariate analysis was
25
26 68 performed to evaluate the association between in-hospital and one-year mortality and
27
28 69 vancomycin MIC phenotype.
29
30

31
32 70 **Results:** 62 cases met the inclusion criteria. VAN MIC was *low* in 28 cases (45%) and
33
34 71 *high* in 34 cases (55%). No significant differences in patient demographic data or
35
36 72 characteristics of infection were observed between patients with IE due to *high* and *low*
37
38 73 VAN MIC isolates. Isolates with *high* and *low* VAN MIC had similar distributions of
39
40 74 virulence genes and clonal lineages. In-hospital and one-year mortality did not differ
41
42 75 significantly between the two groups (32% (9/28) vs. 27% (9/34), $P=0.780$; and 43%
43
44 76 (12/28) vs. 29% (10/34), $P=0.298$, for *low* and *high* VAN MIC, respectively).
45
46

47 77 **Conclusion:** In this international cohort of patients with left-sided MSSA endocarditis
48
49 78 treated with anti-staphylococcal beta-lactams, vancomycin MIC phenotype was not
50
51 79 associated with patient demographics, clinical outcome, or virulence gene repertoire.
52
53

54 80

55
56 81
57
58
59
60

BACKGROUND

The impact of a *high* vancomycin MIC phenotype (HVM; ≥ 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 HVM was identifiable by a genetic “signature” of specific polymorphisms and virulence factors.

107 **METHODS**

108 **Database**

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

116 **Study Sample**

117 **Patients and Settings**

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

124 **Definitions**

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

130 **Geographic regions**

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

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

132

133

Microbiological methods

134

ICE methodology for microbiological procedures has been defined elsewhere [6,10].

135

Detailed microbiological methods can be found in the **Supplemental Appendix**.

136

137

Statistical analysis

138

Categorical variables were expressed as percentage and compared with Fisher's exact

139

test. Continuous variables were expressed as means or medians and compared by non-

140

parametric tests. Survival analysis was performed by Kaplan-Meier analysis, and curves

141

were compared by the log-rank test. A two-sided p-value <0.05 was considered to be

142

statistically significant.

143

144

145

146

147

148

149

150 RESULTS

151 Cases included

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

153

154 Vancomycin MIC distribution in the cohort

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

157

158 Demographics and clinical characteristics

159 Demographics and clinical characteristics of patients with LVM and HVM are shown in

160 **Table 1**.

161

162 Outcomes

163 Complications, surgical rates and mortality according to vancomycin MIC groups are
164 shown in **Table 1**. Differences between the two groups did not reach statistical
165 significance in any of the variables. For LVM, in-hospital and one-year mortality were
166 32% (9/28) and 43% (12/28), respectively, while in-hospital mortality was 27% (9/34) and
167 one-year mortality 29% (10/34) for HVM.

168

169 Genotypic characteristics

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

173

174 Risk factors for mortality

1
2
3 175 The univariate analysis of risk factors for one-year mortality is shown in **Table 2**. HVM,
4
5 176 type of *agr* and CC were not significantly associated with mortality. The analysis for in-
6
7 177 hospital mortality using the same variables did not differ from that of one-year mortality
8
9 178 (data not shown).
10

11 179

12
13
14 180 The Kaplan-Meier survival plot at one-year according to vancomycin MIC group is
15
16 181 displayed in the **Supplemental Appendix**.
17

18 182

19 183

20 184

21 185

22 186

23 187

24 188

25 189

26 190

27 191

28 192

29 193

30 194

31 195

32 196

33 197

34 198

35 199

36

37

38

39

40

1
2
3 200 **Discussion**
4

5 201 Vancomycin MIC was not associated with complications or mortality among patients with
6
7 202 MSSA left-sided IE treated with beta-lactams. Thus, this study was unable to validate
8
9 203 findings from the Cervera et al study [5], from which two main hypotheses were raised: 1)
10
11 204 HVM in MSSA isolates was associated with higher mortality in patients with left-sided IE
12
13 205 due to an increased rate of major embolic events; and 2) a genomic signature identified
14
15 206 MSSA isolates with HVM.
16
17
18 207

