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Correlation between Nasal Microbiome Composition and Remote Purulent Skin and Soft Tissue Infections

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The incidence of skin and soft tissue infections (SSTIs) has increased dramatically over the past decade, resulting in significant morbidity in millions of otherwise healthy individuals worldwide. Certain groups, like military personnel, are at increased risk for SSTI development. Although nasal colonization with *Staphylococcus aureus* is an important risk factor for the development of SSTIs, it is not clear why some colonized individuals develop disease while others do not. Recent studies have revealed the importance of microbial diversity in human health. Therefore, we hypothesized that the nasal microbiome may provide valuable insight into SSTI development. To examine this hypothesis, we obtained anterior-naris samples from military trainees with cutaneous abscesses and from asymptomatic (non-SSTI) participants. We also obtained samples from within abscess cavities. Specimens were analyzed by culture, and the microbial community within each sample was characterized using a 16S sequencing-based approach. We collected specimens from 46 non-SSTI participants and from 40 participants with abscesses. We observed a significantly higher abundance of *Proteobacteria* in the anterior nares in non-SSTI participants ($P < 0.0001$) than in participants with abscesses. Additionally, we noted a significant inverse correlation between *Corynebacterium* spp. and *S. aureus* ($P = 0.0001$). The sensitivity of standard microbiological culture for abscesses was 71.4%. These data expand our knowledge of the complexity of the nasal and abscess microbiomes and potentially pave the way for novel therapeutic and prophylactic countermeasures against SSTI.

Staphylococcus aureus is a leading pathogen in both community and hospital settings. Infections with *S. aureus* range from invasive disease such as bacteremia and pneumonia to generally less severe skin and soft tissue infections (SSTIs) (1–3). SSTIs, especially those caused by USA300 methicillin-resistant *Staphylococcus aureus* (MRSA), have emerged as a common, burdensome, and costly disease (4–6). Individuals in congregate settings (e.g., members of athletic teams, prison inmates, and military trainees) are at increased risk for SSTI (7–10).

Given that approximately one-third of people may exhibit *S. aureus* carriage, it is unclear why some develop SSTI while others do not (11). While it is clear that host genetics, immune responses, and strain differences contribute (12–17), there are likely other important factors. As the anterior nares appear to be a critical *S. aureus* reservoir (1, 18, 19) and because antecedent nasal carriage increases the risk for infection (1, 20, 21), a better understanding of nasal microbial ecology may yield valuable clues regarding SSTI susceptibility.

The anterior nares are a dynamic microbial battleground between pathogens and commensals (22, 23). The concept that changes in the host microbiome may influence human health is well documented (24); however, few studies have investigated how the nasal microbiome is altered in response to human disease (25, 26). Importantly, no study has investigated whether the microbial composition in the anterior nares can influence susceptibility to infection. Therefore, we hypothesized that variations in the nasal microbiome might be associated with purulent SSTIs and *S. aureus* colonization. The purpose of this study was to describe the microbial composition of the anterior nares in persons with and without cutaneous abscesses, as well as the microbial composition within cutaneous abscesses.

MATERIALS AND METHODS

Study participants and study design. This observational investigation was conducted from May 2010 to January 2012 in the setting of an ongoing prospective, field-based, cluster-randomized trial aimed at preventing SSTIs (27). Study participants were all male U.S. Army soldiers, ages 17 to 39, who were undergoing infantry training at Fort Benning, GA. The investigation was approved by the Uniformed Services University Infectious Diseases Institutional Review Board.

Enrollment and data collection. We enrolled two groups of participants: trainees with abscesses and asymptomatic (non-SSTI) controls. Trainees who presented to the Troop Medical Clinic (TMC) with cutaneous abscesses that required incision and drainage were eligible to participate as abscess subjects. Trainees who presented with noninfectious complaints (e.g., ankle sprain) were eligible to participate as non-SSTI subjects. After written informed consent was obtained, all participants underwent anterior-naris sampling with two swabs (BD BBL CultureSwabs [BD Diagnostic, Sparks, MD]). One swab was used for microbiological culture, and the other was used for microbiome analysis. Each swab was inserted about one centimeter into one nostril and rubbed in a circu-

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lar/twirling fashion at least three times along the nasal septum and the superior, lateral, and inferior surfaces of the naris. The same swab was then used to sample the other nostril following the same procedure. For cutaneous abscesses, after skin was prepared with chlorhexidine, incision and drainage were performed in a standard fashion by TMC health care personnel. Two swabs were obtained from within the abscess cavity. One specimen was sent for standard microbiological culture, and the other was used for microbiome analysis. Within 5 min of collection, microbiome specimens were frozen at −20°C at the TMC. These samples were subsequently transported on dry ice and maintained at −80°C until molecular analysis.

Microbiological and molecular analysis. Abscess cultures were processed by the Martin Army Community Hospital microbiology laboratory according to standard protocols. Anterior-naris specimens were processed as previously described (28). *S. aureus* isolates underwent pulsed-field gel electrophoresis (PFGE) to assess relatedness and PCR to assess resistance and virulence determinants, as previously described (27).

DNA extraction. Total genomic DNA was extracted from the samples using the GenElute bacterial genomic DNA kit (Sigma-Aldrich). Briefly, the swab heads were submerged in 500 μl of Gram-positive lysis solution, consisting of lysozyme (45 mg/ml), mutanolysin (125 U/ml), and lysostaphin (0.16 mg/ml) for 30 min at 37°C, followed by the addition of proteinase K (0.95 mg/ml) and 500 μl of lysis solution C for 10 min at 55°C. Column purification of genomic DNA was conducted according to the manufacturer's recommendations.

