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Staphylococcus aureus infective endocarditis versus bacteremia strains: Subtle genetic differences at stake

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ABSTRACT

Infective endocarditis (IE)[(1)] is a severe condition complicating 10–25% of *Staphylococcus aureus* bacteremia. Although host-related IE risk factors have been identified, the involvement of bacterial features in IE complication is still unclear. We characterized strictly defined IE and bacteremia isolates and searched for discriminant features. *S. aureus* isolates causing community-acquired, definite native-valve IE (n = 72) and bacteremia (n = 54) were collected prospectively as part of a French multicenter cohort. Phenotypic traits previously reported

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or hypothesized to be involved in staphylococcal IE pathogenesis were tested. In parallel, the genotypic profiles of all isolates, obtained by microarray, were analyzed by discriminant analysis of principal components (DAPC)[2]. No significant difference was observed between IE and bacteremia strains, regarding either phenotypic or genotypic univariate analyses. However, the multivariate statistical tool DAPC, applied on microarray data, segregated IE and bacteremia isolates: IE isolates were correctly reassigned as such in 80.6% of the cases (C-statistic 0.83, $P < 0.001$). The performance of this model was confirmed with an independent French collection IE and bacteremia isolates (78.8% reassignment, C-statistic 0.65, $P < 0.01$). Finally, a simple linear discriminant function based on a subset of 8 genetic markers retained valuable performance both in study collection (86.1%, $P < 0.001$) and in the independent validation collection (81.8%, $P < 0.01$). We here show that community-acquired IE and bacteremia *S. aureus* isolates are genetically distinct based on subtle combinations of genetic markers. This finding provides the proof of concept that bacterial characteristics may contribute to the occurrence of IE in patients with *S. aureus* bacteremia.

1. Introduction

Infective endocarditis (IE)[1] is a severe complication occurring in 10–25% of *Staphylococcus aureus* bacteremia (Kaasch et al., 2011; Seidl et al., 2011). Despite a stagnation of the global incidence of IE over the past decades in Europe and North America, the epidemiology has evolved, with *S. aureus* being now the predominant causative pathogen (Murdoch et al., 2009; Selton-Suty et al., 2012). Several host-related IE risk factors have been identified, including drug use, congenital heart disease, or the presence of cardiac prosthetic material (Hoen and Duval, 2013; Moreillon and Que, 2004; Que and Moreillon, 2011). Still, approximately 30–50% of IE cases occur without any described risk factors (Hoen and Duval, 2013), suggesting the probable involvement of bacterial features in the occurrence of IE during bacteremia. Several in vitro and animal studies have explored the role of diverse bacterial phenotypes in IE (O'Brien et al., 2002; Piroth et al., 2008; Salgado-Pabón et al., 2013). The few clinical studies attempting to confirm the participation of such bacterial determinants or specific multilocus sequence typing (MLST)[3] clonal complex (CC)[4] yielded contradictory results (Nethercott et al., 2013; Nienaber et al., 2011; Seidl et al., 2011; Xiong et al., 2009). To address the question of a possible distinction between IE and bacteremia *S. aureus* isolates, we conducted a systematic search for genotypic and phenotypic differences between strictly defined community-acquired native-valve IE and bacteremia-related *S. aureus* isolates.

2. Methods

2.1. Patients and strains

Two different sets of isolates were analyzed. The first one, designated hereafter as the training set, included 126 *S. aureus* isolates (72 IE and 54 bacteremia) originating from a French national prospective multicenter cohort, VIRSTA (Le Moing et al., 2015). Briefly, patients with *S. aureus* bacteremia and/or IE were included from 2009–2011 in eight teaching hospitals across the French territory. Patients with community-acquired and non-device related definite native-valve IE, with the presence of echocardiographic vegetation, were defined as cases according to the modified Duke criteria (Li et al., 2000). Patients meeting the community-acquired "possible" or "excluded IE" definition, with negative requested trans-thoracic (TTE)[5] or trans-esophageal echocardiography (TEE)[6], and not meeting post-hospital criteria for IE at a 3-month follow-up visit were defined as bacteremia.