19
20 208 Main findings from studies investigating the relationship between VAN MIC and prognosis
21
22 209 of MSSA and IE, as well as its relationship with *agr* dysfunction are shown in **Table 3**.
23
24
25 210

26
27 211 Higher mortality among patients with LVM than in patients with HVM was found in the
28
29 212 present study, as well as higher rates of systemic embolic events (36% vs. 23%) and
30
31 213 persistent bacteremia (14% vs.12%), neither of them reaching statistical significance.
32
33 214 Given that no significant differences were found in virulence factors between the two
34
35 215 groups, we might speculate that in this dataset, due to the high proportion of patients with
36
37 216 MSSA strains harbouring LVM and CC30, a high rate of detected and not detected
38
39 217 hematogenous complications in the LVM may explain the higher mortality in this group.
40
41
42 218

43
44
45 219 With regard to genotypic features, the analysis of *agr* subgroup did not reveal a
46
47 220 significant association between a specific *agr* polymorphism and HVM or CC. We
48
49 221 expected to find an association between a HVM and *agrII* polymorphism relying on
50
51 222 previous studies performed in MSSA bacteremia [3,11]. In our study, *agrII* was two times
52
53 223 more frequent in the HVM than in the LVM (38% vs. 19%), but did not reach statistical
54
55 224 significance.
56
57
58
59
60

1
2
3 225 We did not identify a specific repertoire of virulence factors in the HVM group, as other
4
5 226 previous studies did [6,7,10]. Although geographical regions are almost equally
6
7 227 represented, changes in the genetic expression and phenotypic pattern in MSSA might
8
9 228 be common over the time and geographical variations are also likely to occur as years
10
11 229 pass. Another factor that could also have influence in the percentage of MSSA strains
12
13
14 230 identified as HVM is the effect of freezer storage. Ludwig et al demonstrated a
15
16 231 progressive decline of VAN MIC determination by Etest in MRSA bloodstream samples
17
18 232 from the moment they were frozen [14].
19
20
21 233

22
23 234 This study has several limitations. First, the low sample size is a major shortcoming,
24
25 235 severely limiting the statistical power of the study and leading to the potential for type II
26
27 236 error in the analysis. Second, the number of isolates tested and available is not
28
29 237 representative of the overall ICE dataset. However, we conducted a subanalysis
30
31 238 comparing ICE left-sided MSSA frozen strains and those that were not frozen, and we did
32
33 239 not detect significant differences regarding in-hospital and one-year mortality and surgery
34
35 240 rates (data not shown). Third, *agrII* dysfunction was not measured, so correlations with
36
37 241 *agr* subgroup, vancomycin MIC, and outcomes were not performed. Fourth, only cases
38
39 242 occurring in the 2000-2006 period were included, which precluded observations
40
41 243 regarding the temporal trends Fifth, the potential variations of MSSA clones between
42
43 244 geographical regions were not investigated. And finally, the specific type of beta-lactam
44
45 245 used was not available.
46
47
48
49 246

50
51 247 In conclusion, in this international cohort of left-sided MSSA IE treated with beta-lactams,
52
53 248 vancomycin MIC ≥ 1.5 $\mu\text{g/mL}$ was not found to be an independent risk factor for
54
55 249 complications of IE or for mortality. Stroke, paravalvular complications, and some *S.*
56
57
58
59
60

1
2
3 250 *aureus* genes were associated with a worse outcome. Differences in clonality and
4
5 251 virulence factors were not found between strains with LVM and HVM. Further studies in
6
7 252 this field are warranted to expand upon these findings and elucidate if the contradictory
8
9 253 results obtained in this field [15] are due to methodological limitations or rather to a
10
11
12 254 difficult-to-interpret phenomenon.
13
14 255

