A quantitative assessment of *Staphylococcus aureus* community carriage in Yuma, Arizona

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Disease control relies on pathogen identification and understanding reservoirs. *Staphylococcus aureus* infection prevention is based upon decades of research on colonization and infection, but diminishing returns from mitigation efforts suggest significant knowledge gaps. Existing knowledge and mitigation protocols are founded upon culture-based detection, with almost no information about pathogen quantities. We employed a qPCR assay on samples from three body sites to characterize colonization more comprehensively than previous studies by describing both prevalence and pathogen quantity. We show a much higher overall prevalence (65.9\%) than previously documented, with higher quantities and prevalence associated with the nares, non-Hispanic males (86.9\%), and correlating with colonization in other body sites. These results

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suggest that research and clinical practices likely misclassify over half of colonized persons, limiting mitigation measures and their impact. This work begins the process of rebuilding foundational knowledge of S. aureus carriage with more accurate and wholistic approaches.

Keywords: carriage of S. aureus, Staphylococcus aureus, molecular detection of S. aureus, SaQuant, Culturing S. aureus, Quantitative carriage

INTRODUCTION

Staphylococcus aureus is a human commensal and opportunistic pathogen that causes skin and soft-tissue infection, bone and joint infection, bacterial endocarditis, and bacteremia [1,2]. Increased effort to control S. aureus infections in hospitals has resulted in a decline in cases over the past 15 years [3]. However, progress in the health-care setting has slowed and is out of step with the rise in cases of community-onset bacteremia [3,4]. These patterns suggest an expanding role of the community as a reservoir for S. aureus transmission and persistence [5].

Self-infection is the most common cause of S. aureus infection [6,7]. Carriage is thus not only a marker for prevalence in the community, but also an important risk factor for infection. Estimates of S. aureus carriage vary widely with geographic location, sex, age, and ethnicity. In the United States, carriage is estimated at approximately one-third of the population [8–12]. Carriage is higher in children than in adults, peaking between the ages of six and twelve. Throughout adulthood, carriage is mostly stable, declining slightly with age [8,9,13–15]. Males are more likely to be colonized than females [8,10,12–17]. Ethnicity-based trends are less clear-cut but suggest that Hispanic and non-Hispanic whites have a similar prevalence that is higher than that of non-Hispanic blacks [8,10,17].

S. aureus colonizes numerous body sites, the most common of which are the nares, hand, perineum, and throat [7]. The nares are commonly reported as the most frequent site of colonization and are thought to provide a reservoir for transfer to other sites [1,18–20]. Indeed, decolonization of the nares decreases colonization at other body sites [21]. Nasal carriage is also an important risk factor for infection during surgery [22,23]. Although the importance of nasal carriage is well-documented, an increasing number of studies have examined throat and nasal carriage in parallel and report similar [24] or greater prevalence in the throat [13,25–27], suggesting that the throat may play an under-appreciated role as a body site reservoir.

Given that the quantity of S. aureus among colonized persons has implications for autoinfection and transmission, current binary S. aureus carriage characterization likely provides an incomplete representation of S. aureus burden. Unfortunately, quantitative aspects of carriage at a given body site have not been widely studied. However, a small number of studies have shown that the quantity of S. aureus in the nares is positively associated with postsurgical infection risk [28,29], with higher likelihood of transmission to household members [30], and with greater
dissemination of *S. aureus* into the environment [31]. Relative to males, nasal carriage in women is characterized by smaller *S. aureus* quantities and a higher likelihood of a false-negative carriage classification using conventional culturing methods [32]. Thus, bacterial quantity is an important yet understudied feature of colonization, and combining independent quantitative and culture-based assessments of *S. aureus* should improve the validity of investigations of possible sex-based disparities in nasal carriage and putative sex-based determinants of *S. aureus* transmission [32]. Most quantitative studies for *S. aureus* have been culture-based which does not lend itself well to quantification due to resource needs and limited throughput.