The second set of isolates, designated hereafter as the test set, included 81 community-acquired methicillin-susceptible *S. aureus* (MSSA)[7] bacteremia isolates collected during a prospective multicenter study from 2006–2007 by 23 representative French hospital laboratories (Grundmann et al., 2010) and 66 community-acquired MSSA definite IE isolates collected during a French population-based survey in 2008 (Selton-Suty et al., 2012). These isolates had been previously genotyped by microarray analysis (Tristan et al., 2012).

All strains were thawed from −80 °C and grown on sheep-blood agar plates at 37 °C.

2.2. DNA microarray assay

Bacterial DNA was extracted using commercial extraction kits (Qiagen, Courtaboeuf, France) according to the manufacturer's recommended protocol. The *S. aureus* microarray genotyping kit (Alere, Jouy-en-Josas, France) used as well as related procedures and protocols have been previously described in detail (Monecke et al., 2009). Briefly, the method uses multiplex PCR covering 332 alleles corresponding to 180 genes. For data interpretation, alleles of a same gene were pooled as one genotypic marker to avoid redundancy; markers that were systematically positive or negative for all strains were excluded to avoid background noise. A total of 138 genotypic markers were thus explored. The assignation of isolates to MLST CCs was determined as previously described (Monecke et al., 2009).

2.3. Phenotypic assays

To assess whether IE and bacteremia isolates could be distinguished by phenotypic traits commonly thought to be relevant in *S. aureus* IE pathophysiology, a subset of 28 isolates (14 IE and 14 bacteremia) was selected, with stratification on CCs to reflect their proportion in the VIRSTA collection. The following phenotypes were assessed using the previously described methods with or without modification: human neutrophil peptide-1 (hNP-1)[8] susceptibility (Xiong et al., 2005), adherence to fibrinogen and fibronectin (Tristan et al., 2009), biofilm formation (Chavant et al., 2007), staphylokinase production (Kwiecinski et al., 2010), platelet aggregation (O'Brien et al., 2002), CD69 superantigen-induced expression (Lina et al., 1998), and adhesion to and internalization by HUVEC[9] cells (Rasigade et al., 2011). Technical details are supplied in Text A.1.

2.4. Statistical analyses

2.4.1. Univariate analysis of microarray data

Univariate analysis was performed on the DNA microarray data with Fisher's exact test and with Bonferroni correction for multiple tests.

2.4.2. Principal component analysis

Principal component analysis (PCA)[10] was performed on the strains' 138 microarray marker data (i) to display inter-strain genetic variability within a strain collection, (ii) to define a set of factors that clusters strains on the factorial map of the PCA and (iii) to compare the genetic variability of two different strain groups (IE and bacteremia).

2.4.3. Discriminant analysis of principal components (DAPC)

DAPC is a linear discriminant analysis method with a built-in dimensionality reduction ability with proven performance for the analysis of

genetically structured populations (Jombart et al., 2010; Weinert et al., 2015). Briefly, DAPC was used to (i) identify the optimal number of principal components (PCs) to use in DAPC to avoid over discrimination; (ii) find the discriminant function (DF)[11] that best separates the two IE and bacteremia groups and calculate individual membership probability and (iii) identify the individual and/or combination markers derived from the DF allowing the discrimination between the IE and bacteremia groups.

The indicator of discrimination performance was defined by the assignment success rate as the ratio of the number of correctly assigned isolates to the total number of isolates. Cross-validation was performed using 70% of the training collection as an evaluation set; variation in the assignment success rate with the number of retained PCs was assessed using the remaining 30% isolates. The assignment success rate of the DAPC was calculated, assuming that a strain was correctly assigned to its original group if its membership probability was higher than 0.50. The contribution of the original variables (i.e., genotypic markers) to the DF was then calculated to identify the most contributing markers. To investigate the robustness of the DAPC model trained on the VIRSTA collection, the same model was applied to the 66 IE and 81 bacteremia isolates of the independent test set.

A DF (without prior PC analysis) was then computed based on the eight most contributing markers with respect to the weight of each marker and was adjusted to yield a decision threshold equal to zero.

C-statistics and Chi^2 P-values of association between observed and predicted values were computed for all models (DAPC and DF).

All analyses were performed with R 3.0.2 software (R development Core Team 2013) using the *ade4* (Dray and Dufour, 2007) and *adegenet* libraries (Jombart and Ahmed, 2011).