15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For Peer Review

256 **Financial disclosures**

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

268

269

270

271 **References**

- 272 [1] Holmes NE, Turnidge JD, Munckhof WJ, Robinson JO, Korman TM, O'Sullivan MV.
273 Antibiotic choice may not explain poorer outcomes in patients with *Staphylococcus*
274 *aureus* bacteremia and high vancomycin minimum inhibitory concentrations. J Infect Dis.
275 2011;204:340-7.
- 276 [2] Aguado JM, San-Juan R, Lalueza A, Sanz F, Rodríguez-Otero J, Gómez-Gonzalez C,
277 et al. High vancomycin MIC and complicated methicillin-susceptible *Staphylococcus*
278 *aureus* bacteremia. Emerg Infect Dis. 2011;17:1099-102.
- 279 [3] Viedma E, Sanz F, Orellana MA, San Juan R, Aguado JM, Otero JR, et al.
280 Relationship between *agr* dysfunction and reduced vancomycin susceptibility in
281 methicillin-susceptible *Staphylococcus aureus* causing bacteraemia. J Antimicrob
282 Chemother. 2014;69:51-8.
- 283 [4] López-Cortés LE, Velasco C, Retamar P, del Toro MD, Gálvez-Acebal J, de Cueto M,
284 et al. Is reduced vancomycin susceptibility a factor associated with poor prognosis in
285 MSSA bacteraemia? J Antimicrob Chemother. 2015;70:2652-60.
- 286 [5] Cervera C, Castañeda X, de la Maria CG, del Rio A, Moreno A, Soy D, et al. Effect of
287 vancomycin minimal inhibitory concentration on the outcome of methicillin-susceptible
288 *Staphylococcus aureus* endocarditis. Clin Infect Dis. 2014;58:1668-75.
- 289 [6] Nienaber JJ, Sharma Kuinkel BK, Clarke-Pearson M, Lamlertthon S, Park L, Rude
290 TH, et al. Methicillin-susceptible *Staphylococcus aureus* endocarditis isolates are
291 associated with clonal complex 30 genotype and a distinct repertoire of enterotoxins and
292 adhesins. J Infect Dis. 2011;204:704-13.
- 293 [7] Bae IG, Federspiel JJ, Miro JM, Woods CW, Park L, Rybak MJ, et al. Heterogeneous
294 vancomycin intermediate susceptibility phenotype in bloodstream methicillin-resistant
295 *Staphylococcus aureus* isolates from an international cohort of patients with infective

- 1
2
3 296 endocarditis: prevalence, genotype, and clinical significance. J Infect Dis. 2009;
4
5 297 200:1355–66
6
7 298 [8] Li JS, Sexton DJ, Mick N, Nettles R, Fowler VG Jr, Ryan T, et al. Proposed
8
9 299 modifications to the Duke criteria for the diagnosis of infective endocarditis. Clin Infect
10
11 300 Dis. 2000;30:633-8.
12
13
14 301 [9] Fowler VG Jr, Miro JM, Hoen B, Cabell CH, Abrutyn E, Rubinstein E, et al.
15
16 302 *Staphylococcus aureus* endocarditis: a consequence of medical progress. JAMA
17
18 303 2005;293:3012 -21.
19
20
21 304 [10] Fowler VG Jr, Nelson CL, McIntyre LM, Kreiswirth BN, Monk A, Archer GL, et al.
22
23 305 Potential associations between hematogenous complications and bacterial genotype in
24
25 306 *Staphylococcus aureus* infection. J Infect Dis. 2007;196:738-47.
26
27
28 307 [11] Holmes NE, Turnidge JD, Munckhof WJ, Robinson JO, Korman TM, O'Sullivan MV,
29
30 308 et al. Genetic and molecular predictors of high vancomycin MIC in *Staphylococcus*
31
32 309 *aureus* bacteremia isolates. J Clin Microbiol. 2014;52:3384-93.
33
34 310 [12] Deodhar D, Varghese G, Balaji V, John J, Rebekah G, Janardhanan J, et al.
35
36 311 Prevalence of Toxin Genes among the Clinical Isolates of *Staphylococcus aureus* and its
37
38 312 Clinical Impact. J Glob Infect Dis. 2015;7:97-102.
39
40
41 313 [13] Argudín MA, Mendoza MC, Méndez FJ, Martín MC, Guerra B, Rodicio MR. Clonal
42
43 314 complexes and diversity of exotoxin gene profiles in methicillin-resistant and methicillin-
44
45 315 susceptible *Staphylococcus aureus* isolates from patients in a Spanish hospital. J Clin
46
47 316 Microbiol. 2009;47:2097-105.
48
49
50 317 [14] Ludwig F, Edwards B, Lawes T, Gould IM. Effects of storage on vancomycin and
51
52 318 daptomycin MIC in susceptible blood isolates of methicillin-resistant *Staphylococcus*
53
54 319 *aureus*. J Clin Microbiol. 2012;50:3383-7.
55
56
57
58
59
60