To better understand binary and quantitative community-based carriage, we apply a quantitative PCR assay to samples collected from a large cohort of community members in Yuma, Arizona [33–35]. Based on data collected during the validation of a new highly sensitive and specific qPCR assay for the accurate detection and quantification of *S. aureus* [36], we expected to achieve higher detection sensitivity compared to culture-based detection. We further hypothesized, in line with existing literature, that the nares would be most frequently colonized with the highest colonization loads; that prevalence and quantity would be greater in males compared to females; that prevalence and quantity between Hispanics and non-Hispanics would be similar; and finally, that prevalence and quantity would decrease slightly with age.

**METHODS**

**Study overview**

This study is part of a larger investigation aimed at understanding carriage and transmission within social groups in a population on the US/Mexico border [33–35]. Study staff recruited participants in naturally occurring social groups of two or more persons encountered at public and private locations [34]. After informed consent, staff closely supervised the self-collection of swabs from three body sites: the nares, throat, and palm [34]. Participants additionally filled out a questionnaire which, among other variables, documented demographic information.

Data were collected from 565 participants from March of 2019 through March of 2020. For the analyses within this manuscript, 17 participants were excluded for incomplete data on sex, age, ethnicity, or assay, leaving a complete sample of 548 participants.

Data were collected as a part of project 1116783 approved by the Northern Arizona University Institutional Review Board. Verbal consent or assent was obtained to maintain anonymity of participants.

**Sample collection**

Two double-tipped BBLCultureSwab swabs were used to sample each of the three body sites (each body site was sampled twice). Study staff demonstrated and explained the swabbing
protocol before providing participants with swabs and assisting when necessary. When sampling the nares, participants were instructed to insert the double tip swab into the anterior nares and rotate it as they moved it around, sampling each nostril for ten seconds with the same swab. For the throat, participants were instructed to swab the back of their tongue, mouth, and cheeks for twenty seconds while rotating the swab. For the palm, participants were instructed to rub the swab over the palm of their hand and between their fingers for twenty seconds. During sampling, study staff counted aloud and reminded participants to rotate the swab and cover the entire targeted body site to maintain sample collection consistency across individuals.

**Sample storage, transportation, and culturing**

After collection, swabs were stored and transported on ice to the laboratory where one of the two swabs from each body site was used for culturing. The selected swab was stored for no longer than 24 hours at 4°C before culturing to maximize the likelihood of cell survival [37]. Each such swab was streaked onto CHROMagar *S. aureus* media and incubated for 24 hours at 37°C. Colonies in the pink to mauve color range were considered to be *S. aureus*. One colony from each swab was isolated and sequenced for species verification and all suspected *S. aureus* colonies were stored at -70°C in 20% glycerol. The other swab from each body site, which did not undergo culturing, was stored at -70°C until direct DNA extraction.

**DNA extraction**

DNA was isolated from suspected positive *S. aureus* colonies using the Qiagen DNEasy Blood and Tissue kit with modified lysis for gram positive bacteria. Each sample was streaked for isolation and a lawn from a single colony was grown for extraction. The remaining swab samples (one from each body site) that were not used for culturing were extracted on a Thermofisher KingFisher Flex instrument using an Applied Biosystems MagMAX™ DNA Multi-Sample Ultra 2.0 Kit with a final elution volume of 50μL.

**QPCR assay**

The SaQuant qPCR assay [36] was run on 1μL aliquots of DNA extract, using Applied Biosystems QuantStudio 7 and 12 real-time PCR machines following the published protocol. Standard curves for quantification were constructed using a minimum of five 10-fold serial dilutions, and at least one standard curve was included in each qPCR run. A quantification value for each sample was generated from its associated standard curve(s) using the QuantStudio software. Data are presented as genome equivalents per reaction.

**Data analyses**

Statistical analyses and data visualization was carried out using R version 1.3.1093 [38]. Violin plots, pie charts, and Venn diagrams were generated using the data visualization package ggplot2 version 3.3.3 [39]. Mann-Whitney tests were conducted to determine significant differences.
between *S. aureus* quantities across dichotomous independent variables (culture detection, sex, ethnicity). Because of correlated data, the Friedman test was used to detect differences in quantity between the three body sites (nares, throat, and palm), with post-hoc Nemenyi pairwise comparisons. Two-proportion z-tests were used to determine statistically significant differences between prevalence among sexes (males and females) and ethnicities (Non-Hispanic and Hispanic). Chi-squared tests were utilized to determine statistically significant differences in prevalence across age classes, while the Kruskal-Wallis rank sum test with post-hoc Mann-Whitney tests, if warranted, were used to compare quantities across age classes. Linear regression models were generated to determine potential associations between colonization and *S. aureus* quantities at one body site with another. Tests yielding a two-tailed p-value ≤ 0.05 were considered statistically significant.