2.5. Ethics

The French National Ethics Committee "Comité de Protection des Personnes Sud-Méditerrannée IV" approved the study. The VIRSTA study is registered in the European Clinical Trials Database (EUDRACT) (number: 2008-A00680-55). Informed written consent was obtained from all patients.

3. Results

A total of 126 community-acquired isolates, collected from 72 IE and 54 bacteremia cases, were available. All 122 MSSA and 4 methicillin-resistant *S. aureus* isolates, were analyzed with the DNA microarray kit. Phenotypes were explored on a subset of 28 isolates (14 IE and 14 bacteremia) selected with stratification on MLST clonal complex to reflect their proportion in the VIRSTA collection (see text in Section 2.3).

3.1. In vitro phenotypes do not discriminate IE isolates from bacteremia isolates

Diverse bacterial phenotypes like *S. aureus* ability to bind fibrinogen and fibronectin (Piroth et al., 2008; Que and Moreillon, 2011; Xiong et al., 2009), resistance to microbicidal peptides (Fowler et al., 2000, 2004; Xiong et al., 2009; Yeaman et al., 1997), biofilm production (Mohamed et al., 2004; Seidl et al., 2011), and the formation of platelet-bacteria thrombi on the valve surface (Heilmann et al., 2004; O'Brien et al., 2002) have been described as critical in IE occurrence. Thus, adhesion to the extracellular matrix (fibrinogen and fibronectin) and endothelial cells, as well as internalization by endothelial cells, resistance to the microbicid human neutrophil peptid, biofilm production, and induction of platelet aggregation were thus tested. Moreover, because staphylokinase production activates plasminogen into plasmin, which in turn degrades fibrin clots, we hypothesized that its production may interfere with platelet-bacteria thrombus formation at IE initiation (Kwiecinski et al., 2013). Given the evidence that enterotoxin C could play a role in IE (Salgado-Pabón et al., 2013), CD69 cell activation, reflecting the superantigen activity of our strains, was also tested (Lina et al., 1998). Considering each of the tested phenotypes, our results showed no difference between the two groups and thus revealed that IE and bacteremia isolates could not be discriminated based on such features (Table 1, Fig A.1).

Table 1
Phenotypic characteristics of IE and bacteremia isolates.

Phenotypes	Mean ± SD		P[a]
	IE (n = 14)	Ba (n = 14)	
Fibrinogen binding (% of binding)	51.7 ± 10.22	47.7 ± 10.14	0.611
Fibronectin binding (% of binding)	45.7 ± 7.31	47.4 ± 5.82	0.826
Endothelial cell adhesion (% of binding)	93.9 ± 28.80	90.1 ± 33.12	0.853
Endothelial cell internalization (% of internalization)	133.1 ± 13.23	140.4 ± 19.45	0.483
Biofilm production (BFI)	12.5 ± 7.21	12.3 ± 7.41	0.968
hNP-1 resistance (% of surviving bacteria)	35.3 ± 9.27	36.6 ± 18.25	0.810
Staphylokinase production (kinetics slope)	0.13 ± 0.031	0.21 ± 0.045	0.130
Platelet aggregation (lag time in minutes)	2.5 ± 2.42	2.2 ± 1.57	0.757
Superantigenic properties (fluorescence arbitrary intensity)	694.6 ± 133.28	631.8 ± 158.83	0.764

Abbreviations: SD, standard deviation; IE, infective endocarditis; Ba, bacteremia; and BFI, biofilm forming index.
[a] Statistical significance was calculated for each gene or allele with the two-tailed Fisher's exact test.

3.2. IE and bacteremia isolates belong to a wide variety of genetic backgrounds

The 126 analyzed isolates fell into 21 different MLST CCs assigned by the DNA microarray (Table 2), reflecting the wide genetic diversity in the IE isolates (16 different CCs) as well as in the bacteremia isolates (16 different CCs). CC5 was the predominant lineage in both the IE (n = 13, 18.1%) and the bacteremia (n = 12, 22.2%) groups. Nearly 50% of IE isolates were represented by only three CCs (CC45, CC5 and CC15) (Table 2). CC30, which has been previously associated with IE (Nienaber et al., 2011), and CC398, which has been described as an emerging lineage in IE (Tristan et al., 2012), accounted for only 6.9% (n = 5) and 8.3% (n = 6) of IE isolates, respectively. None of the CCs was specifically associated with either IE or bacteremia, except CC15, which, although infrequent, was slightly more prevalent in IE than in bacteremia (15.3% vs. 3.8%, respectively) (P = 0.04, two-tailed Fisher's exact test) (Table 2).