DNA amplification and sequencing. After DNA extraction, the V1-V3 region of the 16S rRNA gene was amplified from each sample using the universal primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 534R (5′-ATTACCGCGGCTGCTGG-3′) (29). The reverse PCR primer contained a 6-nucleotide "barcode" sequence that was unique to each sample, which allowed us to multiplex multiple reactions per pyrosequencing run. PCR conditions were in accordance with the 16S 454 sequencing protocol of the Human Microbiome Consortium (http://www.hmpdacc.org/doc/16S_Sequencing_SOP_4.2.2.pdf). Briefly, 0.15 μl of AccuPrime high-fidelity *Taq* DNA polymerase (Invitrogen) was added to a mixture containing 1× AccuPrime PCR buffer II, a 0.2 μM concentration of each primer, and 15 μl of genomic DNA to a final volume of 20 μl. All reactions were performed at least in triplicate, and products were combined after amplicon verification on a 1% agarose gel. The combined reaction products were cleaned using the Qiagen MinElute reaction mixture cleanup kit (per manufacturer's recommendation), quantified using a Nanodrop spectrophotometer (NanoDrop 8000; Thermo Scientific), and added in a 1:1 ratio to a single 1.5-ml Eppendorf vial. One hundred twenty-six samples were multiplexed in two separate pyrosequencing runs (63 samples per run) using the Roche GS FLX Titanium 454 sequencer at the Tufts University Genomics Core Facility. Nasal and abscess samples were randomized between the two sequencing runs to reduce sequencing bias.

Sequence processing. Sequences were processed according to the 454 standard operating procedure (SOP; http://www.mothur.org/wiki/454_SOP) using the open-source software program mothur (v.1.31.2) (30). Sequences greater than 200 bp were denoised using the mothur-adapted PyroNoise algorithm (31) and aligned using the SILVA reference alignment (32). Any sequence containing more than 1 mismatch to the barcode and/or 2 mismatches to the primer sequence was discarded. Sequences within 2 bp of a more abundant sequence were preclustered together. PCR chimeras were identified and removed using the mothur implementation of UCHIME (33). After denoising, trimming, and preclustering of raw sequences, all reads were classified using the Ribosomal Database Project (RDP) Bayesian classifier (34) using an 80% bootstrap confidence level over 100 iterations. Contaminant sequences, including those from mitochondria, chloroplasts, archaea, and eukaryotes and sequences classified as "unknown" at the kingdom level, were removed from the study. All remaining sequences were clustered into operational taxonomic units (OTUs) defined by a 97% similarity level according to the average-neighbor algorithm. To avoid the effects of different sequencing depths, all samples were rarefied to 2,245 reads per sample. Lastly, sequences were classified to the species level using the Greengenes 16S rRNA gene database (May 2013 release) (35).

Diversity analysis and statistics. All alpha and beta diversity analyses were computed using mothur. The inverse Simpson diversity estimator (invsimpson) was used for the alpha diversity analyses, since it can accurately assess diversity levels without being dramatically affected by sampling effort. Phylogenetic diversity was assessed using Faith's index (36). Statistical testing for percent abundance, invsimpson, Faith's index, and observed OTU data was based on the Mann-Whitney test using GraphPad Prism. Linear regression analyses were also performed using GraphPad Prism. To determine if two populations (for example, *S. aureus* carriers versus noncarriers) were significantly different based on bacterial composition, we used the analysis of molecular variance (AMOVA) test for the mothur-generated Yue and Clayton (Θ_{YC}) and Jaccard distance matrices (37, 38). AMOVA was also used to test phylogenetic differences between communities using the unweighted and weighted UniFrac distances generated from a neighbor-joining tree containing all sequences. The neighbor-joining tree was created in mothur using the clear-cut command and was also used when phylogenetic diversity was assessed using Faith's index. The interrelationships between populations were spatially visualized by nonmetric multidimensional scaling (NMDS) using R. When determining differentially represented OTUs between the communities, we utilized the mothur-adapted Metastats software (39). OTUs that differed in abundance had *P* and *q* values below 0.05 and were required to have a mean abundance greater than 0.1% in at least one of the communities.

RESULTS

Baseline participant characteristics. Overall, the study population consisted of 30,209 trainees, and 137 were enrolled in the microbiome portion of the study. From these 137 subjects, 46 non-SSTI and 40 abscess participant samples were sequenced. Of the 40 abscess participants, 2 had received antimicrobials approximately 18 to 26 days prior to sampling and were subsequently removed from the analysis. We categorized non-SSTI participants according to anterior-naris culture results (15 with MRSA, 15 with methicillin-susceptible *S. aureus* [MSSA], and 16 with no *S. aureus* [NoSA]) and abscess participants based on anterior-naris (11 with MRSA, 16 with MSSA, and 11 with NoSA) as well as abscess (16 with MRSA, 15 with MSSA, and 7 with NoSA) culture results. Of the 31 *S. aureus* abscess participants, 15 (48%; 7 MRSA and 8 MSSA) had nasal-abscess concordant isolates as determined by PFGE. Fifteen of the 16 MRSA abscess isolates were USA300. Study participant characteristics are outlined in Table S1 in the supplemental material. Age was shown to not have an influence on the nasal microbiome composition (see Table S2 in the supplemental material). Additionally, the abscess microbiomes appeared similar in structure regardless of broadly defined anatomic location (see Table S2 in the supplemental material).

Sequencing results. Sequencing of 86 participants' samples (46 nasal samples from non-SSTI participants and 40 nasal and 40 abscess cavity samples from abscess participants) was conducted in two separate pyrosequencing reactions, yielding a total of 3,252,212 raw sequences; 66% of these reads remained after quality processing and contaminant removal. In addition to the two patient samples removed due to recent antimicrobial treatment, one abscess sample, which contained only 987 associated reads, was excluded from the analysis. On average, each remaining sample contained 16,538 (range, 2,245 to 44,189) reads with an average read length of 194 (range, 169 to 219) nucleotides. To obviate bias based on sequencing depth, 2,245 reads were randomly subsampled for each specimen. Good's coverage values (≥97%) as