**RESULTS**

**Study participants**

The 548 participants had an average age of 32.1 years. There were 96 participants under the age of 18. Most participants (50.5%) were married, a slight majority (54.9%) were female, and most (72.8%) reported Hispanic ethnicity. Sociodemographic characteristics of participants are included in Supplemental Table 1.

**Comparison of *S. Aureus* detection methods:**

For each body site, we compared culture and qPCR for detection of *S. aureus*. Culturing was not specific for detecting *S. aureus*, resulting in false positives that were excluded after whole genome sequence comparisons. Culturing was also not as sensitive as SaQuant, resulting in 297 out of 608 positive samples (48.8%) detected via culture compared to 553/608 (91.0%) detected by SaQuant (Fig 1A). The SaQuant assay is new with impressive sensitivity and specificity values calculated by running the assay against 533 *S. aureus* isolates and 10 non-*aureus* *Staphylococcus* isolates as well as in-silico against 1,818 *S. aureus* genomes and 1,834 non-*aureus* *Staphylococcus* genomes [36]. However, as this assay had not been assessed outside the initial validation study, we further evaluated the sensitivity and specificity with these data.

Across the three body sites, there were 55 culture positive but PCR negative samples. As only a single replicate of each sample was tested against SaQuant, we expected samples with low *S. aureus* quantities to be stochastically detected, leading to a small proportion of false negatives. As we had culture and whole genome sequences from the paired swab for these 55 samples, we were able to confirm a 100% sequence match with the primer and probe target sites to show that genomic mutations are not responsible for the lack of amplification. Low quantities of *S. aureus* on a sample swab are also likely to account for the 311 samples that were culture negative, but detectable via qPCR as they had a significantly lower quantity of *S. aureus* (Fig 1B).
For assurance that SaQuant was not producing false positive results, we performed amplicon sequencing on samples with the highest amounts of *S. aureus* DNA as detected by the SaQuant assay as these samples were most likely to contain sufficient DNA for sequencing. We targeted 23 *S. aureus* specific amplicons that did not amplify in other *Staphylococcus* species (using *in silico* PCR). The targets were amplified in each of the samples and BLASTn [40] searches of the sequences resulted in hits to *S. aureus* only.

**S. Aureus at different body sites:**

Culture and qPCR detection were used to determine *S. aureus* presence at each sample site (Fig 2A). The SaQuant assay revealed a higher *S. aureus* prevalence than culture, suggesting higher sensitivity. However, culture was able to detect *S. aureus* in some samples that were negative for SaQuant, as well as a subset of samples that did not undergo qPCR testing due to insufficient DNA reserves. Therefore, combining both methods provided the highest level of detection. Overall detection of *S. aureus* was similar at the nares and throat body sites, with respective presence in 233 and 234 of 548 (42.5 and 42.7%) participants compared to only 151/548 (27.6%) for palm samples. Body site results from Fig 2A were used to determine prevalence on a participant basis (Fig 2B). Individuals positive at any site were considered positive for *S. aureus*. The SaQuant assay was more sensitive than culture at all body sites, resulting in a 54.86%, 62.18%, 211.36%, and 63.94% increase in prevalence for the nares, throat, palm, and across individuals, respectively. Combining both methods across three body sites resulted in *S. aureus* prevalence of 65.9% (361/548) of participants. Prevalence results were dissected to indicate the proportion of individuals colonized exclusively at one, two, and three body sites (Fig 2c). Individuals colonized exclusively at a single body site accounted for 49.9% of positive participants (180/361), while 29.1% of positive participants were colonized at two body sites (105/361) and 21.1% (76/361) were colonized at all three sites. A major benefit of a qPCR assay is the ability to quantify the target of interest present in each sample from the nares, throat, and palm (Fig 3). *S. aureus* quantity was significantly different across all three body sites (Friedman test, \( P < 0.001 \)). Nares samples exhibited higher quantities compared to both throat (\( P < 0.001 \)) and palm samples (\( P < 0.001 \)), while throat samples had higher quantities compared to palm samples (\( P < 0.001 \)).