3.3. Univariate analysis and principal component analysis do not discriminate IE isolates from bacteremia isolates

In a first step of assessing whether specific virulence factors could be associated with IE, univariate analysis was performed from 138 markers detected by DNA microarray data (see text in Section 2.2). Known virulence factors encoding genes, such as adhesins, enterotoxins, exfoliatins

Table 2
Distribution of clonal complexes (CC) in the two populations.

Clonal complex (CC)	IE (%) n = 72	Bacteremia (%) n = 54	P[a]
CC5	13 (18.1)	12 (22.2)	0.65
CC8	7 (9.7)	4 (7.4)	0.76
CC15	11 (15.3)	2 (3.8)	*0.04*
CC30	5 (6.9)	7 (13)	0.36
CC45	10 (13.9)	5 (9.3)	0.58
CC398	6 (8.3)	2 (3.8)	0.46
Others[b]	18 (25)	22 (40.7)	0.08

Abbreviations: IE, infective endocarditis.
[a] Statistical association was estimated using Fisher's exact test.
[b] CC1, CC7, CC9, CC12, CC22, CC25, CC59, CC88, CC97, CC101, CC121, CC152, CC182, CC188.

Table 3
Frequency of the genes detected by DNA microarray in *S. aureus* IE and bacteremia isolates.

Gene or allele	IE isolates (%) n = 72	Bacteremia isolates (%) n = 54	P-value[a]
Adhesin encoding genes			
fnbA	72 (100)	54 (100)	1.000
fnbB	65 (90.3)	46 (85.2)	0.416
clfA	72 (100)	54 (100)	1.000
clfB	72 (100)	54 (100)	1.000
cna [b]	32 (44.4)	26 (48.1)	0.718
spa	72 (100)	54 (100)	1.000
sdrC	72 (100)	53 (98.1)	0.432
sdrD	56 (77.8)	43 (79.6)	0.834
bbp	66 (91.7)	49 (90.7)	1.000
ebpS	72 (100)	54 (100)	1.000
map/eap	71 (98.6)	48 (88.9)	0.042
Toxin encoding genes			
eta	1 (1.4)	1 (1.9)	1.000
etb	0 (0)	1 (1.9)	0.433
tst1	7 (9.7)	8 (14.8)	0.416
sea	12 (16.7)	5 (9.3)	0.301
seb	6 (8.3)	8 (14.8)	0.272
sec	10 (13.9)	2 (3.7)	0.070
sed	7 (9.7)	5 (9.3)	1.000
see	0 (0)	0 (0)	1.000
seg	34 (47.2)	35 (64.8)	0.074
seh	2 (3.2)	4 (7.4)	0.400
sei	34 (47.2)	35 (64.8)	0.072
sej	7 (9.7)	5 (9.3)	1.000
*luk*SF-PV	0 (0)	4 (7.4)	0.031
hla [c]	71 (98.6)	49 (90.7)	1.000
hlb^{123} [d]	48 (66.7)	44 (81.5)	0.074
func_hlb [e,f]	9 (12.5)	9 (16.7)	0.601
Other putative virulence factors encoding genes			
icaA	72 (100)	54 (100)	1.000
chp [g]	46 (63.9)	32 (59.3)	0.453
Regulation encoding genes			
agrI	33 (45.8)	22 (40.7)	0.587
agrII	28 (38.9)	17 (31.5)	0.446
agrIII	8 (11.1)	11 (20.4)	0.213
agrIV	3 (4.2)	4 (7.4)	0.462

[a] P-values are calculated for each gene or allele with a two-tailed Fisher's exact test. Bonferronni correction was applied (significant p-value ≤ 0.002).
[b] 2 isolates (1IE, 1 Ba), [c] 5 isolates (1 IE, 4 Ba), [f] 12 isolates (6 IE, 6 Ba) and [g] 3 isolates (3 IE) were excluded because of ambiguous results.
[d] *hlb*^{123} represents *hlb* encoding gene alleles detected by one of the probes 1,2,3.
[e] *func_hlb* accounts for the *hlb* functional encoding gene.