1
2
3 320 [5] Kalil C, van S h neveld TC, Fe PD, Rupp ME. ss iati n between van cin
4
5 321 minimum inhibitory concentration and mortality among patients with *Staphylococcus*
6
7 322 *aureus* bloodstream infections: a systematic review and meta-analysis. JAMA.
8
9 323 2014;312:1552-64.
10
11
12 324
13
14 325
15
16 326
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For Peer Review

1
2
3 327 **Annex I: ICE Micro Investigators**
4
5 328

6 329 **Australia:** Eugene Athan, MD, Owen Harris, MBBS (*Barwon Health*); Tony M. Korman, MD
7 330 (*Monash Medical Centre*), Despina Kotsanas, BS (*Southern Health*); Phillip Jones, MD, Porl
8 331 Reinbott, Suzanne Ryan, MHS (*The University of New South Wales*); **Brazil:** Claudio
9 332 Querido Fortes, MD (*Hospital Universitario Clementino Fraga Filho/UFRJ*); **Chile:** Patricia
10 333 Garcia, MD, Sandra Braun Jones, MD (*Hosp. Clínico Pont. Universidad Católica de Chile*);
11 334 **Croatia:** Bruno Barsic, MD, PhD, Suzana Bukovski, MD (*Univ. Hospital for Infectious*
12 335 *Diseases*); **France:** Christine Selton-Suty, MD, Neijla Aissa, MD, Thanh Doco-Lecompte,
13 336 MD (*CHU Nancy-Brabois*); Francois Delahaye, MD, PhD, Francois Vandenesch, MD
14 337 (*Hopital Louis Pradel*); Pierre Tattevin, MD, PhD (*Pontchaillou University*); Bruno Hoen,
15 338 MD, PhD, Patrick Plesiat, MD (*University Medical Center of Besançon*); **Greece:** Helen
16 339 Giamarellou, MD, PhD, Efthymia Giannitsioti, MD, Ekaterini Tarpatzi, MD (*Attikon University*
17 340 *General Hospital*); **Italy:** Emanuele Durante-Mangoni MD, PhD, Domenico Iossa PhD,
18 341 Serena Orlando MD, Maria Paola Ursi MD, Pia Clara Pafundi PhD, Fabiana D'Amico BSc,
19 342 Mariano Bernardo MSc, Susanna Cuccurullo MSc, Giovanni Dialetto MD, Franco Enrico
20 343 Covino MD, Sabrina Manduca MD, Alessandro Della Corte MD, PhD, Marisa De Feo MD,
21 344 PhD (*Università della Campania*); Marie Françoise Tripodi MD (*University of Salerno*);
22 345 **Lebanon:** Tania Baban, MD, Zeina Kanafani, MD, MS, Souha S.Kanj, MD, Jad Sfeir, MD,
23 346 Mohamad Yasmine, MD (*American University of Beirut Medical Center*); **New Zealand:**
24 347 Arthur Morris, MD (*Diagnostic Medlab*), David R. Murdoch MD, MSc, DTM&H (*University of*
25 348 *Otago*); **Slovenia:** Manica Mueller Premru MD PhD, Tatjana Lejko-Zupanc MD PhD
26 349 (*Medical Center Ljubljana*); **Spain:** Manel Almela, MD, Juan Ambrosioni, MD, PhD, Manuel
27 350 Azqueta, MD, Merce Brunet, MD, PhD, Carlos Cervera, MD, PhD, Elisa De Lazzari, MS,
28 351 Carlos Falces, MD, David Fuster, MD, PhD, Cristina Garcia- de- la- Maria, PhD, Javier
29 352 Garcia-Gonzalez, MS, Jose M. Gatell, MD, PhD, Francesc Marco, MD, PhD, José M. Miró,
30 353 MD, PhD, Asuncion Moreno, MD, PhD, José Ortiz, MD, PhD, Salvador Ninot, MD, J. Carlos
31 354 Paré, MD, PhD, Juan M Pericas, MD, Eduard Quintana, MD, PhD, Jose Ramirez, MD, PhD,
32 355 Elena Sandoval, MD, Marta Sitges, MD, PhD, José M. Tolosana, MD, PhD, Barbara Vidal,
33 356 MD, PhD, Jordi Vila, MD, PhD (*Hospital Clinic – IDIBAPS. University of Barcelona*) Emilio
34 357 Bouza MD, PhD, Marta Rodríguez-Créixems, MD, PhD, Victor Ramallo, MD, (*Hospital*
35 358 *General Universitario Gregorio Marañón*); **USA:** Suzanne Bradley, MD (*Ann Arbor VA*
36 359 *Medical Center*); Dannah Wray, MD, Lisa Steed, PhD, MHS, Robert Cantey, MD, (*Medical*
37 360 *University of South Carolina*); Gail Peterson, MD, Amy Stancoven, MD (*UT-Southwestern*
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 361 *Medical Center*); Christopher Woods, MD, MPH, G. Ralph Corey, MD, L. Barth Reller, MD,
4 362 Vance G. Fowler Jr., MD, MHS, Vivian H. Chu, MD, MHS, (*Duke University Medical Center*).
5
6
7 363

