RESEARCH ARTICLE

Staphylococcus aureus nasal carriage and microbiome composition among medical students from Colombia: a cross-sectional study [version 1; peer review: 2 approved]

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Abstract

Background: The anterior nares are the main ecological niche for *Staphylococcus aureus*, an important commensal and opportunistic pathogen. Medical students are frequently colonized by a variety of pathogens. Microbial interactions in the human nose can prevent or favor colonization by pathogens, and individuals colonized by pathogens have increased risk of infection and are the source of transmission to other community members or susceptible individuals. According to recent studies, the microbiome from several anatomic areas of healthy individuals varies across different ethnicities. Although previous studies analyzed the nasal microbiome in association with *S. aureus* carriage, those studies did not provide information regarding ethnicity of participants. Our aim was to assess *S. aureus* nasal carriage patterns and prevalence among medical students from Colombia, a country of Hispanic origin, and to investigate possible associations of colonization and nasal microbiome composition (bacterial and fungal) in a subgroup of students with known *S. aureus* carriage patterns.

Methods: Nasal swabs from second-year medical students were used to determine prevalence and patterns of *S. aureus* nasal carriage. Based on microbiological results, we assigned participants into one of three patterns of *S. aureus* colonization: *persistent*, *intermittent*, and *non-carrier*. Then, we evaluated the composition of nasal microbial communities (bacterial and fungal) in 5 individuals from each carriage category using 16S rRNA and Internal-Transcribed-Spacer sequencing.

Results: Prevalence of *S. aureus* nasal carriage among medical

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students was 28%. Carriage of methicillin-resistant strains was 8.4% and of methicillin-sensitive strains was 19.6%. We identified 19.6% persistent carriers, 17.5% intermittent carriers, and 62.9% non-carriers.

Conclusions: Analysis of nasal microbiome found that bacterial and fungal diversity was higher in individuals colonized by *S. aureus* than in non-carriers; however, the difference among the three groups was non-significant. We confirmed that fungi were present within the healthy anterior nares at substantial biomass and richness.

Keywords

Microbiome, mycobiome, microbiota, *Staphylococcus aureus*, bacterial communities.

This article is included in the **Pathogens** gateway.

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Introduction

The anterior nares are significant because they represent the main ecological niche for *Staphylococcus aureus*, a bacterium that behaves both as commensal and opportunistic pathogen¹. Asymptomatic carriage of *S. aureus* in healthy individuals has high prevalence, especially in children, young adults, and healthcare workers, including medical students^{2–4}. Due to their frequent contact with the general community and healthcare environment, medical students commonly encounter a variety of pathogens that may colonize them. Individuals colonized by pathogens have increased risk of infection, but also they become a source of transmission to other community members or susceptible patients^{1,5–7}.

Microorganisms residing in a particular anatomical site engage in complex interactions that prevent or favor colonization by pathogens⁸. The complete collection of microbes colonizing the anatomical areas of the human body constitute the microbiota, which includes bacteria, archaea, viruses, and fungi, intertwined in a complex network of interactions among them and the host⁹. The genes and genomes harbored by these microbial communities make up the human microbiome^{10,11}. According to recent studies, the microbiome from several anatomic areas of healthy individuals varies across different ethnicities^{12–15}. Although previous studies have analyzed the nasal microbiome in association with *S. aureus* carriage, these studies have not provided information on the ethnicity of participants, or have used individuals from different ethnic populations^{16,17}. Differences in microbiome composition linked to ethnic background highlight the need to consider and potentially account for ethnic diversity in microbiome research¹². This study sought to determine the nasal prevalence and long-term carrier patterns of *S. aureus* in second-year medical students with Hispanic background. Additionally, we aimed to explore biodiversity of the nasal microbiome (bacterial and fungal) and possible differential abundance of specific taxa among the three categories of *S. aureus* long-term carriage.

Methods

Study design and population

The Ethics Review Boards of the University of Cartagena (Approval #280313) and New York Medical College approved this study (Protocol # 12697; IRB ID: 12697). The study was conducted between January and June of 2018 and enrolled second-year medical students from University of Cartagena, Colombia, who had not yet engaged in clinical rotations. To prevent sampling bias, we aimed to enroll the complete population of second-year medical students of our institution. Students were recruited via fliers and lecturer announcements. Those who agreed to participate signed an informed consent and completed a written questionnaire on demographics and medical history. Exclusion criteria were recent infections, allergies and other non-infectious pathologies, smoking habits, antibiotic usage in the previous three months, surgeries and hospitalizations in the previous six months. In total 143 out of 158 second-year medical students completed the study. Thus, we enrolled 90.5% of the total population of second-year medical students in our institution in this study. We followed the STROBE cross sectional reporting guidelines¹⁸.

Specimen collection, prevalence of colonization and carriage categories

Nasal swabs were obtained from both nostrils, inoculated into Stuart transport medium (OXOID, England), transported to the microbiology laboratory and processed within 8–18 hours according to described protocols⁶. *S. aureus* was identified based on colony morphology, Gram-stain, catalase-test, tube coagulase-test, and latex agglutination-test. Genomic DNA was obtained from each isolate with Wizard® Genomic DNA Purification Kit (Promega, USA) and *S. aureus* molecular confirmation and methicillin-resistance were assessed by PCR-amplification using specific primers for *nuc* and *mecA* genes, respectively. Detailed protocols for all these methods, including primer sequences, have been previously described⁶. To determine prevalence of *S. aureus* nasal carriage, each participant was classified either as carrier or non-carrier based on laboratory results obtained from the first nasal swab survey. To establish *S. aureus* long-term carriage categories, four additional consecutive nasal swabs were obtained from each participant, in three-week intervals. According to definitions proposed by *Khuytmas et al.*¹⁹, participants that yielded five negative cultures for *S. aureus* were classified as non-carriers; those yielding one to three positive cultures were classified as intermittent carriers; and those yielding four or five positive cultures were classified as persistent carriers.