and superantigens were analyzed but none of these markers was significantly associated with IE in univariate analysis with Bonferroni correction (Table 3). To explore the possibility that multiple genetic markers contribute to the discrimination between IE and bacteremia isolates, principal component analysis was performed on microarray data of the 126 strains. Projection on the first factorial plan of the IE and bacteremia groups did not lead to any obvious clustering (Fig. 1), confirming the close genetic relationship between IE and bacteremia strains. All in all, univariate and PCA statistical methods did not evidence clear genetic distinctions between IE and bacteremia strains.

3.4. Combining genotypic markers discriminates IE from bacteremia isolates

We used the multivariate statistical method DAPC to generate a discriminant function allowing the discrimination between several populations (Jombart et al., 2010). Cross-validation showed an optimum number of 30 PCs (93.7% of the total variance), and subsequent DAPC was therefore performed with 30 PCs. The distribution of the DF scores for IE and bacteremia isolates is shown in Fig. 2.

The DAPC model based on these 30 PCs yielded a reassignment success rate of 80.6% and 68.5% for IE (58/72) and bacteremia isolates (38/54), respectively (C-statistic 0.83, $P < 0.001$). The variability of the assignment success rates between CC groups was very low (CC5, 72.0%; CC8, 81.8%; CC15, 76.9%; CC30, 66.7%; CC45, 73.3%; all contingency χ^2, $P > 0.64$), suggesting that the CC groups did not affect the power of the DF. Nearly half of IE isolates (n = 33/72, 45.8%) were assigned to the IE group with a high membership probability (>0.80) and few IE isolates (n = 7/72) showed an individual membership probability below 0.40 (Fig A.2A).

3.5. Validation of the statistical model on an independent collection

The robustness of the statistical model was tested on the independent set of 81 bacteremia and 66 definite-IE isolates (see Section 2.1). The assignment success rate of IE isolates remained as high as 78.8% (52/66 IE isolates) whereas it decreased to 44.4% for bacteremia isolates (36/81 bacteremia isolates) (C-statistic 0.65, $P < 0.01$) (Fig A.2B), probably due, at least in part, to the fact that IE had not been strictly ruled out by systematic TTE/TEE in this collection of bacteremia cases, contrary to the VIRSTA collection (Grundmann et al., 2010). Plus, the proportion of IE isolates assigned with a high membership probability (>0.80) was similar in the training and test sets (43.9% vs. 45.8%) (Fig A.2B), corroborating the robustness of this combination of markers to distinguish IE from bacteremia isolates.

3.6. An eight-marker linear model retains good performance in discriminating IE isolates

The DAPC model, which allows the determination of the markers most contributing to the DF (Fig. 3), revealed that a subset of eight markers, namely, *erm*A, *sea*, *seb*, *sec*, *sel*, *setC*/*selx*, *hlb*^{123} and Q2YUB3, could be retained in a simplified model. Of note, the *erm*A product confers resistance to the macrolide antibiotic family; *sea*, *seb*, *sec* and *sel* encode enterotoxins A, B, C and L, respectively; *setC*/*selx* encodes a superantigen-like protein; Q2YUB3 encodes a putative efflux protein; and *hlb*^{123} represents three alleles of the β-hemolysin-encoding gene detected by DNA microarray.

The analysis performed on this limited number of markers, whose normalized weights are given in Table 4, exhibited a reassignment success rate of IE isolates as high as 86.1% (62/72) in the training set and 81.8% (54/66) in the test set.

4. Discussion

Understanding the pathophysiology of *S. aureus* IE and the participation of both the host and the bacteria is a major challenge in the field of infectious diseases. The previous identification of several bacterial phenotypes associated with IE in in vitro studies or in animal models using isogenic strains (Salgado-Pabón et al., 2013) (Que, 2005) (Piroth et al., 2008) had raised the hope of determining phenotypic characteristics contributing to IE occurrence. In our collection of well-defined native-valve IE and bacteremia isolates with both temporal and geographical matching, possible discrimination based on phenotypic characteristics or single genetic features was excluded. However, we showed that the genetic profile of the causative strain obtained by DNA microarray, allowed the discrimination of *S. aureus* IE from *S. aureus* bacteremia isolates.