**S. Aureus colonization and sex, ethnicity, and age:**

We evaluated sex and ethnicity-based disparities in colonization and quantity. Males showed significantly higher *S. aureus* prevalence compared to females at all individual body sites, as well as overall colonization prevalence (Fig 4A). Differences in *S. aureus* quantity between males and females at the nares (\( P = 0.094 \)), throat (\( P = 0.695 \)), and palm (\( P = 0.152 \)) were not statistically significant (Fig 4B).
Our data revealed that *S. aureus* colonization was more prevalent for non-Hispanic participants compared to Hispanic participants at all body sites, although the difference was only statistically significant at the nares, throat, and participant level (Fig 5A). qPCR results showed significantly higher quantities for non-Hispanic individuals compared to Hispanic individuals at the nares (*P* = 0.028), but not in the palm or throat samples (Fig 5B).

Lastly, participants were split into three age classes (0-19, 20-49, and ≥50) to compare prevalence and quantity across ages. *S. aureus* prevalence between age classes yielded significant differences between certain classes only for the throat, although the data generally show higher prevalence among participants aged 20-49, and lower prevalence among participants aged 50 or greater (Fig 6A). Similarly, comparison of *S. aureus* quantity between age classes resulted in no significant difference at any body site (Fig 6B).

**Cross-site influences:**

Sampling at multiple sites enabled cross-site comparisons using data obtained from the SaQuant assay. These comparisons reveal that a high *S. aureus* quantity in the nares is associated with concurrent colonization at other sites (Fig 7A). Conversely, throat and palm quantity comparisons yielded no association with the number of sites colonized. There was no significant association between bacterial quantity for any pairwise comparison of body sites (Fig 7B).

**DISCUSSION**

We combined culture and quantitative PCR-based methods on samples from three different body sites to characterize *S. aureus* colonization in a cohort of 548 participants in southwestern Arizona. Overall, >65% of participants were colonized with *S. aureus*. Males and non-Hispanics were more likely to be colonized than females and Hispanics. Our current understanding of *S. aureus* carriage in the US population is largely based on the 2001-2002 National Health and Nutrition Examination Survey (NHANES) which showed an overall prevalence of ~32%, with higher prevalence among males, but no clear difference between Hispanics and non-Hispanics [8–10,17]. Slightly lower nasal prevalence, varying from 12.1% to 29.4%, was documented across 9 European countries, with higher prevalence in males and in younger age groups [15].

Our results are not consistent with other studies, showing a much higher overall prevalence and clear sex and ethnic-based differences. However, both the US and European estimates, as well as those in other studies, were derived from culture-based detection from a single site, the anterior nares. In the present data, if we restrict analyses to results from anterior nares swabs, overall prevalence using only culture for detection (26.3%) is in line with previous estimates. However, prevalence estimates in the nares based on qPCR (40.7%) and a combination of culture and qPCR (42.5%) suggest that these previous studies based only on culture are likely to have lacked sensitivity and underestimated prevalence.
Comparing prevalence across multiple body sites can improve insights into reservoirs and sources for spread to other body sites and other people. The anterior nares are most consistently identified as a site of colonization, providing evidence for the epidemiological importance of this body site. However, studies that consider both anterior nares and throat colonization show similar or even higher prevalence in the throat, suggesting that the throat should not be overlooked as a principal reservoir or dispersion site [13,24–27]. Consistent with these studies, our data show that *S. aureus* was most prevalent in the nares (42.5%) and the throat (42.7%): *S. aureus* on the palm was least prevalent (27.6%), however 30 individuals (8.3%) were colonized exclusively on the palm, suggesting that the role of other unsampled body sites in maintenance and spread should be thoroughly investigated [41].