8 364 **ICE Coordinating Center:** Khaula Baloch, MPH, Vivian H. Chu, MD, MHS, G. Ralph Corey,
9 365 MD, Christy C. Dixon, Vance G. Fowler, Jr., MD, MHS, Tina Harding, RN, BSN, Marian
10 366 Jones-Richmond, Paul Pappas, MS, Lawrence P. Park, PhD, Thomas Redick, MPH, Judy
11 367 Stafford, MS
12
13
14
15 368

16 369 **ICE Publications Committee:** Kevin Anstrom, PhD, Eugene Athan, MD, Arnold S. Bayer,
17 370 MD, Christopher H. Cabell, MD, MHS, Vivian H. Chu, MD, MHS, G. Ralph Corey, MD,
18 371 Vance G. Fowler, Jr., MD, MHS, Bruno Hoen, MD, PhD, A W Karchmer MD, José M. Miró,
19 372 MD, PhD, David R. Murdoch, MD, MSc, DTM&H, Daniel J. Sexton, MD, Andrew Wang, MD
20
21
22
23 373

24 374 **ICE Steering Committee:** Arnold S. Bayer, MD, Christopher H Cabell, MD, MHS, Vivian
25 375 Chu, MD, MHS, G. Ralph Corey MD, David T. Durack, MD, D Phil, Susannah Eykyn, MD,
26 376 Vance G. Fowler, Jr., MD, MHS, Bruno Hoen, MD, PhD, José M. Miró, MD, PhD, Phillipe
27 377 Moreillon, MD, PhD, Lars Olaison, MD, PhD, Didier Raoult, MD, PhD, Ethan Rubinstein MD,
28 378 LLB, Daniel J. Sexton, MD
29
30
31
32
33 379

34 380

35 381

36 382

37 383

38 384

39 385

40 386

41 387

42 388

43 389

44 390

45 391

46 392

47 393

48 394

49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 395 **FIGURE LEGENDS**

4 396
5
6 397 **Figure 1.** Flowchart of the patients included in the study. Abbreviation: ICE, International
7
8 398 Collaboration on Endocarditis; IE, infective endocarditis; MIC, Minimum Inhibitory
9
10 399 Concentration; MSSA, methicillin-susceptible *Staphylococcus aureus*; VAN, vancomycin.