Our results showed no significant difference between IE and bacteremia isolates regarding bacterial phenotypes like adherence to extracellular matrix components (fibrinogen, fibronectin), adhesion and internalization into endothelial cells, biofilm production or susceptibility to microbicidal peptide hNP-1. Furthermore, we tested phenotypes that are likely to be involved in IE but that they had never been explored before on a large series of clinical isolates. For instance, given the evidence that enterotoxin C could play a role in IE (Salgado-Pabón et al., 2013), we tested the superantigenic activity of our strains. Since staphylokinase production activates plasminogen into plasmin, which in turn degrades fibrin clots, we hypothesized that its production may interfere with the platelet-bacteria thrombi at IE initiation. However, neither CD69-cell activation reflecting the superantigenic properties of *S. aureus* strains nor staphylokinase production led to any significant

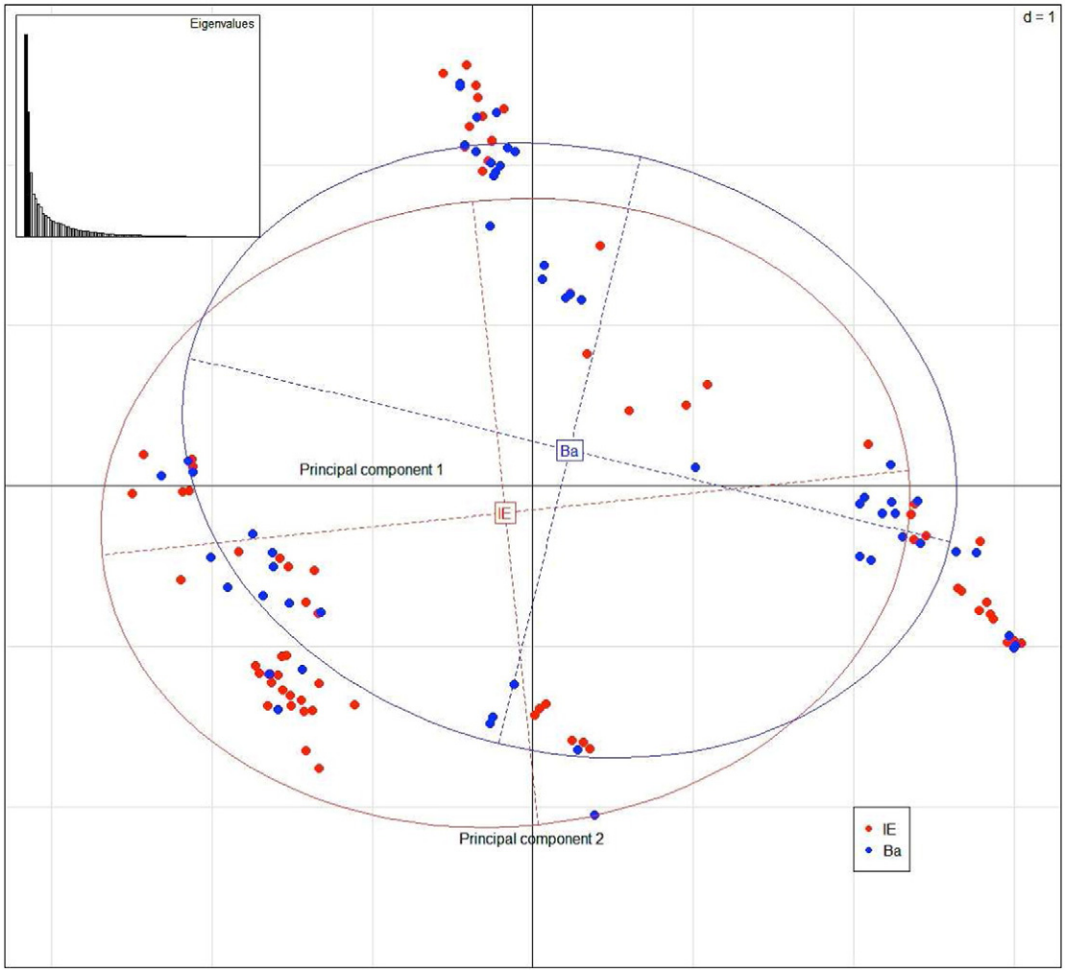

Fig. 1. First factorial plan of principal component analysis (PCA) of the DNA microarray data of the VIRSTA isolates. Each strain was plotted on the two first principal components. Each IE (IE) and bacteremia (Ba) strain is represented by a blue and red dot, respectively; the centroid and variability of IE and bacteremia groups are displayed by blue and red ellipses, respectively.

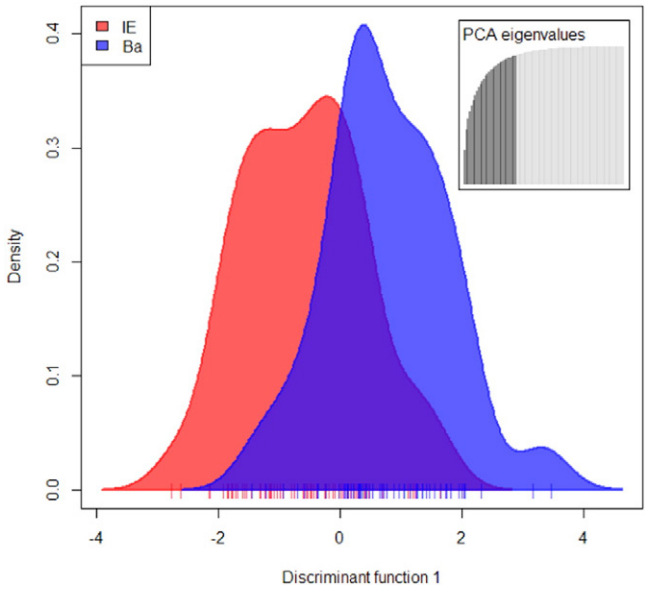

Fig. 2. Distribution of VIRSTA isolates according to their individual DF scores. IE (red) and bacteremia (blue) isolates are plotted according to their DF scores (X-axis); the density of isolates with the same DF score is represented on the Y-axis.