Nasal microbiome (bacterial and fungal) analysis

This study aimed to describe the microbiome composition in a small group of second-year medical students with a known *S. aureus* carriage status. At the end of the study, 15 participants with known *S. aureus* long-term carriage status (5 non-carriers, 5 intermittent-carriers, and 5 persistent-carriers) were randomly selected from the cohort of 143 participants. The 15 selected participants provided an additional nasal swab that was stored at room temperature in Amies-Transport-Medium with Charcoal (Copan Diagnostics, Inc., Murrieta, CA), and sent to the laboratory of Robert D. Burk at Albert Einstein College of Medicine (AECOM) for microbiome analysis. Sample processing was performed according to protocols described by *Usyk et al.*²⁰.

16S rRNA gene and Internal Transcribed Spacer (ITS) PCR-amplification

The V4 hypervariable region of 16S rRNA gene was amplified using primers 16SV4_515F (GTGYCAGCMGCCGCGGTA) and 16SV4_806R (GGACTACHVGGGTWTCTAAT), with a unique 12-bp barcode Golay-barcoding^{21,22}. PCR conditions were: initial 5min denaturation at 95°C, followed by 15-cycles of 95°C for 1min, 55°C for 1min, and 68°C for 1min, and final extension for 10min at 68°C. For the fungal component of microbiome, barcoded amplicons were generated covering the ITS gene region using ITS1-30F/ITS1-217R primer pair, as previously described by *Usyk et al.*²⁰ (ITS1-30F: 5'-GTCCCTGCCCTTTGTA-CACA-3' and ITS1-217R: 5'-TTTCGCTGCGTTCTTCATCG-3'). PCR conditions were: 3min initial denaturation at 95°C, followed by 35-cycles of 95°C for 30sec, 55°C for 30sec, and 68°C for 2min, followed by final extension at 68°C for 10min. PCR reagents were obtained from Affymetrix (Affymetrix, Santa Clara, CA). PCR reactions were run in GeneAmp PCR-System 9700 (Applied Biosystems).

High-throughput sequencing

PCR products were purified using QIAquick Gel Extraction Kit (QIAGEN) and quantified using Qubit™ dsDNA High-Sensitivity Assay kit (Life Technologies). Next-generation sequencing library preparation was performed using KAPA-LTP library preparation kit (KAPA Biosystems, Wilmington, MA). Size integrity of isolated amplicons was validated with 2100 Bioanalyzer (Agilent-Technologies, Santa Clara, CA). High-throughput sequencing of libraries was performed using Illumina HiSeq2500 Sequencing System (Illumina, San Diego, CA) with a 2×250-bp paired-end read kit at the Genomics Core Facility of AECOM.

Bacterial microbiome bioinformatics analysis

Illumina reads were pre-processed to remove bases that fell below PHRED quality score of 25 using PRINSEQ²³. Processed reads were de-multiplexed using sample specific barcode combinations with Novobarcode V1.00. This can also be performed with deML, a program freely available for use under the GPL license. Paired-end reads were merged using free open source PANDAseq v1.20 with default settings²⁴. OTU-clustering and quality filtering was performed using the Quantitative Insights Into Microbial Ecology (QIIME v1.9) software package²⁵. Removal of sequencing noise and sequence chimeras was done with USEARCH v8.0²⁶. Sequences were de-multiplexed and clustered into operational taxonomic units (OTUs) with 97% minimum cluster similarity using UCLUST²⁶. Sequences were assigned using UCLUST with Greengenes 13.8 microbial database²⁷. The resulting BIOM table was rarefied to 29,000 reads/sample and statistical analyses were performed after collapsing OTUs at genus level.

Fungal microbiome bioinformatics analysis

Sequence reads were processed using open-reference OTU-picking with QIIME v1.9 against the targeted host-associated fungi ITS database (THF1)²⁸ for the reference-based clustering component. VSEARCH v1.4.0²⁹ was used to de-replicate reads, cluster reads into OTUs and remove chimeric sequences. OTU-clustering threshold was set at 99% sequence identity to account for fungal heterogeneity. Sequence de-replication and chimera removal were performed using QIIME quality-control protocol. Representative sequences for each OTU-cluster were chosen based on sequence abundance. BLAST was used to assign taxonomy using the UNITE database³⁰. The default behavior of BLAST in QIIME was changed to minimum of 99% sequence identity for taxonomic assignment. Data were processed in R v3.3.1³¹. QIIME outputs were imported into R using phyloseq package v1.22.3³² and further processed with vegan v2.5-3³³, coin v1³⁴, and reshape2³⁵. Data visualization was performed using ggplot2³⁶.