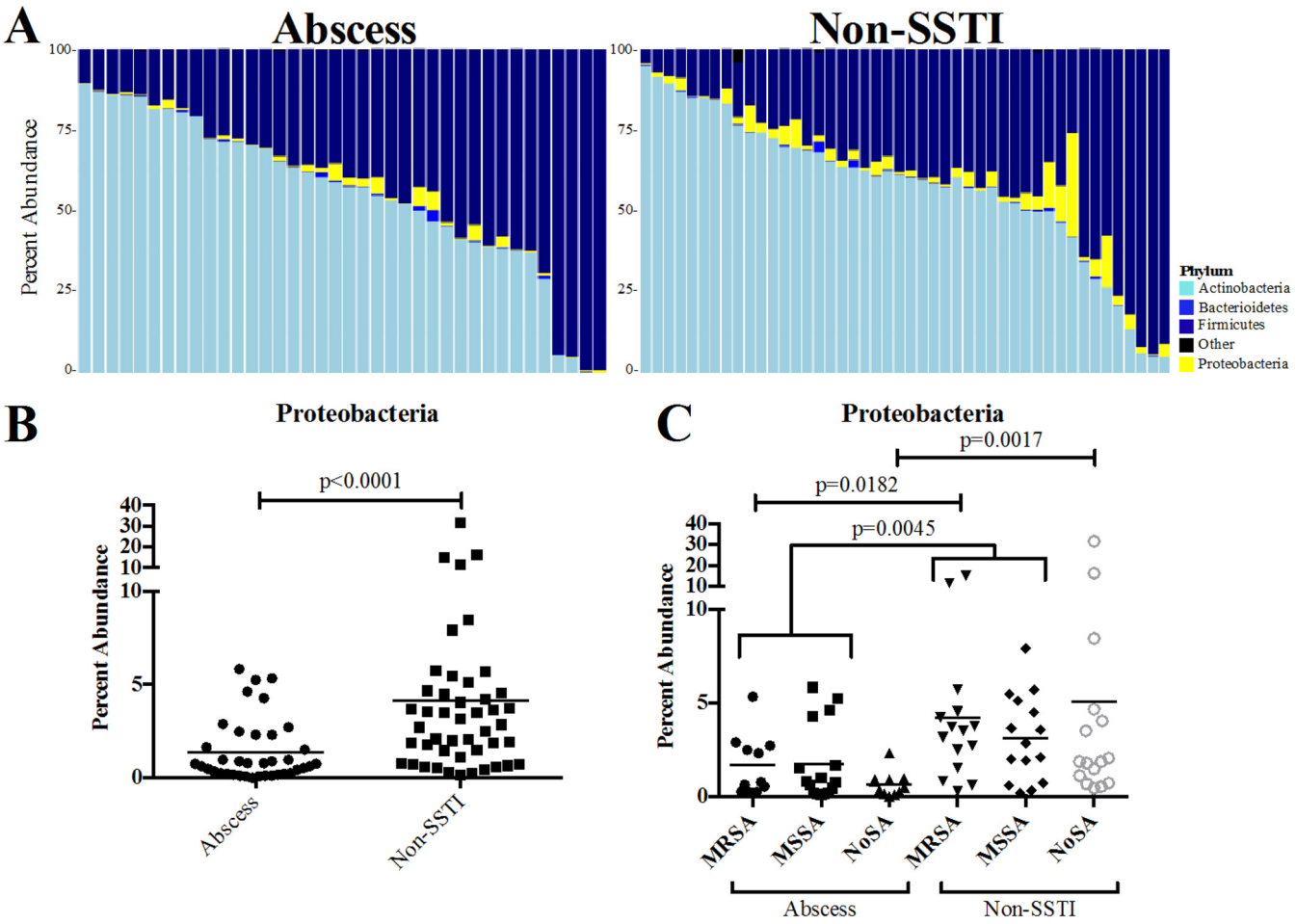

FIG 1 Characterization of the nasal microbiomes for those that did (abscess) and did not (non-SSTI) develop SSTI. (A) Breakdown of each nasal specimen by phylum. Each column represents one swab. (B) Percent abundance of *Proteobacteria* in the nose for the abscess and non-SSTI groups. (C) *Proteobacteria* abundance broken down by nasal culture results (MRSA, MSSA, and NoSA) for the abscess and non-SSTI groups. Horizontal lines represent the average percent abundance. Significant comparisons were calculated using the Mann-Whitney statistical test.

well as rarefaction curves (data not shown) suggested that this sampling size adequately represented the total observed biodiversity for each specimen. Table S3 in the supplemental material shows the percent abundance of each taxon for each sample.

Phylum-level anterior-naris microbiome. Analysis of the relative abundance of bacteria present within the 84 anterior-naris samples revealed that the microbiome was largely composed of four phyla (*Actinobacteria*, *Firmicutes*, *Proteobacteria*, and *Bacteroidetes*) and was predominantly composed of bacteria from the phyla *Actinobacteria* and *Firmicutes* (average abundances, 56.8% and 39.7%, respectively) (Fig. 1A). The presence of *Actinobacteria* and that of *Firmicutes* were inversely correlated in the anterior nares (see Fig. S1A in the supplemental material). There was a significantly higher percent abundance of *Proteobacteria* in the anterior nares of the non-SSTI group than in the abscess participants (average abundance, 1.4% versus 4.1%; $P < 0.0001$) (Fig. 1B). This increase in abundance of the *Proteobacteria* was not attributed to any single bacterium but was due to an overall increase in multiple genera, including *Neisseria* and *Haemophilus* (Table 1). When we analyzed abundance based on *S. aureus* nasal carriage status (MRSA, MSSA, or NoSA), we found that the non-SSTI participants that did not carry *S. aureus* (NoSA) showed a significantly higher abundance of *Proteobacteria* than the NoSA abscess participants ($P = 0.0017$) (Fig. 1C). The NoSA abscess participants showed exceptionally low levels of nasal *Proteobacteria*. A significant difference was not observed when the MSSA groups were compared between the non-SSTI and abscess groups ($P >$

TABLE 1 Abundance of *Proteobacteria* in the nares

Genus[a]	Avg % abundance (mean ± SD) in group	
	Abscess[b]	Non-SSTI[c]
Dyella	0.41 ± 0.88	1.08 ± 2.63
Rhodanobacter	0.28 ± 0.49	0.99 ± 1.04
Haemophilus	0.01 ± 0.05	0.46 ± 2.09
Vitreoscilla	0.16 ± 0.43	0.79 ± 2.42
Neisseria	0.06 ± 0.15	0.24 ± 0.54
Moraxella	0.02 ± 0.11	0.15 ± 0.36
Burkholderia	0.03 ± 0.05	0.10 ± 0.14

[a] As determined by the Greengenes database.
[b] Individuals that developed skin and soft tissue infections.
[c] Individuals that did not develop skin and soft tissue infections.