*S. aureus* quantity at any given site has direct implications on the likelihood of spread and transmission, and the likelihood of autoinfection [30,31], however we know almost nothing about quantitative aspects of community carriage. With one exception [32], the few studies that quantified *S. aureus* colonization relied on culture-based enumeration, but the expense and time-consuming nature of these methods have severely limited such studies. To address this deficit, we utilized SaQuant, a recently developed assay for the detection and quantification of *S. aureus* [36]. A small number of other PCR assays are available for *S. aureus*; however, they are not ideal for detection and quantification [36]. Culture is less sensitive than SaQuant, mostly due to a failure of culture to detect low quantities of *S. aureus*. Our quantitative assessment of colonization reveals some interesting characteristics of carriage in the anterior nares compared to the throat and palm. For example, quantity in the anterior nares is highly variable, with values ranging from less than a single genome equivalent per 1 µL to over 310,386. The average quantity in the nares was significantly greater than the throat, which in turn, was greater than the palm. Unlike the other body sites, the average quantities in the nares were greater in males (compared to females) and non-Hispanics (compared to Hispanics), although the sex-based difference was not statistically significant. Quantities in the nares were also greater when other sites were colonized, however quantities in those other sites were not correlated with nares quantity. These characteristics of nares colonization were not observed for the throat and suggest that the epidemiological role of nares colonization may be significantly distinct from other sites.

Our work has some important limitations. Firstly, culture-based detection methods are varied and culture-based detection sensitivity may have been improved by broth enrichment for example. While we cannot speculate about the comparative sensitivity of broth enrichment and the SaQuant qPCR assay, the value of quantitative data should be considered. Similarly, qPCR-based detection sensitivity would be increased by testing replicates to reduce stochasticity associated with detecting low quantities. Our prevalence values for both culture and qPCR are thus undoubtedly underestimated. Secondly, our sampling was cross-sectional, and thus does not provide any insights into carriage over time, whether low quantities detected at any site indicate colonization (ecological establishment) for any length of time, or relationships between pathogen quantities and likelihood of subsequent infection as has been shown previously [28,42]. Lastly,
unlike other studies [8,9,13,14], we did not observe clear age-based differences in colonization. This may be due to small sample sizes in our age groups, or underestimation of detection in these other studies.

CONCLUSIONS

Effective disease control and prevention is dependent on identifying and understanding pathogen reservoirs. Our prevalence results are much higher than have been documented in a general population [15,19] and likely explained by higher detection sensitivity (assessing 3 body sites and utilizing direct culture in combination with qPCR). The failure of culture-based methods to capture a substantial portion of carriers has been previously suggested [32] and the gains in sensitivity by using PCR and sampling multiple body sites for the detection of MRSA has been demonstrated [43]. Previous works formed the foundation of our current understanding of community carriage, longitudinal carriage within individuals, carriage at different body sites, assessments of infection risk, and infection mitigation practices, but these studies are likely to have lacked sensitivity, underestimated carriage, and need to be reassessed.

Few studies have investigated the epidemiological importance of carriage quantity and this work helps fill this knowledge gap using a new high-throughput quantitative tool. We are aware of only a single study of carriage quantity in a non-clinical population [32]. While providing profound insights, the few previous studies do not provide a comprehensive understanding of the epidemiological role of carriage quantity. This study documents how carriage quantity varies across body sites, sex, and ethnicity in a large non-clinical cohort.

Efforts to mitigate S. aureus infections have been met with diminishing returns. Progress has certainly been made, but perhaps the relatively high remnant infection rates are due to the persistence of small quantities in various body sites that go undetected despite screening and decolonization efforts. Our work demonstrates the importance of sensitive and quantitative detection in understanding carriage towards more effective mitigation.

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FOOTNOTE PAGE

Conflicts of interest

None of the authors report any conflict of interest.

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FIGURE LEGENDS

Fig 1. Comparison of culture and SaQuant for *S. aureus* detection. A) Proportion of samples (n=608) that were positive with only culture, both culture and SaQuant qPCR, and only SaQuant qPCR. A large proportion of samples were exclusively detected by SaQuant while only a few were exclusively picked up by culture. B) Distribution of *S. aureus* quantity in 553 samples positive for the SaQuant assay shows higher quantities in samples that were culture positive compared to culture negative samples. Detection and quantitative comparisons suggest that the SaQuant qPCR assay is more reliable for *S. aureus* detection due to a higher likelihood of detecting low quantities. Differences in *S. aureus* quantities between culture positive and negative samples were statistically significant according to the Mann-Whitney test (*P* < 0.001). *S. aureus* amounts below the limit of detection (LOD) of between 3 and 5 genomic copies can still be detected, albeit at less than a 95% confidence level. Likewise, amounts below the limit of quantification (LOQ) of 8.27 genomic equivalents will be quantified with less accuracy.