Alpha/Beta diversity analysis of 16S rDNA-V4 and ITS sequences

Statistical analyses were performed to assess differences in OTU distribution and abundance between samples and groups. Microbial diversity for bacterial and fungal communities was evaluated within samples (α -diversity) or between samples (β -diversity) using QIIME. Rarefaction to subsampling depth of 29,000 reads/sample or 9,000 reads/sample, for bacteria or fungi respectively, and 5 iterations were performed on all samples to

standardize the sequencing effort. Alpha-diversity was measured with Chao1 (richness) and Shannon entropy (OTU-based diversity) index. Beta-diversity was calculated using Bray-Curtis dissimilarity coefficient. To test for dissimilarities in the microbial composition between *S. aureus* carrier groups, non-metric multidimensional scaling (NMDS) was performed with Bray-Curtis dissimilarity.

Results

S. aureus nasal carriage

The first nasal swab isolated *S. aureus* from 40 out of 143 participants, for a prevalence of 28%. Methicillin-resistant *S. aureus* (MRSA) was carried by 12 participants (8.4%) and methicillin-sensitive *S. aureus* (MSSA) by 28 (19.6%). The longitudinal study identified 28 (19.6%) persistent carriers, 25 (17.5%) intermittent carriers, and 90 (62.9%) non-carriers. MRSA strains were isolated from 6 persistent carriers and 6 intermittent carriers. Table 1 lists the characteristics of students used in the microbiome study. Underlying data: Table S1³⁷ lists the main results obtained from the complete study population.

Diversity and abundance of resident bacterial and fungal communities

Figure 1 shows clustering analysis of bacterial genus (A) and fungal species (B) compositions of nasal specimens from the cohort of 15 healthy medical students with known *S. aureus* carriage patterns. Bacterial microbiome analysis, sequencing, quality filtering and mapping resulted in 1,424,972 mapped V4-region sequences, ranging between 29,971-217,540 copies per sample (average 120,062; SD = 53,027), corresponding to 600 OTUs. We identified 57 of the 600 OTUs (9.5%) at the genus level, while

Table 1. Characteristics of participants of the microbiome study.

Participant code	Long-term carriage category	Age	Gender
JG06	Intermittent	22	M
JG07	Intermittent	19	F
JG08	Intermittent	19	F
JG09	Intermittent	20	M
JG10	Intermittent	18	M
JG11	Non carrier	18	F
JG12	Non carrier	21	F
JG13	Non carrier	18	F
JG14	Non carrier	28	M
JG15	Non carrier	19	M
JG01	Persistent	22	F
JG02	Persistent	21	F
JG03	Persistent	20	F
JG04	Persistent	20	M
JG05	Persistent	28	M

Figure 1. Cluster analysis of bacterial genus (A) and fungal species (B) compositions in the nares of healthy medical students with a known *S. aureus* carriage pattern. Heatmap was constructed using normalized log10 abundance of each OTU in each sample type. Data are presented only for the 20 most abundant taxa. Colored bar above heatmap indicates *S. aureus* carriage status. P: persistent (red), I: intermittent (blue), NP: non-carrier (green).

the remaining OTUs mapped to unclassified genera (70.8%) or upper taxonomic groups (96.8%). Three phyla were identified, *Proteobacteria* being the most abundant (78.3% of 16S rRNA sequences) and the most diverse (42.2% of all identified OTUs), followed by *Firmicutes* and *Actinobacteria*. In general, the nasal bacterial microbiome was dominated by the Class *Gammaproteobacteria* (order *Pseudomonadales* and genera *Citrobacter* and *Acinetobacter*), which is consistent with a 2016-review by Lee *et al.*³⁸. Table 2 shows the predominant bacterial genera isolated from the nostrils of healthy medical students.

Alpha-diversity analysis found that bacterial diversity was greater in the persistent group followed by the intermittent and then by the non-carriers, as shown in Figure 2A. However, the difference in diversity among the three carrier groups was non-significant. Beta-diversity analysis of bacterial communities showed non-significant separation of groups (Figure 2B). Analysis of differential abundance identified that order *Pseudomonadales* was more abundant in non-carriers followed by intermittent and persistent categories (Figure 3), a finding that may have implications regarding microbial antagonism. However, this trend did not reach statistical significance ($p > 0.05$, *Kruskal–Wallis test*).

For fungal microbiome analysis, sequencing, quality filtering and mapping resulted in 4,274,743 mapped ITS-region sequences, ranging between 9,543–743,278 copies per sample

(average 284,983; SD = 237,339), corresponding to 8,346 fungal OTUs (average 556; SD = 333) (Table 3). We were able to classify 4,453 of the 8,346 OTUs (53.3%) down to species level. Out of the seven recognized major phylum of fungi, three phyla were identified in the nostrils, being *Ascomycota* the most abundant (90.8% of fungal sequences) and the most diverse (88.7% of all identified OTUs), followed by *Basidiomycota* and *Neocallimastigomycota* (containing anaerobic fungi). In general, the nasal mycobiome was dominated by species of the phylum *Ascomycota*, which is consistent with a 2013-publication by Findley, *et al.*³⁹. Table 4 shows the predominant fungi species identified from the nostrils of healthy medical students. The most frequent fungal species detected was *Penicillium oxalicum*, representing 19.0% of the total 4,274,743 sequence reads in the chimera-filtered OTU table. *Malassezia restricta* was the second most frequent fungus (12.2% of all sequences). 4.4% of all sequences were unassigned sequences, thought to represent non-fungal contamination.

Based on the identified OTUs and number of sequences in this study, we report a substantial diversity and amount of fungal biomass in the anterior nares of this group of medical students with Colombian ethnicity. Consistent with the 16S analysis, alpha-diversity of fungal communities showed that fungal diversity was greater in the persistent group followed by the intermittent and then by the non-carrier groups. However, this

Table 2. Predominant bacterial genera identified from the nostrils of healthy medical students.