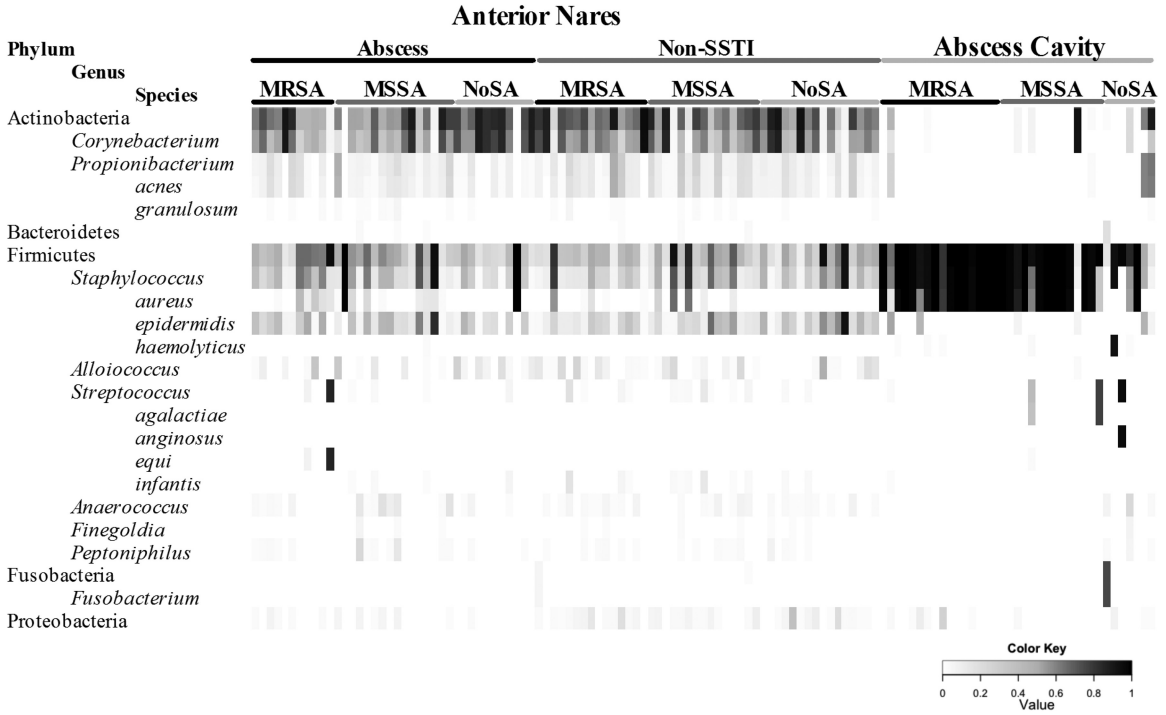

FIG 2 Heatmap showing the percent abundance for the predominant phyla, genera, and species for all nasal and abscess cavity samples. The anterior-naris specimens are separated according to subjects that did (abscess) and did not (non-SSTI) develop SSTI. Additional subgroupings are based on nasal culture results (MRSA, MSSA, or no *S. aureus* [NoSA]). The darker the shading, the more abundant the taxon. Each column represents a single sample.

0.05); however, MRSA carriers showed a significantly higher percent abundance of *Proteobacteria* in the non-SSTI group ($P = 0.0182$) (Fig. 1C). Additionally, *S. aureus* carriers (MRSA + MSSA) had a significantly greater abundance of *Proteobacteria* in the non-SSTI group ($P = 0.0045$; Fig. 1C).

Genus- and species-level anterior-naris microbiome. Visualization of the predominant phyla, genera, and species across nasal samples suggested significant differences between the abscess and non-SSTI samples (Fig. 2). Indeed, we observed a significantly higher abundance of *Propionibacterium* ($P = 0.0292$) and *Staphylococcus epidermidis* ($P = 0.0221$) in the non-SSTI NoSA group compared to the NoSA abscess group (Fig. 3A and B). Additionally, among the abscess participants, we found that the NoSA group contained a significantly higher percentage of *Corynebacterium* compared to the MRSA and MSSA groups (Fig. 3C). However, this correlation was not observed for the non-SSTI groups.

Nasal microbiome composition analysis. We next investigated the overall variation in bacterial composition between the non-SSTI and abscess participants' nasal microbiomes. Among the non-SSTI and abscess participants, we observed a total of 977 and 1,392 OTUs, respectively. Only 507 OTUs were shared across the groups (see Fig. S1B in the supplemental material). In keeping with this, we observed a significant difference in the Jaccard distance matrix (AMOVA, $P < 0.001$), which suggests a difference in

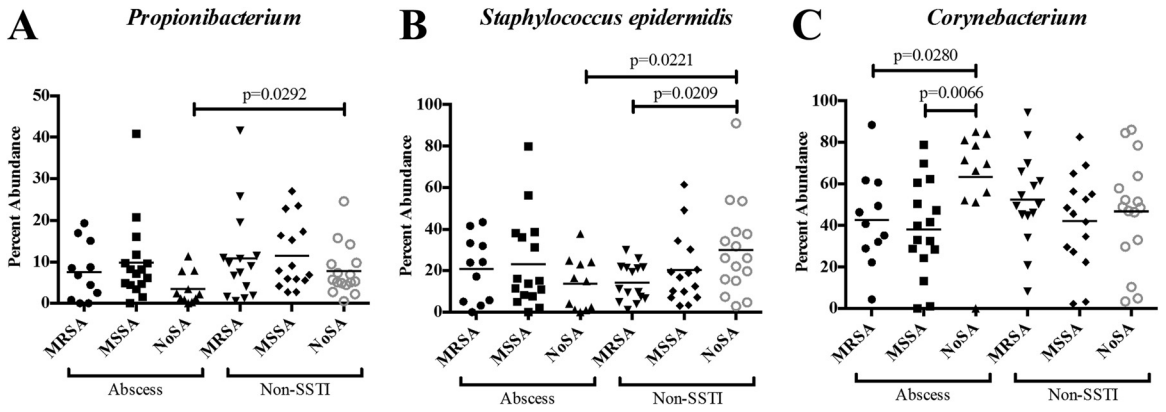

FIG 3 Percent abundance in the anterior nares for *Propionibacterium*, *Staphylococcus epidermidis*, and *Corynebacterium* (A, B, and C, respectively). Samples are categorized based on SSTI status (abscess or non-SSTI) as well as *S. aureus* nasal culture results (MRSA, MSSA, and NoSA). Horizontal lines represent the average percent abundance. Significant comparisons were calculated using the Mann-Whitney statistical test.