Fig 2. Prevalence across sites for 548 participants. A) Consistent with Fig 1, SaQuant shows greater sensitivity in detecting *S. aureus* at each body site. Combining culture and SaQuant results provides the highest detection sensitivity. A subset of samples could not be evaluated with SaQuant, as DNA extraction failed. B) When data are combined across all 3 body sites, detection with SaQuant is greater than for culture, but combining SaQuant with culture provides the greatest overall detection. For culture, combining data across 3 sites results in a prevalence estimation that is slightly greater than most published population accounts. When detection methods and body sites are combined, the overall prevalence is 65.9%. C) The number of individuals with carriage at single or multiple body sites. Individual samples that did not have a pair at the other body sites were excluded.

Fig 3. Quantities at different body sites. Quantities differ at each body site (Friedman test, *P* < 0.001). Specifically, the quantity in nares is greater than the throat and palm (Nemenyi test, *P* < 0.001) and throat quantities are greater compared to the palm (Nemenyi test, *P* < 0.001).

Fig 4. Colonization across sex and body site. A) Prevalence at different body sites for males and females shows a higher prevalence of *S. aureus* in males compared to females at all individual sites as well as on a participant level. Differences in prevalence between males and females were statistically significant according to the two-proportion z-tests (Nares: *Z* = 2.78, *P* = 0.006, Throat: *Z* = 3.39, *P* < 0.001, Palm: *Z* = 3.83, *P* < 0.001, Participant: *Z* = 4.58, *P* < 0.001). B) Differences in quantity between males and females were not statistically significant using the Mann-Whitney test. Quantity plots were made with a subset of the data used for the presence bar plots, as there are samples that were exclusively detected by culture, and hence have no associated quantity value.

Fig 5. Colonization across ethnicity and body site. A) Non-Hispanic individuals had higher prevalence of *S. aureus* colonization compared to Hispanic individuals at the nares, throat, and...
participant level according to the two-proportion z-tests (Nares: $Z = 2.65$, $P = 0.008$, Throat: $Z = 2.60$, $P = 0.009$, Palm: $Z = 1.92$, $P = 0.055$, Participant: $Z = 3.01$, $P = 0.003$) (two-proportion z-test). Colonization was also higher in the palm for non-Hispanic individuals, but this difference was not statistically significant. B) *S. aureus* quantity was significantly higher for non-Hispanic individuals at the Nares body site.

**Fig 6. Prevalence and quantities at different body sites across three age classes.** A) Prevalence patterns across different age groups suggests a lower prevalence in the oldest age group and a higher prevalence in the middle age group, but these differences are only significant at the throat and participant level between age groups 20-49 and $\geq 50$ (Throat: $X^2 = 9.68$, $P = 0.002$, Participant: $X^2 = 4.62$, $P = 0.032$). All other comparisons between age groups 0-19 and 20-49 (Nares: $X^2 = 0$, $P = 1$, Throat: $X^2 = 1.43$, $P = 0.232$, Palm: $X^2 = 1.01$, $P = 0.316$, Participant: $X^2 = 0.50$, $P = 0.478$), 20-49 and $\geq 50$ (Nares: $X^2 = 1.36$, $P = 0.243$, Palm: $X^2 = 0$, $P = 0.967$), as well as 0-19 and $\geq 50$ (Nares: $X^2 = 0.92$, $P = 0.339$, Throat: $X^2 = 2.86$, $P = 0.091$, Palm: $X^2 = 0.30$, $P = 0.584$, Participant: $X^2 = 1.31$, $P = 0.253$), were not significantly different. B) Quantity of *S. aureus* at each body site does not differ according to age group.

**Fig 7. Association of colonization and bacterial quantity at one body site with colonization and bacterial quantity at other body sites.** A) Quantity at each body site when different sites are also colonized. Quantity in nares was significantly higher when other sites were also colonized, however, B) quantities are not associated among sites.
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