Genus	Order	Phylum	Relative abundance (%)
<i>Citrobacter</i>	Enterobacteriales	Proteobacteria	10.3%
<i>Acinetobacter</i>	Pseudomonadales	Proteobacteria	9.2%
<i>Corynebacterium</i>	Corynebacteriales	Actinobacteria	7.2%
<i>Paenibacillus</i>	Bacillales	Firmicutes	4.7%
<i>Bacillus</i>	Bacillales	Firmicutes	4.2%
<i>Pseudomonas</i>	Pseudomonadales	Proteobacteria	3.0%
<i>Lysinibacillus</i>	Bacillales	Firmicutes	2.7%
<i>Morganella</i>	Enterobacteriales	Proteobacteria	2.4%
<i>Rhodococcus</i>	Actinomycetales	Actinobacteria	2.0%
<i>Cellulomonas</i>	Actinomycetales	Actinobacteria	1.9%

Figure 2. Alpha and beta diversity of bacterial composition of nasal samples. (A) Alpha-diversity, measured by Chao1 and Shannon diversity Index, is plotted for individuals with different *S. aureus* carrier status: non-carriers (NP, dark green), intermittent carriers (I, purple) and persistent carriers (P, light green). The Chao1 index (left panel) and Shannon index (right panel) were computed for all 15 subjects. The line inside the box represents the median, while the whiskers represent the lowest and highest values within the 1.5 interquartile range (IQR). Statistical testing showed no significant differences among the groups: Chao1 $p = 0.0675$; Shannon $p = 0.108$. (B) Comparison of beta-diversity of bacterial composition between *S. aureus* carriage groups with NMDS ordination calculated from Bray-Curtis distance estimation. *I*: Intermittent, *NP*: non-carrier, *P*: persistent.

Figure 3. Boxplots representing relative abundance analysis of the bacterial taxa identified in nasal samples of individual from the different *S. aureus* carrier groups. (A) Differential abundance at the genus level. (B) Differential abundance for the order *Pseudomonadales*. Taxa with minimum median abundance of 1% were used for the comparison. There was non-significant difference in abundance among the three carrier groups ($p > 0.05$, Kruskal-Wallis test). NP: non-carrier, I: Intermittent, P: persistent.

trend did not reach statistical significance (Figure 4A). Beta-diversity analysis of fungal communities showed non-significant separation of groups (Figure 4B).

Analysis of differential abundance showed increased abundance for several fungi species in the persistent group compared to the intermittent and non-carriers (Figure 5A). However, the species *Candida orthopsilosis* was the only one with significant difference in abundance between the persistent and non-carrier groups ($p < 0.05$, Kruskal-Wallis test) (Figure 5B).

Discussion

Prevalence of *S. aureus* nasal carriage was 28%, which is consistent with other studies⁷. We also found that 37.1% of second-year medical students carried *S. aureus* in their nares, persistently or intermittently. The distinction in carriage category is important, as persistent carriers are at higher risk of developing active autoinfection than intermittent and non-carriers^{40–43}.

MRSA was carried by 8.4% of participants, which represents an important increase from the 1.6% MRSA carriage that we previously reported for our institution in 2012⁶.

We sought to analyze both bacterial and fungal composition in individuals with a known long-term nasal carriage pattern for *S. aureus*. We found that nasal bacterial microbiome had low diversity at the phylum level, with three dominating phyla: Proteobacteria, Firmicutes, and Actinobacteria^{44,45}. The top five most abundant genera were *Citrobacter*, *Acinetobacter*, *Corynebacterium*, *Paenibacillus* and *Bacillus*, all of which contain pathogenic species, evidencing the potential of the anterior nares as reservoir for pathogens^{46–48}. An interesting finding was that abundance of the genus *Staphylococcus* in the nares was generally low, even in nasal carriers of *S. aureus*. The estimated bacterial richness (# of species) found in our study is consistent with a former study that reported an estimate of 2,264 species in the anterior nares based on V3-V5 16S rRNA sequencing⁴⁹.

Table 3. Number of fungal sequences and OTUs from medical students with known *Staphylococcus aureus* carriage status.

Sample	Carriage Category	^a OTUs Clustered	^b Fungal sequences
JG11	Non carrier	125	9,543
JG12	Non carrier	245	25,175
JG13	Non carrier	727	743,278
JG14	Non carrier	381	160,273
JG15	Non carrier	720	621,267
Average:		440	311,907
JG06	Intermittent	491	265,710
JG07	Intermittent	154	14,612
JG08	Intermittent	740	341,059
JG09	Intermittent	578	289,560
JG10	Intermittent	502	366,262
Average:		493	255,441
JG01	Persistent	272	57,915
JG02	Persistent	855	499,815
JG03	Persistent	725	281,963
JG04	Persistent	404	48,946
JG05	Persistent	1427	549,365
Average:		737	287,601
Total in the cohort:		8,346	4,274,743

Table 4. Predominant fungi species identified from the nostrils of healthy medical students.