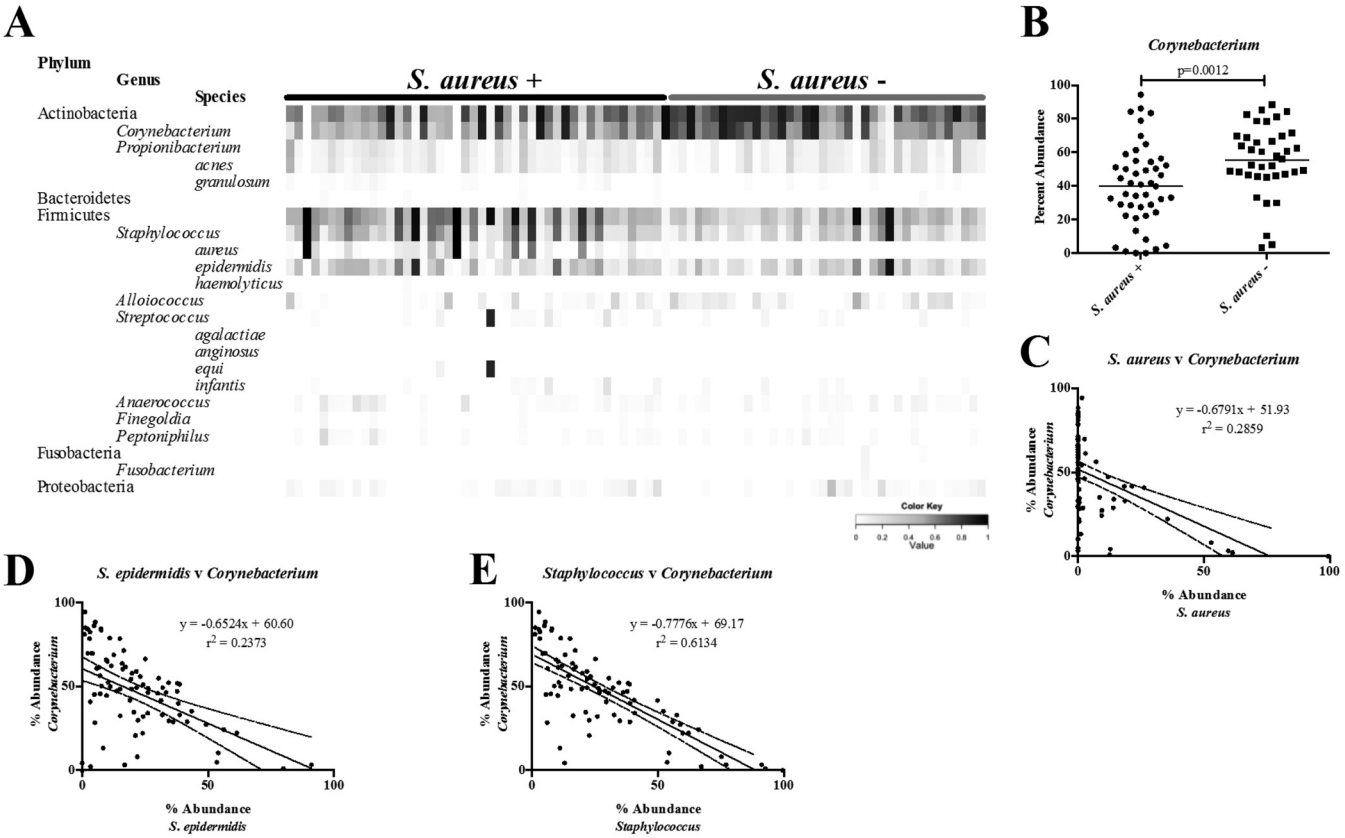

FIG 4 Impact of *Staphylococcus* on the nasal microbiome. (A) Heatmap showing the percent abundance for the predominant phyla, genera, and species for nasal samples separated by *S. aureus* colonization status (based on sequencing results). The darker the shading, the more abundant the taxa. Each column represents a single sample. (B) Percent abundance of *Corynebacterium* in the nose for individuals that were sequence positive or negative for *S. aureus*. Horizontal lines represent average percent abundance. Inverse correlation between *Corynebacterium* and *S. aureus* (C), *S. epidermidis* (D), and *Staphylococcus* (E). Solid lines and inset equations represent the best fit line [y = slope(x) + y intercept]. Dashed lines represent the 95% confidence interval. r^2, coefficient of determination.

OTU membership between the non-SSTI and abscess participants' nasal microbiomes. This OTU membership difference was present between the non-SSTI and abscess MRSA groups (Jaccard AMOVA, $P = 0.023$; Θ_{YC} AMOVA, $P = 0.152$), MSSA groups (Jaccard AMOVA, $P = 0.01$; Θ_{YC} AMOVA, $P = 0.928$), and NoSA groups (Jaccard AMOVA, $P = 0.014$; Θ_{YC} AMOVA, $P = 0.225$), suggesting that there is a bacterial membership difference between the abscess and non-SSTI groups' nasal microbiomes, regardless of *S. aureus* colonization status. Metastats analysis of the differentially represented OTUs between the abscess and non-SSTI nasal microbiomes showed that many were very low in abundance. However, a substantial proportion of them were from the phylum *Proteobacteria* (see Table S4 in the supplemental material). Phylogenetic analysis using the unweighted UniFrac distances supported the bacterial membership difference between the abscess and non-SSTI nasal microbiomes (unweighted UniFrac AMOVA, $P < 0.001$). However, when the relative abundance of OTUs was considered, we did not detect differences between the abscess and non-SSTI nasal communities (Θ_{YC} AMOVA, $P = 0.825$; weighted UniFrac AMOVA, $P = 0.603$).

