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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