Species	Order	Phylum	Relative abundance (%)
<i>Penicillium oxalicum</i>	Eurotiales	Ascomycota	19.0%
<i>Malassezia restricta</i>	Malasseziales	Basidiomycota	12.2%
<i>Capnobotryella renispora</i>	Capnodiales	Ascomycota	7.5%
<i>Nectria cinnabarinata</i>	Hypocreales	Ascomycota	7.4%
<i>Calonectria asiatica</i>	Hypocreales	Ascomycota	6.6%
<i>Cladosporium phaenocomae</i>	Capnodiales	Ascomycota	5.2%
<i>Lipomyces doorenjongii</i>	Saccharomycetales	Ascomycota	4.0%
<i>Knufia perforans</i>	Chaetothyriales	Ascomycota	3.8%
<i>Rhodospiridium lusitaniae</i>	Ustilaginales	Basidiomycota	3.7%
<i>Serpula himantoides</i>	Boletales	Basidiomycota	3.6%

Recent studies suggest that composition of the nasal microbiota greatly influences *S. aureus* nasal colonization^{50,51}. However, the mechanisms used by the nasal microbiota to antagonize *S. aureus* colonization are not completely understood⁴⁶. Our results are in concordance with those from the Human Microbiome Project in the sense that microbiome composition varies by anatomical site and that interpersonal variation is significant^{52,53}.

Recently, researchers started to focus on the fungal component of the microbiome, revealing the remarkable diversity of the human mycobiome. Fungi were detected at varying abundance in our three carriage groups. The most abundant fungus identified was *Penicillium oxalicum*, a common environmental fungi that has been recently identified as a cause of invasive mycosis in immunocompromised patients⁵⁴. Although not

Figure 4. Alpha and beta diversity of fungal composition of nasal samples. (A) Alpha-diversity, measured by Chao1 and Shannon diversity Index is plotted for individuals with different *S. aureus* carrier status: non-carriers (NP, dark green), intermittent carriers (I, purple) and persistent carriers (P, light green). The Chao1 index (left panel) and Shannon index (right panel) were computed for 15 subjects. The line inside the box represents the median, while the whiskers represent the lowest and highest values within the 1.5 interquartile range (IQR). Statistical testing showed no significant differences among the groups: Chao1 $p = 0.13$; Shannon $p = 0.566$. **(B)** Comparison of beta-diversity of fungal composition between *S. aureus* carriage groups with NMDS ordination calculated from Bray–Curtis distance estimation. I: Intermittent, NP: non-carrier, P: persistent.

statistically significant, we identified a trend towards higher richness and evenness of both bacteria and fungi in the persistent group compared to the intermittent and non-carrier groups. Other studies have suggested that a more diverse microbiota may be associated with resistance to colonization by pathogens; however, we did not observe this phenomenon in our study. Here, a more diverse bacterial and fungal microbiome in the anterior nares seems to favor *S. aureus* carriage. A similar finding was reported for the pathogen *Streptococcus pneumoniae*, where a more diverse nasopharyngeal microbiome appeared to facilitate pneumococcal carriage in this human niche⁵⁵.

We could not evaluate completely the involvement of specific OTUs in *S. aureus* carriage due to the small sample size. However, we could identify that the order Pseudomonadales was enriched in non-carriers and that the fungi species

Candida orthopsilosis was significantly enriched in the persistent group. These results may suggest that some species in the Pseudomonadales antagonize long-term colonization by *S. aureus* or that *S. aureus* colonization may impact the composition of the underlying bacterial communities in the nares, displacing other microbial communities, as has been proposed by others¹⁶. Our results also suggest that *Candida* species may favor the long-term *S. aureus* colonization of human nares; however, additional studies are required to test these suggestions. Further studies with larger sample size and defined ethnic background are required to identify the interactions between specific members of the resident microbiota that favor or antagonize the colonization process of the bacterium *S. aureus* in the anterior nares of specific ethnic groups of the human population. This study is the first to analyze simultaneously the bacterial and fungal communities in the nostrils of healthy medical students with a

A.

B.

Figure 5. Boxplots representing relative abundance analysis of the fungal taxa identified in nasal samples of individual from the different *S. aureus* carrier groups. (A) Differential abundance at the species level. (B) Differential abundance for the species *Candida orthopsilosis* showed a significant difference in abundance between the persistent and non-carrier groups ($p < 0.05$, Kruskal-Wallis test). Taxa with minimum median abundance of 1% were used for the comparison. NP: non-carrier, I: Intermittent, P: persistent.

Hispanic/Latino background, and their association with *S. aureus* nasal colonization.

Data availability

Underlying data

Nasal Microbiome. Raw sequencing data of the nasal microbiome of a set of medical school students, Accession number, PRJNA600228: <https://www.ncbi.nlm.nih.gov/bioproject/600228>.

Open Science Framework: Nasal Microbiome of Medical Students UdeC. <https://doi.org/10.17605/OSF.IO/UDNWA37>.

This project contains the following underlying data:

- Table S1: Data for complete study population
- JG_16S_OTU_L6: Bacterial species found for microbiome study of 15 participants.
- JG_ITS_OTU_L7: Fungal species found for microbiome study of 15 participants.

Data are available under the terms of the [Creative Commons Zero "No rights reserved" data waiver](#) (CC0 1.0 Public domain dedication).