Although we did not detect any differences in bacterial diversity between the abscess and non-SSTI nasal microbiomes using the inverse Simpson calculator (data not shown), at the phylogenetic level, we found that the abscess group was significantly more diverse than the non-SSTI nasal communities (see Fig. S2A in the supplemental material). However, we did not detect any significant differences when the MRSA, MSSA, and NoSA groups were compared individually between the abscess and non-SSTI groups (see Fig. S2B in the supplemental material).

***S. aureus* impact on anterior-naris bacterial composition.** Given that our data suggested that *S. aureus* colonization affects the nasal architecture (Fig. 2 and 3), we next assessed the overall impact of *S. aureus* carriage on the nasal microbiome. As determined by the Jaccard and Θ_{YC} distances, we did not observe significant differences in anterior-naris microbiome structure between MRSA and MSSA carriers among the abscess and non-SSTI participants (data not shown). Therefore, we grouped all participants together (abscess and non-SSTI) and subsequently reorganized them into *S. aureus*-positive and *S. aureus*-negative groups based on the sequencing results (>0% abundance = *S. aureus* positive). Upon visual observation of the most abundant phyla, genera, and species, there appeared to be a higher abundance of bacteria from the phylum *Actinobacteria*, particularly from the genus *Corynebacterium*, which was present in the anterior nares of *S. aureus*-negative participants (Fig. 4A and B). This inverse correlation was confirmed by regression analysis (coefficient of determination [r^2] = 0.2859, slope [m] = −0.6791, $P < 0.0001$) (Fig. 4C). Of note, the inverse correlation was also present between *S. epidermidis* and *Corynebacterium* ($r^2 = 0.2373$, $m = -0.6524$, $P < 0.0001$) but was strongest when all species of *Staph-*

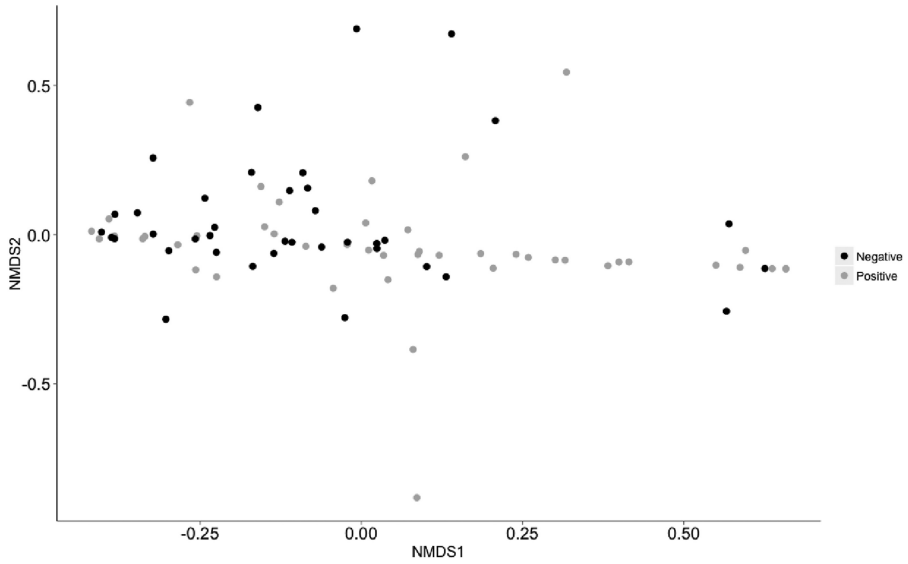

FIG 5 Nonmetric multidimensional scaling (NMDS) plot showing the spatial variation between *S. aureus*-positive and -negative nasal communities using the Θ_{YC} distances, indicating that these communities are compositionally distinct. The spatial separation between the positive and negative groups is statistically significant (AMOVA, $P < 0.001$).

ylococcus were considered ($r^2 = 0.6134$, $m = -0.7776$, $P < 0.0001$) (Fig. 4D and E). Thus, *Staphylococcus* and *Corynebacterium* appear to compete for nasal landscape.

When we analyzed the overall bacterial composition between *S. aureus*-negative and -positive nasal microbiomes using the Θ_{YC} and weighted UniFrac distance matrices, we observed a significant difference in OTU abundance levels between *S. aureus*-positive and *S. aureus*-negative groups (Θ_{YC} AMOVA, $P = 0.003$; weighted UniFrac AMOVA, $P < 0.001$). This difference could be visualized by nonmetric multidimensional scaling (NMDS) (Fig. 5), and was shown by Metastats analysis to be largely driven by differential representation of staphylococcal and corynebacterial OTUs (see Table S4 in the supplemental material). These data support our finding that the presence of *Staphylococcus* and that of *Corynebacterium* are inversely correlated (Fig. 4) and suggest that these two genera alone can greatly impact the overall diversity of the anterior nares.

Abscess microbiome. We next characterized the 37 abscess swabs obtained from abscess participants. As shown in Fig. 6A, we found that the majority of bacteria present within the abscess belonged to the phylum *Firmicutes* (87.6% average abundance). Of note, many of the abscesses were polymicrobial; 10 of the 37 abscesses had no single bacterial species with more than 90% abundance (Fig. 2 and 6A). At the species level, *S. aureus* dominated the majority of abscesses (73.7% average abundance). Other common bacteria found within the abscesses included *Staphylococcus haemolyticus*, *Streptococcus agalactiae*, *Streptococcus anginosus*, *Propionibacterium acnes*, and *Corynebacterium*. We observed one sample highly rich in *Fusobacterium* (Fig. 2). For the seven NoSA abscesses, we collected basic microbiological information for the organisms that were cultured from the abscess cavity. These included coagulase-negative *Staphylococcus*, *Escherichia coli*, group A and G *Streptococcus*, and *Enterobacter*. Although it is difficult to draw any correlations between nasal and abscess colonization, we note that for the individual in which group A *Streptococcus* was cultured from the abscess, we observed an exceptionally high level of *Streptococcus* in the nares (>80% abundance).