difference between IE and bacteremia strains. Taken together, our data rule out the possibility of a single specific bacterial phenotype responsible for the occurrence of IE in the course of *S. aureus* bacteremia. These results are in line with previous studies that either reported no significant differences between IE isolates and non-IE isolates comparing their ability to adhere or to be internalized into endothelial cells or to produce biofilm (Seidl et al., 2011; Xiong et al., 2009), either led to conflicting results in clinical strain studies (Xiong et al., 2009; Ythier et al., 2010). Importantly, our results do not preclude the significance of certain phenotypes that have been clearly individually established in vitro or in animal models under experimental conditions optimizing the function of specific determinants (i.e., heterologous expression in *Lactococcus lactis*) (Que, 2005).

Considering the isolates' genetic background, univariate analysis did not reveal any specific MLST CC or genotypic markers associated with IE isolates compared with strictly defined bacteremia isolates (Tables 2 & 3). These findings are consistent with a study by Tristan et al. comparing IE and bacteremia isolates (Tristan et al., 2012). Of note, in works by Nethercott et al., CC12 and CC20 were associated with IE isolates compared with bacteremia and skin and soft tissue infections (SSTI)[12] isolates (Nethercott et al., 2013). Similarly, CC30 in Nienaber's study, and several adhesins and enterotoxins in Rasmussen's study were associated with IE compared with skin and soft-tissue infection isolates and nasal carriage isolates, respectively, but not with bacteremia isolates (Nienaber et al., 2011; Rasmussen et al., 2013). Given the comparator groups of these studies, we hypothesize that these data reveal the role