References

1. Kluytmans JA, Wertheim HF: **Nasal carriage of *Staphylococcus aureus* and prevention of nosocomial infections.** *Infection.* 2005; **33**(1): 3–8. [PubMed Abstract](#) | [Publisher Full Text](#)
2. Busato CR, Carneiro Leão MT, Gabardo J: ***Staphylococcus aureus* Nasopharyngeal Carriage Rates and Antimicrobial Susceptibility Patterns Among Health Care Workers and Their Household Contacts.** *Braz J Infect Dis.* 1998; **2**(2): 78–84. [PubMed Abstract](#)
3. Lin YC, Lauderdale TL, Lin HM, *et al.*: **An outbreak of methicillin-resistant *Staphylococcus aureus* infection in patients of a pediatric intensive care unit**

- and high carriage rate among health care workers. *J Microbiol Immunol Infect.* 2007; **40**(4): 325–34.
[PubMed Abstract](#)
4. Uhlemann AC, Knox J, Miller M, *et al.*: The environment as an unrecognized reservoir for community-associated methicillin resistant *Staphylococcus aureus* USA300: a case-control study. *PLoS One.* 2011; **6**(7): e22407.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
  5. Bhatta DR, Hamal D, Shrestha R, *et al.*: Nasal and Pharyngeal Colonization by Bacterial Pathogens: A Comparative Study between Preclinical and Clinical Sciences Medical Students. *Can J Infect Dis Med Microbiol.* 2018; **2018**: 7258672.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
  6. Bettin A, Causil C, Reyes N: Molecular identification and antimicrobial susceptibility of *Staphylococcus aureus* nasal isolates from medical students in Cartagena, Colombia. *Braz J Infect Dis.* 2012; **16**(4): 329–34.
[PubMed Abstract](#) | [Publisher Full Text](#)
  7. Carmona-Torre F, Torrellas B, Rua M, *et al.*: *Staphylococcus aureus* nasal carriage among medical students. *Lancet Infect Dis.* 2017; **17**(5): 477–478.
[PubMed Abstract](#) | [Publisher Full Text](#)
  8. Sakr A, Brégeon F, Mège JL, *et al.*: *Staphylococcus aureus* Nasal Colonization: An Update on Mechanisms, Epidemiology, Risk Factors, and Subsequent Infections. *Front Microbiol.* 2018; **9**: 2419.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
  9. de Steenhuijsen Pters WA, Sanders EA, Bogaert D: The role of the local microbial ecosystem in respiratory health and disease. *Philos Trans R Soc Lond B Biol Sci.* 2015; **370**(1675): pii: 20140294.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
  10. Turnbaugh PJ, Ley RE, Hamady M, *et al.*: The human microbiome project. *Nature.* 2007; **449**(7164): 804–10.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
  11. Ursell LK, Metcalf JL, Parfrey LW, *et al.*: Defining the human microbiome. *Nutr Rev.* 2012; **70** Suppl 1: S38–44.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
  12. Brooks AW, Priya S, Blekhan R, *et al.*: Gut microbiota diversity across ethnicities in the United States. *PLoS Biol.* 2018; **16**(12): e2006842.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
  13. Mason MR, Nagaraja HN, Camerlengo T, *et al.*: Deep sequencing identifies ethnicity-specific bacterial signatures in the oral microbiome. *PLoS One.* 2013; **8**(10): e77287.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
  14. Fettweis JM, Brooks JP, Serrano MG, *et al.*: Differences in vaginal microbiome in African American women versus women of European ancestry. *Microbiology.* 2014; **160**(Pt 10): 2272–2282.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
  15. Kaplan RC, Wang Z, Usyk M, *et al.*: Gut microbiome composition in the Hispanic Community Health Study/Study of Latinos is shaped by geographic relocation, environmental factors, and obesity. *Genome Biol.* 2019; **20**(1): 219.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
  16. Frank DN, Feazel LM, Bessesen MT, *et al.*: The human nasal microbiota and *Staphylococcus aureus* carriage. *PLoS One.* 2010; **5**(5): e10598.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
  17. NIH HMP Working Group, Peterson J, Garg S, *et al.*: The NIH Human Microbiome Project. *Genome Res.* 2009; **19**(12): 2317–23.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
  18. von Elm E, Altman DG, Egger M, *et al.*: The Strengthening of Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *J Clin Epidemiol.* 2008; **61**(4): 344–9.
[PubMed Abstract](#) | [Publisher Full Text](#)
  19. Kluytmans J, van Belkum A, Verbrugh H: Nasal carriage of *Staphylococcus aureus*: epidemiology, underlying mechanisms, and associated risks. *Clin Microbiol Rev.* 1997; **10**(3): 505–20.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
  20. Usyk M, Zolnik CP, Patel H, *et al.*: Novel ITS1 Fungal Primers for Characterization of the Mycobiome. *mSphere.* 2017; **2**(6): pii: e00488-17.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
  21. Caporaso JG, Lauber CL, Walters WA, *et al.*: Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proc Natl Acad Sci U S A.* 2011; **108** Suppl 1: 4516–22.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
  22. Wang Y, Qian PY: Conservative fragments in bacterial 16S rRNA genes and primer design for 16S ribosomal DNA amplicons in metagenomic studies. *PLoS One.* 2009; **4**(10): e7401.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
  23. Schmieder R, Edwards R: Quality control and preprocessing of metagenomic datasets. *Bioinformatics.* 2011; **27**(6): 863–4.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