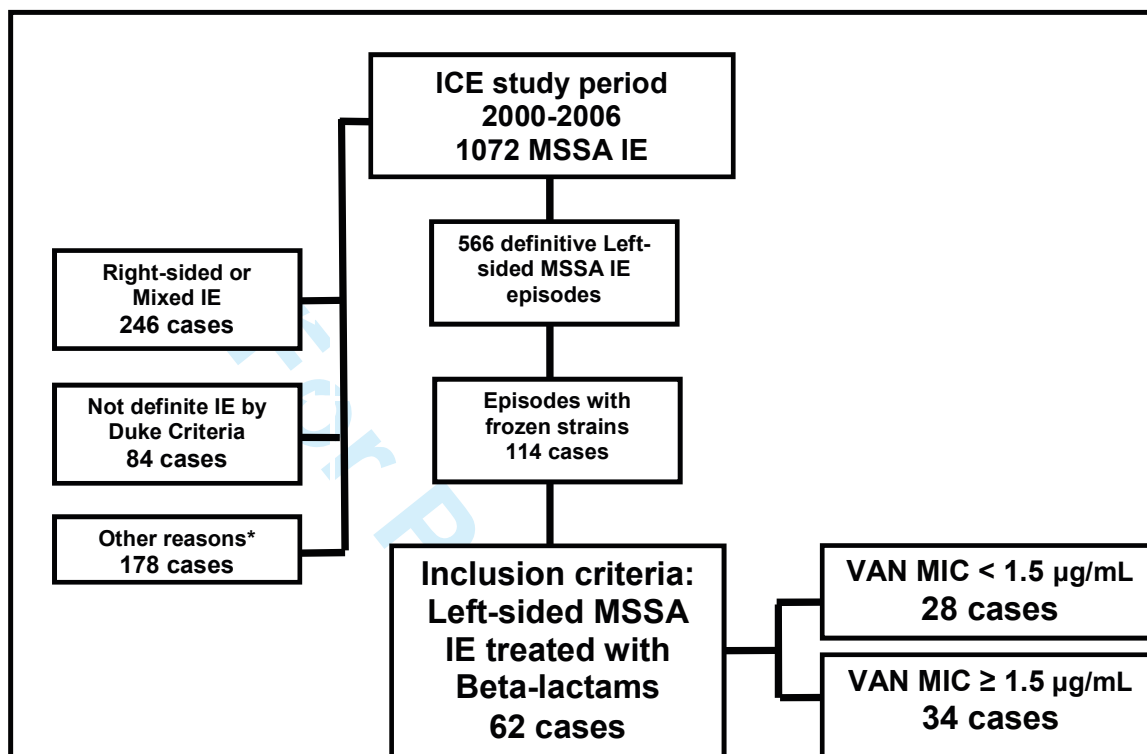
11 400
12
13 401 **Figure 2.** Distribution of the vancomycin MIC within the cohort of 62 left-sided MSSA
14
15 402 Infective endocarditis and overall one-year mortality rates according to vancomycin MIC
16
17 403 determination.