Our study design also allowed us to directly compare the microbiomes of abscesses from the MRSA, MSSA, and no-*S. aureus* (NoSA) groups. This analysis revealed that NoSA abscesses showed a significantly greater number of observed OTUs than MRSA ($P = 0.0273$) and MSSA ($P = 0.0097$) abscesses (Fig. 6B). In addition, the NoSA group was significantly more diverse than the *S. aureus*-positive abscesses (MRSA + MSSA; $P = 0.0261$) (Fig. 6C). Using Faith's index, we also found that at the phylogenetic level, the NoSA groups were more diverse than the MRSA ($P = 0.0469$) and MSSA ($P = 0.0309$) abscesses (see Fig. S2C in the supplemental material). Comparison of the Θ_{YC} and weighted UniFrac distances among groups revealed that the NoSA abscesses were significantly different from the MRSA (Θ_{YC} AMOVA, $P < 0.001$; weighted UniFrac AMOVA, $P = 0.002$) and MSSA (Θ_{YC} AMOVA, $P = 0.004$; weighted UniFrac AMOVA, $P = 0.035$) abscesses. This suggests that bacteria found among the *S. aureus*-positive and -negative abscess communities were different in abundance levels. There were no OTU abundance differences between the MRSA and MSSA abscesses. Membership-based Jaccard and unweighted UniFrac distance analysis revealed that the MRSA and MSSA abscesses were significantly different from the NoSA abscesses in regard to which OTUs were present (Jaccard AMOVA, $P = 0.005$ and $P = 0.021$, respectively; unweighted UniFrac AMOVA, $P = 0.002$ and $P = 0.001$, respectively).

Sensitivity of culture for *S. aureus* detection. For individuals that were considered *S. aureus* negative (NoSA) by anterior-naris culture, we observed a high level of concordance with sequencing data. Only one out of the 27 culture-negative anterior-naris specimens demonstrated a *S. aureus* abundance greater than 0.1% in the sequencing results. On the other hand, abscess culture appeared significantly less reliable; 2 of the 7 culture negative abscesses were sequence positive and showed a relatively high abundance of *S. aureus* (>49%). Thus, this culture sensitivity of 71.4%

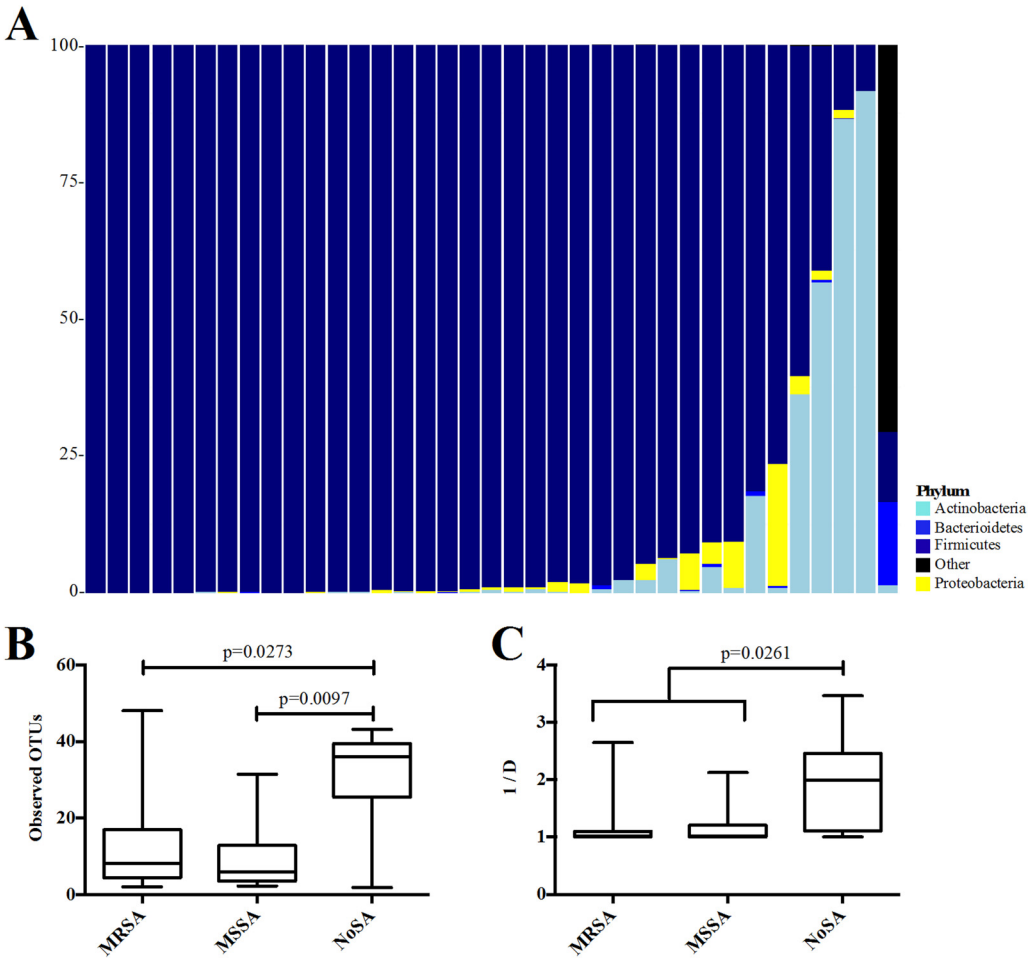

FIG 6 Characterization of the abscess cavity microbiomes. (A) Breakdown of each abscess cavity specimen by phylum. Each column represents one abscess swab. (B and C) Number of observed OTUs for each abscess (B) and inverse Simpson index (1/*D*) (C). Each box represents the interquartile range, with the mean shown within. The whiskers spread from the minimum to maximum values. Significant comparisons were calculated using the Mann-Whitney statistical test.

for abscess samples may indicate that standard microbiology culture alone has limitations for determining abscess etiology.

DISCUSSION

In this investigation involving a large community-based population at high risk for SSTI, we made several significant observations that help describe a healthy nasal microbial composition and may help inform future prevention strategies. We observed a greater abundance of *Proteobacteria* in the anterior nares of the non-SSTI participants than in nares of those who had developed cutaneous abscesses. Additionally, with regard to anterior-naris carriage, we noted an inverse correlation between *Corynebacterium* spp. and *S. aureus*.