  24. Masella AP, Bartram AK, Truszkowski JM, *et al.*: PANDAseq: paired-end assembler for illumina sequences. *BMC Bioinformatics.* 2012; **13**: 31.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
  25. Caporaso JG, Kuczynski J, Stombaugh J, *et al.*: QIIME allows analysis of high-throughput community sequencing data. *Nat Methods.* 2010; **7**(5): 335–6.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
  26. Edgar RC: Search and clustering orders of magnitude faster than BLAST. *Bioinformatics.* 2010; **26**(19): 2460–1.
[PubMed Abstract](#) | [Publisher Full Text](#)
  27. DeSantis TZ, Hugenholtz P, Larsen N, *et al.*: Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Appl Environ Microbiol.* 2006; **72**(7): 5069–72.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
  28. Tang J, Iliev ID, Brown J, *et al.*: Mycobiome: Approaches to analysis of intestinal fungi. *J Immunol Methods.* 2015; **421**: 112–121.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
  29. Rognes T, Flouri T, Nichols B, *et al.*: VSEARCH: a versatile open source tool for metagenomics. *PeerJ.* 2016; **4**: e2584.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
  30. Abarenkov K, Henrik Nilsson R, Larsson KH, *et al.*: The UNITE database for molecular identification of fungi—recent updates and future perspectives. *New Phytol.* 2010; **186**(2): 281–5.
[PubMed Abstract](#) | [Publisher Full Text](#)
  31. Team R: R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria. 2018.
[Reference Source](#)
  32. McMurdie PJ, Holmes S: phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One.* 2013; **8**(4): e61217.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
  33. Oksanen J, Kindt R, Legendre P, *et al.*: The vegan package. Community ecology package, R.F.f.S. Computing, Editor. Vienna, Austria. 2007; **10**: 631–637.
[Reference Source](#)
  34. Batdorf C: Coin-package. US. 1903.
  35. Wickham H: Reshape2: flexibly reshape data: a reboot of the reshape package. R package version 1. R.F.f.S. Computing, Editor. Vienna, Austria. 2013.
  36. Wickham H, Chang W: An implementation of the Grammar of Graphics. R package version. R.F.f.S. Computing, Editor. Vienna, Austria. 2013.
  37. Reyes N: Nasal Microbiome of Medical Students Udec. OSF. 2020.
<http://www.doi.org/10.17605/OSF.IO/UDNWA>
  38. Lee JT, Frank DN, Ramakrishnan V: Microbiome of the paranasal sinuses: Update and literature review. *Am J Rhinol Allergy.* 2016; **30**(1): 3–16.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
  39. Findley K, Oh J, Yang J, *et al.*: Topographic diversity of fungal and bacterial communities in human skin. *Nature.* 2013; **498**(7454): 367–70.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
  40. Wertheim HF, Melles DC, Vos MC, *et al.*: The role of nasal carriage in *Staphylococcus aureus* infections. *Lancet Infect Dis.* 2005; **5**(12): 751–62.
[PubMed Abstract](#) | [Publisher Full Text](#)
  41. Wertheim HF, Vos MC, Ott A, *et al.*: Risk and outcome of nosocomial *Staphylococcus aureus* bacteraemia in nasal carriers versus non-carriers. *Lancet.* 2004; **364**(9435): 703–5.
[PubMed Abstract](#) | [Publisher Full Text](#)
  42. Nouwen JL, Fieren MW, Snijders S, *et al.*: Persistent (not intermittent) nasal carriage of *Staphylococcus aureus* is the determinant of CPD-related infections. *Kidney Int.* 2005; **67**(3): 1084–92.
[PubMed Abstract](#) | [Publisher Full Text](#)
  43. von Eiff C, Becker K, Machka K, *et al.*: Nasal carriage as a source of *Staphylococcus aureus* bacteremia. Study Group. *N Engl J Med.* 2001; **344**(1): 11–6.
[PubMed Abstract](#) | [Publisher Full Text](#)
  44. Human Microbiome Project Consortium: Structure, function and diversity of the healthy human microbiome. *Nature.* 2012; **486**(7402): 207–14.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
  45. Costello EK, Lauber CL, Hamady M, *et al.*: Bacterial community variation in human body habitats across space and time. *Science.* 2009; **326**(5960): 1694–7.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
  46. Krüger B, Weidenmaier C, Zipperer A, *et al.*: The commensal lifestyle of *Staphylococcus aureus* and its interactions with the nasal microbiota. *Nat Rev Microbiol.* 2017; **15**(11): 675–687.
[PubMed Abstract](#) | [Publisher Full Text](#)
  47. Saez-Nieto JA, Medina-Pascual MJ, Carrasco G, *et al.*: *Paenibacillus* spp. isolated from human and environmental samples in Spain: detection of 11 new species. *New Microbes New Infect.* 2017; **19**: 19–27.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
  48. Pan H, Cui B, Huang Y, *et al.*: Nasal carriage of common bacterial pathogens among healthy kindergarten children in Chaoshan region, southern China: a cross-sectional study. *BMC Pediatr.* 2016; **16**(1): 161.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
  49. Huse SM, Ye Y, Zhou Y, *et al.*: A core human microbiome as viewed through 16S rRNA sequence clusters. *PLoS One.* 2012; **7**(6): e34242.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
  50. Andersen PS, Pedersen JK, Fode P, *et al.*: Influence of host genetics and environment on nasal carriage of *Staphylococcus aureus* in danish middle-aged and elderly twins. *J Infect Dis.* 2012; **206**(8): 1178–84.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
  51. Liu CM, Price LB, Hungate BA, *et al.*: *Staphylococcus aureus* and the ecology of the nasal microbiome. *Sci Adv.* 2015; **1**(5): e1400216.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

