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Differing lifestyles of *Staphylococcus epidermidis* as revealed through Bayesian clustering of multilocus sequence types

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ABSTRACT

Staphylococcus epidermidis is part of the normal bacterial flora of human skin and a leading cause of infections associated with indwelling medical devices. Previous phylogenetic analyses of subgenomic data have been unable to distinguish between *S. epidermidis* strains with nosocomial or commensal lifestyles, despite the identification of specific phenotypes and accessory genes that may contribute to such lifestyles. To attempt to better define the population structure of this species, the international *S. epidermidis* multilocus sequence typing database was analyzed with the Bayesian clustering programs STRUCTURE and BAPS. A total of six genetic clusters (GCs) were identified. A local population of *S. epidermidis* from clinical specimens was classified according to these six GCs, and further characterized for antibiotic susceptibilities, biofilm, and various genetic markers. GC5 was abundant and significantly enriched for isolates that were resistant to four classes of antibiotics, high biofilm production, and positive for the virulence markers *icaA*, IS*256*, and *sesD*/*bhp*, indicating its potential clinical relevance. In contrast, GC2 was rare and contained the only isolates positive for the putative commensal marker, *fdh*. GC1 and GC6 were abundant but not significantly associated with any of the examined characteristics, except for *sesF*/*aap* and GC6. GC3 was rare and identified as a potential genetic sink that received, but did not donate, core genetic material from other GCs. In conclusion, population genetics analyses were essential for identifying clusters of strains that may differ in their adaptation to nosocomial or commensal lifestyles. These results provide a new, population genetics framework for studying *S. epidermidis*.

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1. Introduction

Staphylococcus epidermidis is a ubiquitous resident of the human skin. However, over the past 30 years, this species has emerged as an important opportunistic pathogen that causes infections associated with indwelling medical devices such as intravenous catheters and prosthetic heart valves (Fang et al., 1993; Richet et al., 1990; Zandri et al., 2012). *S. epidermidis*, along with other coagulase-negative staphylococci, is now a leading cause of nosocomial bloodstream infections (Luzzaro et al., 2011). In neonates and immunocompromised patients, *S. epidermidis* is more prevalent as a cause of infection than its more pathogenic relative, *S. aureus* (Villari et al., 2000).

S. epidermidis virulence factors have been identified through comparisons of nosocomial isolates with isolates colonizing the skin of healthy individuals in the community and healthy individuals with varying degrees of hospital contact (Kozitskaya et al., 2005; Rohde et al., 2004; Rolo et al., 2012). Some *S. epidermidis* isolates appear to be high biofilm producers and, hence, may better persist on catheter tips and other indwelling medical devices (Gill et al., 2005; Mekni et al., 2012). Many *S. epidermidis* virulence factors are involved in biofilm formation, such as the production of the polysaccharide intercellular adhesin encoded by the *ica* locus (Heilmann et al., 1996), and the accumulation-associated protein encoded by *sesF*/*aap* (Rohde et al., 2005). In addition, IS*256* has been associated with isolates from nosocomial infections (Gu et al., 2005; Kozitskaya et al., 2004; Yao et al., 2005), and *fdh*, which encodes a formate dehydrogenase, has been associated with commensal isolates (Conlan et al., 2012).

While *S. epidermidis* may not carry a wide array of virulence factors, the species has become increasingly antibiotic-resistant; up to 50% of isolates were multidrug-resistant in some studies (Mendes et al., 2012). Oxacillin resistance has been reported in up to 70% of nosocomial isolates (Flamm et al., 2013; Mendes et al., 2012). Indeed, Rolo et al. (2012) have noted that *S. epidermidis* isolates from the hospital environment more frequently carry an SCC*mec* genetic element, which harbors the *mecA* gene that is responsible for broad β-lactam resistance.

The population structure of *S. epidermidis* has not been fully revealed. eBURST analysis of multilocus sequence typing (MLST) data places the majority of *S. epidermidis* sequence types (STs) into one

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clonal complex (Miragaia et al., 2007; Wisplinghoff et al., 2003; Wong et al., 2010), which confounds attempts to identify lineages that might be associated with more or less virulent lifestyles. Lineages that are enriched for isolates with virulence or commensal markers have not been identified by earlier studies that have used less portable, but potentially more discriminatory, strain typing tools (Begović et al., 2013; Kozitskaya et al., 2005; Rohde et al., 2004). Some data support the hypothesis that relatively high rates of recombination occur between strains of *S. epidermidis* (Kozitskaya et al., 2005; Miragaia et al., 2007), which may impede the differentiation of lineages, but this hypothesis requires further testing with data that is more powerful than MLST data (Zhang et al., 2012).