18 404
19
20 405
21
22 406
23
24
25 407

For Peer Review

26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

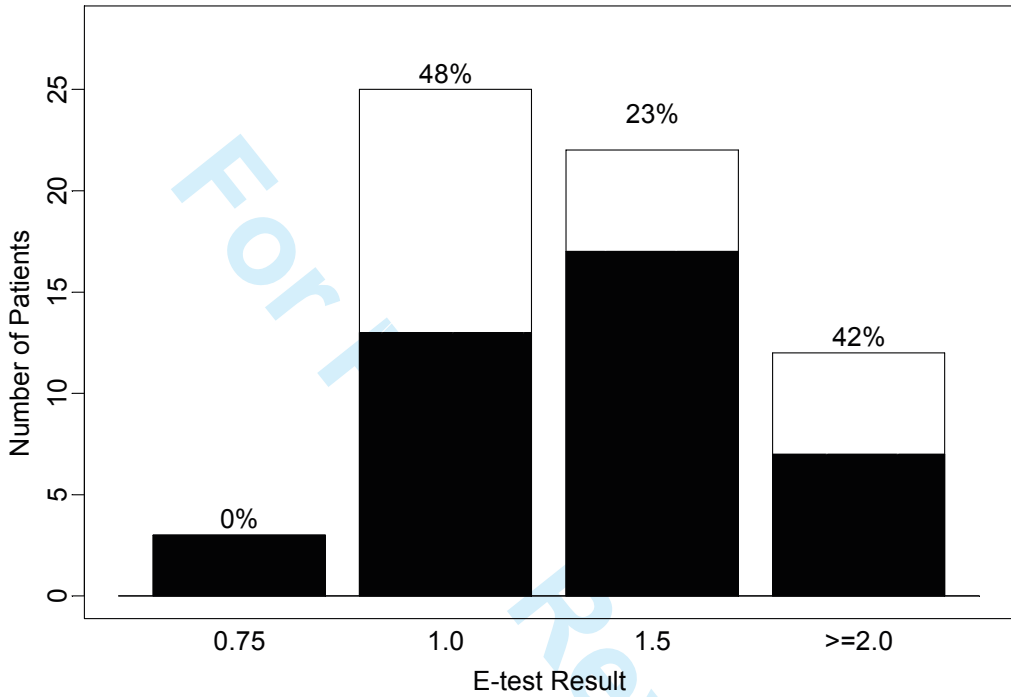
Figure 1.



*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).

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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.

For Peer Review

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 \geq 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
<i>see</i>	8 (20%)	10 (46%)	3.33 (0.9-12.2)	0.044
<i>sei</i>	33 (83%)	22 (100%)	2.94 (1.1-13.7)	0.044
<i>chp</i>	39 (98)	16 (73%)	0.07 (0.0-0.7)	0.006
<i>eta</i>	8 (20%)	6 (27%)	1.5 (0.4-5.9)	0.539
<i>pvl</i>	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, <i>agr</i> dysfunction, <i>agr</i> genotype II, <i>blaZ</i> , <i>sea</i> , <i>clfA</i> , <i>sp/A</i> and ACME locus. Associated to LVM: CC22, CC88 and CC188	Associated to HVM: CC8 P < 0.001), <i>agr</i> dysfunction (P= 0.014), <i>agr</i> genotype II (P=0.043), <i>blaZ</i> (P=0.002), <i>sea</i> (P < 0.001), <i>clfA</i> (P < 0.001), <i>sp/A</i> (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 <i>agr</i> 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: <i>agr II</i> polymorphism: 17.2%; average levels of RNAIII gene expression: Δ Ct 1.5 \pm 2.11 LVM: <i>agr II</i> polymorphism: 41.8%; average levels of RNAIII gene expression: Δ Ct 4.05 \pm 3.29	In-hospital mortality: P=0.057 <i>agr</i> 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: <i>agrII</i> polymorphism: 19% LVM: <i>agrII</i> polymorphism: 38%	In-hospital mortality: P=0.780. <i>agrII</i> 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.

Supplement Appendix

1. Microbiological Methods

E-test for Vancomycin MIC

MSSA strains were stored at -80° 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

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

(*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

[1] Campbell SJ, Deshmukh HS, Nelson CL, Bae IG, Stryjewski ME, Federspiel JJ, et al. Genotypic characteristics of *Staphylococcus aureus* isolates from a multinational trial of complicated skin and skin structure infections. *J Clin Microbiol.* 2008;46:678-84.

[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

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

53 **Table 1. Genotypic Characteristics and Distribution of Clonal Complexes**
54 **of 62 Episodes of Left-sided Methicillin-susceptible *Staphylococcus***
55 ***aureus* Infective Endocarditis Treated with Beta-lactams According to**
56 **High or Low Vancomycin Minimum Inhibitory Concentration (MIC).**
57

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