Association between vancomycin MIC with virulence genes expression and clonal complexes of methicillin-susceptible *Staphylococcus aureus* (MSSA) strains isolated from left-sided endocarditis

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Background. The impact of a high vancomycin (VAN) minimum inhibitory concentration (MIC) phenotype (HVM; >1.5 mg/L) in methicillin-susceptible *Staphylococcus aureus* (MSSA) bacteraemia and infective endocarditis (IE) is poorly known. MSSA IE isolates are known to possess a distinct repertoire of virulence genes and clonal complexes (CC) that differentiates them from other type of MSSA isolates [1].

Several studies reported higher rates of complications and mortality in patients with MSSA bacteraemia caused by strains with HVM [2,3], as well as a correlation with *agr* dysfunction, *agr* type II polymorphism, and other specific findings shaping a repertoire of virulence factors in the HVM group [4-7]. Our group demonstrated the association between HVM and significantly higher mortality in a cohort of 93 patients with MSSA IE treated with cloxacillin [8], demonstrating higher mortality rates and systemic emboli in the HVM group. Mortality of MSSA IE was three-fold higher among patients with high VAN MIC isolates. Nonetheless, some recent studies did not find significant differences either on *agr* subgroup and function in MSSA bacteremia and IE [9,10] nor on left-MSSA IE treated with beta-lactams' clinical outcomes [10] according to vancomycin MIC.

The **aim of this study** was to investigate whether the strains belonging to the cohort of patients from the Cervera et al study [8] presented specific patterns of virulence factors, CC types or ability to form biofilms in the presence or not of vancomycin.

Methods. The cohort included 53 isolates with HVM and 40 with HVM collected from 2000 to 2006. Isolates underwent spa typing to infer CC, and multiplex polymerase chain reaction for the presence of virulence genes as described in [1]. Biofilm formation was determined following the methodology presented in [11]. The association between CC, virulence factors and VAN phenotype, as well as in-hospital mortality and symptomatic systemic emboli, were analyzed.

Results. We found no differences in adhesins [fibronectin-binding proteins (fnbA, fnbB), clumping factors (clfA, clfB), collagen-binding gene (cna), serine-aspartate repeat proteins for adhesion (sdrC, sdrD, sdrE), sialoprotein (bbp) and elastin-binding protein (ebps), and MHC class II analog proteins (MAP/EAP)], toxins [exfoliative toxins (eta, etb), enterotoxins (tst), staphylococcal enterotoxins (sea, seb, sec, sed, see, seg, seh, sei, sej), Panton-Valentin leucocidin (PVL), and hemolysin (hlg)] or other putative virulence [fibrinogen-binding protein (*efb*), adhesion intracellular protein A (icaA), chemotaxis-inhibiting protein (chp), and serine endopeptidase (V8)] genes between MSSA isolates according to VAN MIC (**Table 1**).

Agr subgroups I and III predominated, with no association with VAN MIC. Isolates with lower VAN MICs exhibited **higher ability to form biofilm with and without the presence of VAN** (p<0.001 and p=0.022, respectively). **There was no association of CC type with VAN MIC** (CC30, CC34; CC45 = 50% of isolates).

Table 1. Genotypic Characteristics, Clonal Complexes and Biofilm Productin of MSSA Isolates from Patients With IE According to Vancomycin (VAN) MIC

	Vancomycin MIC < 1.5 µg/mL N=53	Vancomycin MIC ≥ 1.5 µg/mL N=40	*P*
Adhesins:			
▪ *fnbA*	53 (100%)	40 (100%)	-
▪ *fnbB*	41 (77%)	37 (93%)	0.049
Toxins:			
▪ *eta*	4 (8%)	7 (18%)	0.197
▪ *sei*	34 (64%)	26 (65%)	0.932
▪ *sej*	20 (38%)	13 (33%)	0.601
▪ *pvl*	0	1 (2.5%)	0.430
▪ *hlg*	41 (77%)	28 (70%)	0.422
Other putative virulence genes:			
▪ *efb*	35 (66%)	29 (73%)	0.505
▪ *icaA*	53 (100%)	40 (100%)	-
▪ *chp*	27 (51%)	20 (50%)	0.928
▪ *V8*	10 (19%)	8 (20%)	0.891
Agr subgroup:			
▪ I	17 (32%)	15 (38%)	0.586
▪ II	12 (23%)	9 (23%)	0.987
▪ III	20 (38%)	12 (30%)	0.437
▪ IV	2 (4%)	0	0.504
Clonal complex:			
▪ 5	3 (6%)	6 (15%)	0.166
▪ 30	12 (23%)	9 (23%)	0.987
▪ 34	7 (13%)	3 (8%)	0.507
▪ 45	8 (15%)	7 (18%)	0.755
▪ Other	23 (43%)	15 (38%)	0.567
Biofilm formation			
▪ without VAN (SD)	2,871 (0.685)	2,602 (0.417)	0.022
▪ with VAN (SD)	2,482 (0.509)	2,034 (0.421)	<0.001
tPMP			
▪ 50 mcg/mL (SD)	64,442 (18,595)	72,764 (23,918)	0.062
▪ 25 mcg/mL (SD)	80,183 (21,271)	87,298 (20,607)	0.109
▪ 12.5 mcg/mL (SD)	76,665 (18,593)	80,845 (18,861)	0.289