Difficulties in defining population structure for *S. epidermidis* may arise from an insufficient number of informative polymorphisms and the confounding effects of recombination, but there is also the broader challenge of defining a bacterial "population" (Robinson et al., 2011). Multiple definitions of a population have been proposed for non-bacterial species (Waples and Gaggiotti, 2006). For bacterial species with relatively low rates of recombination, populations may be defined by their clonal lineages (Rannala et al., 2000). However, for species such as *S. epidermidis* that may be more recombinant, Bayesian model-based clustering may be used to define populations. The Bayesian clustering programs STRUCTURE and BAPS have been applied to study the population structures of a diverse array of bacterial species such as *Burkholderia pseudomallei*, *Chlamydia trachomatis*, *Enterococcus faecium*, *Escherichia coli*, *Helicobacter pylori*, *Salmonella enterica* and *Streptococcus pneumoniae* (Dale et al., 2011; Didelot et al., 2011; Falush et al., 2003b; Gordon et al., 2008; Hanage et al., 2009; Joseph et al., 2012; Willems et al., 2012). In both programs, the strain composition of *K* clusters, where *K* is defined *a priori,* is determined essentially by either minimizing the within-cluster genetic diversity or maximizing the between-cluster genetic diversity, while allowing for admixture to occur between clusters. Here, both STRUCTURE and BAPS were used to elucidate the population structure of *S. epidermidis*, at global and local scales. In addition, the distribution of antibiotic susceptibilities, biofilm, and a variety of genetic markers was examined, which leads to a new understanding of *S. epidermidis* population structure and how it may be related to the bacteria's lifestyle.

2. Materials and methods

2.1. Bacterial isolates

The isolate collection used in this study was described previously by Smyth et al. (2011). Briefly, for a 6-month period between January and June 2007, all staphylococcal isolates from clinical specimens were collected on a weekly basis from the Microbiology Laboratory of Westchester Medical Center (WMC) in Valhalla, NY, USA. The 129 *S. epidermidis* isolates used here were previously typed by MLST (Zhang et al., 2012), according to published methods for *S. epidermidis* (Thomas et al., 2007). Bacteria were grown overnight on tryptic soy agar plates at 37 °C. Isolates were stored long-term at −80 °C in tryptic soy broth (TSB) with 15% glycerol (v/v). Bacterial genomic DNA was extracted with a DNeasy kit (Qiagen), as per the manufacturer's instructions. Characteristics of all study isolates are listed in Supplemental Table 1.

2.2. Antibiotic susceptibilities

Susceptibilities to clindamycin, erythromycin, gentamicin, oxacillin and trimethoprim–sulfamethoxazole (TMP–SMX) were determined at the time of isolation by MicroScan Gram-positive MIC susceptibility panel and a MicroScan WalkAway system (Dade Behring, Inc.), or by broth microdilution. All susceptibilities were checked by disk diffusion tests for this study, with the exception of oxacillin that had been checked previously (Smyth et al., 2011). Susceptibilities were interpreted according to the Clinical and Laboratory Standards Institute (CLSI, 2007). To detect inducible clindamycin resistance, "*D*-tests" were performed by placing an erythromycin disk 15 mm from the clindamycin disk. A positive *D*-test was indicated by a flattening of the zone of inhibition bordering the erythromycin disk (CLSI, 2007). Isolates positive for inducible clindamycin resistance were considered to be resistant for the purposes of analysis.

2.3. Biofilm assay

The degree of biofilm formation of each *S. epidermidis* isolate was determined using the method of Christensen et al. (1985) with the modifications of O'Neill et al. (2007). In brief, isolates were randomly assigned to Nunclon™ ΔSurface, flat-bottomed, 96-well polystyrene plates and grown in brain heart infusion (BHI) medium at 37 °C. Optical density was measured at 600 nm after 24 h growth. Plates were rinsed with sterile distilled water four times. Plates were subsequently dried at 60 °C for 1 h, stained with a 0.4% crystal violet solution, and optical density at 492 nm was determined with a Bio-Rad xMark™ microplate spectrophotometer. *S. epidermidis* strain RP62a was a positive control and sterile BHI medium was a negative control on each plate. Three technical replicates were performed for each isolate per plate, and three biological replicates for each isolate were performed on separate plates to provide the average optical density for each isolate (A_{OD}). Due to the negative correlation between biofilm production ($A_{OD}492$) and growth ($A_{OD}600$), as described in Results Section 3.6, biofilm values for each isolate were first normalized to their respective growth values, before being normalized to the positive control for each plate. Biofilm values were then averaged across the three biological replicates. High biofilm production was defined as an $A_{OD}492$ greater than the median $A_{OD}492$ for all isolates.

2.4. Detection of various genetic markers

Isolates had been screened previously by PCR for the presence of the arginine catabolic mobile element (ACME) (Zhang et al., 2012). Here, all isolates were screened by PCR for the presence of the virulence markers *icaA*, IS*256* and *mecA*, as well as the surface protein-encoding genes *sesA-I*, and the putative commensal marker *fdh*. The genes *sesD* and *sesF* are also known as *bhp* and *aap*, respectively (Bowden et al., 2005; Gill et al., 2005). PCR was repeated for all isolates where a gene was variably present within an ST, and where results for three or fewer STs differed from the remainder of their genetic cluster. Primers and PCR conditions are listed in Supplemental Table 2.

2.5. Bayesian and non-Bayesian clustering

Allelic and sequence data for all 437 sequence types (STs) from the international MLST database as of 29th June 2012 were assigned to clonal complexes using eBURST (Feil et al., 2004) and goeBURST (Francisco et al., 2009) and to genetic clusters (GCs) using the Bayesian clustering programs STRUCTURE v2.3.3 (Falush et al., 2003a) and BAPS v5.4 (Corander et al., 2008). For the two BURST analyses, which were based on allelic data, all STs in a clonal complex differed by no more than one allele from at least one other ST in the clonal complex.

Sequence data were analyzed with STRUCTURE using the admixture model with correlated allele frequencies. Each run consisted of a Monte Carlo Markov Chain (MCMC) of 200,000

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