S. aureus nasal colonization is a risk factor for the development of SSTIs (18, 19, 40); however, as demonstrated in our investigation, it is not always associated with SSTI. Therefore, we set out to classify the nasal microbiomes of people who did and who did not develop cutaneous abscess in order to identify a "marker" microbiome associated with abscess development. In agreement with other nasal microbiome studies that looked primarily at healthy individuals (22, 23, 41, 42), we found that *Actinobacteria* and *Firmicutes* dominated the nares of all patients (Fig. 1A). In particular, *Corynebacterium* and *Staphylococcus* were the most abundant genera present (Fig. 2). Intriguingly, we found that participants without abscesses carried a higher abundance of *Proteobacteria* in their anterior nares (Fig. 1B). This finding suggests that a loss of *Proteobacteria* in the nares either may be associated with development of SSTI or may be a marker of the SSTI state. While the most significant reduction of *Proteobacteria* was seen in NoSA abscess individuals, lower abundance was also seen in *S. aureus*-colonized abscess participants (Fig. 1C). Thus, even in the presence of *S. aureus* carriage, low levels of *Proteobacteria* appear to correlate with SSTI. In a recent study by Lemon et al., an inverse correlation between *Firmicutes* presence and *Proteobacteria* abundance was observed in the oropharynx (42). Their finding suggests that *Proteobacteria* may inhibit *Firmicutes* colonization in that niche. However, when we compared abundance of *Proteobacteria* to that of *Firmicutes* in our patients, we did not find any significant correlations (data not shown). Thus, SSTI incidence is not due solely to increased abundance of *S. aureus* (Fig. 1C). It is intriguing to speculate that for individuals who are not *S. aureus* carriers, *Proteobacteria* occupy the nasal niche in such a way as to prevent

colonization by other pathogenic microbes. Therefore, it would be interesting to determine if *Proteobacteria* in the nares may in fact be protective against SSTI and could be used prophylactically to prevent SSTI.

We observed a significant inverse correlation between *S. aureus* and *Corynebacterium* abundance in the nares (Fig. 4C). A similar finding has been documented by other studies (41–43). Indeed, *Corynebacterium* has been shown to directly inhibit *S. aureus* growth (42), as well as to foster clearance of *S. aureus* from the anterior nares (43). The molecular mechanism by which this occurs is not clear. Perhaps *Corynebacterium* produces a product that is toxic to *S. aureus*. Alternatively, *Corynebacterium* may successfully compete for limited nutrients required for *S. aureus* growth. If the former is the case, and the toxic molecule can be isolated and purified, it could potentially serve as a novel therapeutic agent to prevent *S. aureus* colonization.

Our data indicate that *S. aureus* colonization has a major impact on the overall composition of the nasal microbiota in our study population (Fig. 5). However, our cross-sectional sampling limits our ability to discern between persistently or nonpersistently colonized individuals. In a recent study that monitored the nasal microbiomes of 12 healthy individuals over 3 weeks, it was determined that while *S. aureus* carriers and noncarriers had similar nasal microbiomes, nonpersistent carriers displayed a significant drop in bacterial diversity (41). *Proteobacteria* levels in the nares were also shown to significantly vary among persistent and nonpersistent carriers (41). Additionally, microbiota changes at other body locations (axilla, groin, and perianal sites), as well as the site of infection prior to abscess formation, may also contribute to SSTI susceptibility (44).

Although vaccine studies have been largely ineffective at preventing *S. aureus* carriage in humans (17, 45, 46), a greater understanding of the nature of the apparent competition between *S. aureus* and other taxa may hold promise for future therapeutic design to prevent *S. aureus* carriage and SSTI development. Although bacterial antagonism against *S. aureus* has previously been investigated (41, 43, 47, 48), our data suggest that there are additional variables to consider when prophylactic countermeasures are being designed; in order to lower the risk of development of SSTI, one must lower *S. aureus* colonization levels but must be cognizant of other nasal bacterial inhabitants, especially from the phylum *Proteobacteria*. Additionally, a reduction of specific bacteria in noncarriers, including *Propionibacterium* and *S. epidermidis*, correlates with SSTI development (Fig. 3). Given these data, nasal administration of a single bacterium may not be sufficient to protect against SSTIs; a medley of bacteria may be needed.

Given that culture has been the primary method used to identify the etiological agent of abscesses (49), our study provides the first in-depth characterization of abscesses from individuals infected with MRSA and MSSA and those not infected with *S. aureus*. Not surprisingly, we found that the vast majority of MRSA and MSSA culture-positive abscesses were dominated by *S. aureus* (Fig. 2). Interestingly, we identified a high number of polymicrobial infections, especially in the NoSA abscess group (Fig. 2 and 6). It is possible that bacteria within a polymicrobial infection may possess a distinct transcriptional profile compared to a monomicrobial abscess. Indeed, *S. aureus* in the context of a polymicrobial infection displays enhanced pathogenesis compared to a *S. aureus* monoinfection (50).

There are limitations to our investigation. First, this study was conducted in the context of a prospective cluster-randomized trial that involved hygiene measures. Although we did not detect any differences in the nasal microbiomes of the participants in different study groups (data not shown), it is possible that hygiene interventions (51), especially chlorhexidine use, may have impacted the anterior-naris microbiome. However, given that some of our findings are congruent with other studies (the inverse correlation between *S. aureus* and *Corynebacterium* in the nares has also been observed elsewhere [41–43]), this suggests that chlorhexidine use did not have a major impact on the nasal microbiome. Nevertheless, our large sample provides a firm hypothesis-generating foundation upon which to build. Second, because all abscesses were drained based on clinical criteria, these culture swabs were processed in the microbiology lab using standard techniques, which is different from the enriched technique employed for the anterior-naris specimens. This may in part explain the discrepancy between culture-negative and sequence-positive abscesses.

In summary, we have characterized the nasal microbiomes of over 80 individuals and have described significant differences in microbial composition in the anterior nares between those with and without abscesses and between *S. aureus* carriers and noncarriers. We also used high-throughput sequencing techniques to unveil the microbiome of abscesses. These observations expand our understanding of the complexity of the nasal and abscess microbiomes and will potentially be useful in the design of future therapeutic and prophylactic countermeasures against *S. aureus* carriage and subsequent disease.

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