Neither CC, biofilm formation nor virulence factors were identified as risk factors for **in-hospital mortality,** except for *efb*, which was associated with lower mortality. As for clinically evident **systemic emboli**, *sei, hlg, efb*, *V8*, and a lower ability for biofilm formation in the presence of VAN were associated with significantly higher rates of emboli (**Table 2**). On **multivariate analysis**, the presence of the ***hlg*** **gene was associated with almost 20-fold higher risk of emboli** (OR 19.5, 95%CI 1.78-212.51; P=0.015), whereas **the ability to form biofilm in presence of VAN was associated with reduced risk of emboli** (OR 0.21, 95%CI 0.06-0.80; P=0.022).

Table 2. Univariate Analysis: In-hospital Mortality; Symptomatic Embolic Events

	Survivors (n=56)	In-hospital mortality (n=37)	*P*	No embolic events (n=74)	Embolic events (n=19)	*P*
Adhesins[1]:						
▪ *fnbA*	56 (100%)	37 (100%)	-	74 (100%)	19 (100%)	-
▪ *fnbB*	46 (82%)	32 (87%)	0.577	63 (85%)	15 (79%)	0.499
Toxins[2]:						
▪ *sei*	38 (68%)	22 (59%)	0.407	44 (60%)	16 (84%)	0.044
▪ *sej*	16 (29%)	17 (46%)	0.087	27 (37%)	6 (32%)	0.792
▪ *pvl*	0	1 (3%)	0.398	0	1 (5%)	0.204
▪ *hlg*	42 (75%)	27 (73%)	0.827	50 (68%)	19 (100%)	0.002
Other putative virulence genes:						
▪ *efb*	43 (77%)	21 (57%)	0.041	47 (64%)	17 (90%)	0.029
▪ *icaA*	56 (100%)	37 (100%)	-	74 (100%)	19 (100%)	-
▪ *chp*	29 (52%)	18 (49%)	0.767	35 (47%)	12 (63%)	0.217
▪ *V8*	10 (18%)	8 (22%)	0.653	11 (15%)	7 (37%)	0.048
Agr subgroup:						
▪ I	20 (36%)	12 (32%)	0.744	25 (34%)	7 (37%)	0.802
▪ II	11 (20%)	10 (27%)	0.405	16 (22%)	5 (26%)	0.759
▪ III	22 (39%)	10 (27%)	0.223	26 (35%)	6 (32%)	0.771
▪ IV	2 (4%)	0	0.516	1 (1%)	1 (5%)	0.369
Clonal complex:						
▪ 5	4 (7%)	5 (14%)	0.316	6 (8%)	3 (16%)	0.467
▪ 30	12 (21%)	9 (24%)		19 (26%)	2 (11%)	
▪ 34	9 (16%)	1 (3%)		7 (10%)	3 (16%)	
▪ 45	9 (16%)	6 (16%)		13 (18%)	2 (11%)	
▪ Other	22 (39%)	16 (43%)		29 (39%)	9 (47%)	
Biofilm formation						
▪ without VAN (SD)	2.75 (0.66)	2.76 (0.49)	0.915	2.75 (0.58)	2.79 (0.66)	0.811
▪ with VAN (SD)	2.30 (0.50)	2.27 (0.55)	0.820	2.35 (0.53)	2.06 (0.41)	0.028
tPMP						
▪ 50 mcg/mL (SD)	69.34 (21.7)	66.03 (20.9)	0.467	67.3 (21.1)	70.8 (22.3)	0.549
▪ 25 mcg/mL (SD)	84.59 (23.1)	81.20 (17.9)	0.453	82.5 (21.6)	86.1 (19.7)	0.508
▪ 12.5 mcg/mL (SD)	78.92 (18.6)	77.77 (19.1)	0.772	78.4 (19.0)	78.8 (18.2)	0.930

Conclusions

- MSSA with **higher** vancomycin MICs had **lower ability to form biofilms,** which was associated with **higher embolic rates**.
- No association was found between adhesins, toxins, *agr* or other virulence gene expression and CC according to vancomycin MIC or **in-hospital mortality,** except for *efb*, which was associated with lower mortality.
- **Hemolysin** (*hlg*) and **reduced biofilm formation** in the presence of vancomycin were associated with **systemic emboli.**

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