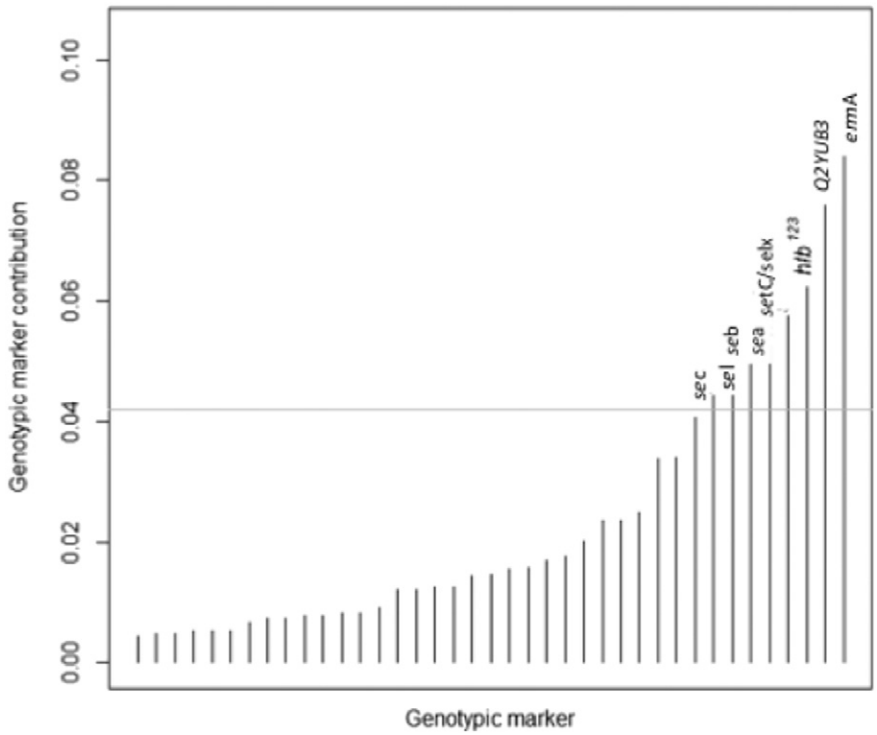

Fig. 3. Contribution of each genotypic marker to the DF. Upon visual inspection of the plot of marker contributions, an inflection point (horizontal line) located after the eight most contributing markers was identified, suggesting that markers with a lower contribution could be of little benefit to the performance of the model.

of these markers in the invasion step, rather than in the cardiac localization. Taken together, our results indicate that no single specific bacterial phenotype or genotypic marker seems to be individually responsible for the occurrence of IE in the course of *S. aureus* bacteremia.

Turning to a more powerful multivariate model using DNA microarray assay data, we showed that DAPC was able to discriminate IE from bacteremia isolates with robust performance, even when applied to an independent collection. Finally, a simple linear DF based on a subset of 8 markers showed valuable performance both in the study and in independent validation collection. This finding clearly uncovers subtle genetic differences between IE and bacteremia isolates. This is in line with complex eukaryotic phenotypes, which are presumed to result from the additive effects and interactions among multiple causative alleles, for which individual effect varies from imperceptible to significant, rather than to monogenic traits (Marian, 2012). Of note, five of the eight markers retained in the final model are superantigens or superantigen-like proteins (Fig. 3), some of them being positively associated with IE, others negatively (Table 4), which may explain that the overall superantigen activity did not differ between IE and bacteremia isolates (Table 1 and Fig A.1). Moreover, the functional role of *erm*A, a macrolide-resistance gene, or Q2YUB3, a putative efflux protein, in causing IE remains elusive. These associations may well be surrogate markers of genome rearrangements affecting, for instance, core-genome expression or causing epigenetic alterations (Borrell and Gagneux, 2011), rather than causative elements.

To conclude, we here provide the proof of concept that IE and bacteremia isolates are distinct based on subtle genetic differences. This drives forward the understanding of *S. aureus* IE and may open up to promising use of such combinations of genetic markers to predict the risk for IE.

Table 4
Predictive model for infective endocarditis based on eight genetic markers of *Staphylococcus aureus*.

Marker	Weight [a]
setC/selx	1.000
sea	0.620
seb	−0.510
hlb^{123}	−0.463
Q2YUB3	−0.460
*erm*A	0.442
sec	0.412
sel	0.412
Constant	−0.527

[a] Presence or absence of the genetic markers coded as 1 or 0, respectively. *S. aureus* isolates with a weight sum of >0 are assigned to the infective endocarditis group.

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Potential conflicts of interest

None of the other authors have any conflict of interest to report.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.meegid.2015.08.029.

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