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Genetic assessment of *Staphylococcus aureus* in an underreported locality: Ambulatory care clinic

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ABSTRACT

Background: Staphylococcus aureus has strong association with anthropogenic environments. This association has not been well supported by use of genetic tools. The aim of this study was to phylogenetically relate numerous isolates from three environments — NCBI samples from hospitals, a community, and a previously unexplored healthcare environment: an ambulatory care clinic (ACC).
Methods: This study incorporated hospital samples from NCBI, a community database from the University of Central Florida (UCF), and newly added samples taken from employees of an ambulatory care clinic located at UCF. Samples were collected from nasal swabs of employees, and positive samples were cultured, extracted, and sequenced at seven MLST loci and one virulence locus (spa). MLST sequences were used in eBURST and TCS population structure analyses and all sequences were incorporated into a phylogenetic reconstruction of relationships.
Results: A total of 185 samples were incorporated in this study (15 NCBI sequences from hospital infections, 29 from the ACC, and 141 from the community). In both phylogenetic and population genetics analyses, samples proved to be panmixic, with samples not segregating monophyletically based on sample origin.
Conclusion: Samples isolated from ambulatory care clinics are not significantly differentiated from either community or hospital samples at the representative loci chosen. These results strengthen previous conclusions that *S. aureus* may exhibit high genetic similarity across anthropogenic environments.

Introduction

Staphylococcus aureus is a widespread human pathogen of high concern to human health globally [1]. This pathogen is most often associated with healthcare settings [2]. In addition, *S. aureus* is commonly found community sampling, where asymptomatic carriage is associated with eventual infection [3]. *S. aureus* literature delineates strains into one of two categories: hospital-acquired (HA) strains, and strains that are community acquired (CA) [4,5,6]. Phenotypic variation in pathogenesis factors of strains and the symptoms of resulting infections are suggestive of such categories [7]. Despite a strong historic focus on hospital samples, there is growing interest into the epidemiological consequences of community carriage, emphasizing the HA/CA divide [8,9,10].

Abbreviations: ACC, ambulatory care center; CA, community acquired; HA, hospital acquired; BF, Bayes Factor; MLST, multi locus sequence type; PFGE, pulse field gel electrophoresis; ST, sequence type.

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Table 1
Demographic information of participants (a) and positive donors (b) by race. Asterisks denote positive numbers that were significantly different than expected ($p \leq 0.05$).

a)

Racial categories	Females	Males	Total	Percentage
White	49	25	74	52.48
Asian Pacific Islanders	8	3	11	7.80
Black or African American	22	8	30	21.28
American Indian or Alaskan Native	0	0	0	0.00
More than one race	6	1	7	4.96
Unknown	16	3	19	13.48
Total of all subjects	101	40	141	100.00

b)

Racial categories	Females	Males	Total	Percentage
White	13	6	19	65.5
Asian Pacific Islanders	4*	0*	4	13.7
Black or African American	1*	2	3	10.3
American Indian or Alaskan Native	0	0	0	0.00
More than one race	0*	0	0	0
Unknown	0*	3*	3	10.3
Total of all subjects	18	11	29	100.00

Systematic investigations into the genetics of hospital and community isolates of *S. aureus* have failed to recover evidence to differentiate these categories [11]. Though phylogenetic investigations suggest little divergence across healthcare environments, protein expression does vary across [12,13]. Furthermore, the loci coding for certain proteins are entirely present or absent in the genomes of strains associated with one environment or the other [7]. In order to address this disconnect, further genetic investigations of *S. aureus* are necessary. Increased taxon sampling, especially from varied origins, is beneficial in refining the results of investigations of relationships between sampled organisms and may serve to resolve conflicting conclusions [14]. As the current nomenclature of *S. aureus* relationships is centered on the locality of strain isolation, additional sampling schemes encompassing novel localities may serve to clarify evolutionary relationships.

Classically, samples of *S. aureus* representing the 'clinical' group have been drawn from hospital settings [15]. Medical clinics, also known as ambulatory care centers (ACCs), are facilities that share similarities between both traditional hospital settings as well as nonclinical, community environments [16]. Little research has been conducted into pathogen assemblage within ACCs. This is especially relevant in the case of *S. aureus*, given importance of origin of isolation for categorization of isolates. Epidemiological studies into *S. aureus* within ACCs have been performed, though no phylogenetic information incorporating these centers is available [17]. Ambulatory care clinics may act as interfaces between hospitals and communities, as it has been previously demonstrated that medical attendants (such as those operating in ACCs) can bridge *S. aureus* outbreaks between distinct environments [18]. Studies that include *S. aureus* isolates from ACCs will explore previously uninvestigated environment, as well as aid in resolving confusion between HA and CA nomenclature and *S. aureus* genetics overall.