52. Eckburg PB, Bik EM, Bernstein CN, *et al.*: **Diversity of the human intestinal microbial flora.** *Science*. 2005; **308**(5728): 1635–8.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
53. Bogaert D, Keijser B, Huse S, *et al.*: **Variability and diversity of nasopharyngeal microbiota in children: a metagenomic analysis.** *PLoS One*. 2011; **6**(2): e17035.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
54. Chowdhary A, Kathuria S, Agarwal K, *et al.*: **Voriconazole-Resistant *Penicillium oxalicum*: An Emerging Pathogen in Immunocompromised Hosts.** *Open Forum Infect Dis*. 2014; **1**(2): ofu029.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
55. Cremers AJ, Zomer AL, Gritzfeld JF, *et al.*: **The adult nasopharyngeal microbiome as a determinant of pneumococcal acquisition.** *Microbiome*. 2014; **2**: 44.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

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Anne-Sofie Furberg

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The authors aim to assess the prevalence of *S. aureus* nasal carriage and *S. aureus* nasal carriage phenotypes among medical students in Colombia, and to examine whether *S. aureus* nasal carriage is associated with composition of the nasal microbiome. *S. aureus* nasal carriage among medical students and health care workers may represent a source for transmission of the microbe and infection in susceptible patient groups. *S. aureus* carriage is partly determined by interactions with other members of the microflora. According to the authors, this is the first study analyzing simultaneously the bacterial and fungal communities in the nares of healthy medical students with a Hispanic/Latino background. The study was done among second-year medical students at the same university during a six months period in 2018. The authors collected five repeated nasal swabs for *S. aureus* culturing from 143 students, and an additional nasal swab for microbiome analysis from a random sample of 15 students having different *S. aureus* carriage phenotypes. Using only data from the first set of nasal swabs, they report a prevalence of *S. aureus* carriage of 28%. Using data from all five time points, they report that 20% are persistent carriers. They show that nasal samples from persistent carriers had higher bacterial and fungal diversity than intermittent carriers and non-carriers, however not statistically significant. *S. aureus* carrier groups could not be separated by bacterial or fungal diversity.

The manuscript is generally well written and the results are easy to read from tables and figures. The classification of *S. aureus* carriage phenotypes by repeated sampling in a relatively large sample is a major strength. The analysis of *S. aureus* carriage in relation to the microbiome is timely and relevant. However, the present study has some limitations that should be addressed.

Microbiome data was collected at the end of the study among 15 students within the three *S. aureus* carriage phenotype groups. It would be interesting to know more about the stability of the nasal microbiome over time. Did the authors consider taking nasal swabs for microbiome analysis at another time point, e.g. at "baseline" when the first set of swabs for *S. aureus* culturing were taken? Please, consider rephrasing the sentences in Discussion page 9 "Here, a more diverse...microbiome ..seems to favor *S. aureus* carriage." and "Our results also suggest that

Candida species may favor the long-term *S. aureus* colonization of human nares.”, as the design of the present study may not be in line with these interpretations. Please, explain why the microbiome analysis was limited to five students in each group. Did the authors perform any power analysis?

S. aureus nasal colonization was assessed at five different time points. Could these data have been used to estimate *S. aureus* prevalence?

Abstract: The authors may consider a more consistent use of terminology; e.g. patterns of *S. aureus* nasal carriage or patterns of *S. aureus* colonization. First part of conclusion can be moved to results.

Introduction: The first sentence may be modified as the anterior nares are important due to several functions.

Methods: Five nasal swab samples for *S. aureus* culturing were collected with three weeks intervals. Please, describe how information about factors defined in exclusion criteria was collected and updated during the minimum 12 weeks period; e.g. recent infections, antibiotic use. Please, describe the procedure for specimen collection in more detail; i.e. how did the authors standardize the collection method, who did the sampling and where? The nasal swab for microbiome analysis was kept at room temperature and transported to New York, USA, for analysis. Please, include information about transport time and stability of the material from collection to laboratory analysis.

Results: It would be interesting to know more about the characteristics of the study population for comparison with other studies; e.g. sex, age- and BMI distribution. Results and figures: Please, include “statistically” when referring to the results of statistical tests; i.e. statistically non-significant.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Infectious disease epidemiology.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 09 Apr 2020

NIRADIZ REYES, University of Cartagena, Cartagena, Colombia

The authors are thankful for the Reviewer's recommendations. We greatly value your comments and consider that they have improved the quality of our article. We have followed them and modified the article accordingly.

Competing Interests: No competing interests were disclosed.

Reviewer Report 18 March 2020

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Marcelo Brocchi

Tropical Disease Laboratory, Department of Genetics, Evolution, Microbiology and Immunology, Institute of Biology, University of Campinas, Campinas, Brazil

This is an interesting study describing the *Staphylococcus aureus* carrying state among medical students in Colombia. The prevalence of MRSA strains was investigated and the association of the *S. aureus* carrying state with microbiome composition investigated in a minor number of individuals. It was observed that bacterial and fungal diversity was higher in individuals colonized by *S. aureus* than in non-carriers although the differences were non-significant. These results contributed with epidemiological information on *S. aureus* carrier state in Colombian medical students. Maybe, microbiome analysis in a greater number of individuals could reveal a more robust association with *S. aureus* carrier state. However, the data presented suggest some interesting associations that deserve publication. My only suggestion to the authors is the inclusion of a paragraph in the discussion section about microbiome composition and ethnic groups. Are there differences among Hispanic and other ethnic groups regarding microbiome composition and diversity?

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Microbiology and Genetics of Microorganisms.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 09 Apr 2020

NIRADIZ REYES, University of Cartagena, Cartagena, Colombia

The authors are thankful for the Reviewer's recommendations.
We have followed them and modified the article accordingly.

Competing Interests: No competing interests were disclosed.

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