Staphylococcus aureus population structure and systematics has an extensive history. Pulse field gel electrophoresis (PFGE) results in the highest discriminatory power between *S. aureus* strains, but cannot be reliably reproduced between laboratories and across studies [19,4]. Conversely, Multi Locus Sequence Typing (MLST) produces standardized results, but a reliance on slowly evolving housekeeping genes limits discriminatory power at local spatial scales [20–24]. Attempts to find a standardized method with a high discriminatory power have incorporated virulence factors such as Staphylococcal protein A (spa) and clumping factors (clf) typing [24,25]. While *spa* typing and MLST have been used in the thorough investigation of *S. aureus* population structure within the community and hospitals [24,21], no investigation has yet sought to leverage these tools in exploring novel environments such as ACCs.

Here, we have performed an evolutionary analysis of seven MLST gene fragments and *spa*, incorporating samples taken from a representative example of previously uninvestigated ACC, with the aim of better understanding how population structure of *S. aureus* varies across environments. Isolates were taken from employees of the University of Central Florida's Health Center, a representative ACC. This sample site was additionally attractive as it was correlated to a previous community cohort, allowing us to eliminate the effects of geographic distance, which can influence the resulting phylogeny [25,10]. We confirm that neither HA or CA strains demonstrate monophyly, while additionally showing ambulatory care centers contain an admixture of samples associated with both other sources.

Material and methods

Ethics statement

Samples were collected from willing donors, under the guidance of University of Central Florida's Institutional Review Board (IRB) approved procedures. Written, informed consent was acquired from all donors prior to sampling. All investigators involved in sample collection were properly instructed and granted Collaborative Institutional Training Initiative (CITI) certification.

Bacterial isolates

One hundred and forty one healthy employees of the University of Central Florida's Health Center underwent pre-screening for bacterial isolates. Donors' volunteered demographic information is provided in Table 1. Isolates were collected via donor insertion of a cotton swab into both nostrils and circulation for approximately 5–10 s. Of the screened donors, 29 (20.5%) resulted in positive identification of *S. aureus*. Positive samples were correlated with demographic information (Table 1). Swabs were immersed in glycerol-Trypticase™ Soy Broth (TSB) solution during transport, followed by plating on Trypticase™ Soy Agar (TSA) containing 5% sheep's blood (Becton, Dickinson and Company, Franklin Lakes, New Jersey, USA). Isolates were incubated at 37 °C for 16 h. Resultant colonies were tested with Staphyloslide™ Latex Test (Becton, Dickinson and Company, Franklin Lakes, New Jersey, USA) reagent to positively identify cultures as *S. aureus*. Verified *S. aureus* colonies were isolated and inoculated in TSB for an additional 16 h at 37 °C at 250 rpm in preparation for DNA extraction.

DNA extraction and sequence analysis

1.5 mL of each bacterial inoculate was centrifuged at 16,000 g for two minutes. Supernatant was removed, and the remaining pellet was utilized in the extraction protocol. DNA was extracted utilizing GenElute Bacterial Genomic DNA kits (Sigma–Aldrich Co., St. Louis, Missouri), in accordance with manufacturer's instructions. Fragments of seven MLST loci (*arc, aroE, glpF, gmk, pta, tpi, yqiL)* ranging from 402 to 512 base pairs were amplified [21]. Additionally, approximately 500 base pair fragments of *spa* were amplified [24].

Following amplification, PCR products were purified utilizing QiaQuick PCR Purification Kit (QiaGen, Redwood City, Ca). All Sanger Sequencing was performed at University of Arizona's Genetics Core. Forward and reverse reads were visualized with Sequencher 5.1 (Gene Codes Co., Ann Harbor, Michigan). Sequences were organized in MEGA 5.2 and aligned with ClustalW. Sequence Types (STs) were determined for each sample based on alleles identified for each of the seven MLST loci. Alleles were cross-referenced

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