**Microbial Ecology**

**Staphylococcus aureus** and the ecology of the nasal microbiome

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The human microbiome can play a key role in host susceptibility to pathogens, including in the nasal cavity, a site favored by *Staphylococcus aureus*. However, what determines our resident nasal microbiota—the host or the environment—and can interactions among nasal bacteria determine *S. aureus* colonization? Study of 46 monozygotic and 43 dizygotic twin pairs revealed that nasal microbiota is an environmentally derived trait, but the host’s sex and genetics significantly influence nasal bacterial density. Although specific taxa, including lactic acid bacteria, can determine *S. aureus* colonization, their negative interactions depend on thresholds of absolute abundance. These findings demonstrate that nasal microbiota is not fixed by host genetics and opens the possibility that nasal microbiota may be manipulated to prevent or eliminate *S. aureus* colonization.

**REPORT**

Our familiarity with the exterior of the nose belies the intriguing puzzle within. Individuals can have distinctive susceptibilities to nasal colonization by *Staphylococcus aureus*, a major pathogen (1); yet, it also appears that host genetics is not a significant determinant of *S. aureus* nasal colonization (2). How can this be? One potential explanation is that an individual’s susceptibility to *S. aureus* nasal colonization is driven by an environmentally determined phenotype. To satisfy this explanation, the phenotype should have limited association with host genetics, but it should predict *S. aureus* nasal colonization. Because the human microbiome is increasingly considered a host phenotype (3–5), we examined the potential role of nasal microbiota in *S. aureus* nasal colonization. Testing our hypothesis provided useful insight into the malleability of nasal microbiota and explanations for previous contradictory findings regarding *S. aureus* negative association with nasal bacteria such as *Propionibacterium* and *Staphylococcus epidermidis* (6–10). To test our hypothesis, we enrolled and analyzed the nasal microbiome of 46 monozygotic and 43 dizygotic twin pairs from The Danish Twin Registry.

Nasal bacterial density (that is, the total amount of nasal bacteria present) and microbiota composition (that is, the types and proportions of bacteria present in the nasal microbiota) were highly diverse among 178 healthy, community-dwelling middle-aged adults (Table 1). The median nasal bacterial density was $4.4 \times 10^6$ 16S ribosomal RNA (rRNA) gene copies per swab, and it spanned nearly four orders of magnitude, from $6.7 \times 10^5$ to $2.1 \times 10^9$ 16S rRNA gene copies per swab [interquartile range (IQR): $1.6 \times 10^6$ to $1.7 \times 10^7$]. Many bacteria were found in large proportions of subjects, such as *Corynebacterium* ($n = 157/178$, 88.2%), *Propionibacterium acnes* ($n = 149/178$, 83.7%), and *S. epidermidis* ($n = 161/178$, 90.4%), but proportional abundance varied substantially across individuals, contributing to distinctive microbiota compositions. We identified seven major nasal community state types (CST1 to CST7) among our participants (Fig. 1A to C). Each CST had a uniquely high prevalence and proportional abundance of specific nasal bacteria, as identified by indicator analysis: *S. aureus* defined CST1, *Enterobacteriaceae*—including *Escherichia* spp., *Proteus* spp., and *Klebsiella* spp.—defined CST2, *S. epidermidis* defined CST3, *Propionibacterium* spp. defined CST4, *Corynebacterium* spp. defined CST5, *Moraxella* spp. defined CST6, and *Dolosigranulum* spp. defined CST7 (table S1). The most prevalent nasal CST was CST4 ($n = 51/178$, 28.7%), followed by CST3 ($n = 40/178$, 22.5%) and CST1 ($n = 22/178$, 12.4%). CST6 was the least common, with only 5.6% prevalence ($n = 10/178$) (table S2). Thus, our study revealed distinctive nasal CSTs and greater nasal microbiota heterogeneity than previously reported (6–10), particularly among *Enterobacteriaceae*, of which *Proteus* and *Serratia* were not previously known to dominate the nasal microbiota.

**Was nasal microbiota significantly associated with host genetics?**

Host genetics played no significant role in nasal microbiota composition. Among monozygotic twin pairs, only 26.1% had the same nasal CSTs ($n = 12/46$) (Fig. 1C), which was comparable to the 25.6% among dizygotic twin pairs ($n = 11/43$) (fig. S1, A to C). We confirmed the limited similarity in nasal microbiota composition of monozygotic twin pairs by ecological distance–based analysis, where we found that nasal microbiota of monozygotic twins were not more similar than all or same-sex dizygotic twins, or than unrelated same-sex pairs (table S3).

In contrast, host genetics and nasal bacterial density were significantly linked. Nasal bacterial densities of monozygotic twin pairs were significantly more correlated than those of dizygotic twin pairs [sex- and age-adjusted intraclasse correlation coefficient (ICC) in monozygotic twins: 0.42, 95% confidence interval (CI): 0.12 to 0.65, and in dizygotic twins: $-0.06$, 95% CI: $-0.35$ to $0.23$]. The variations in nasal bacterial density were best explained by a model that comprised additive genetic and nonshared environmental effects (table S4). About 30% of the variation in nasal bacterial density was heritable (95%...
The sex of the host also significantly influenced nasal bacterial density, as indicated by the significantly different densities across CSTs (ANOVA, \( P < 0.001 \)). Bacterial density was highest in the two least prevalent CSTs: Enterobacteriaceae-dominated CST2 and Moraxella-dominated CST6; in contrast, bacterial densities were lowest in the two most prevalent CSTs: CST3 and CST4 (table S2). The distinctive densities across nasal CSTs indicate that density may be a unique feature of the individual nasal CSTs.

The sex difference in nasal bacterial densities was not due to men’s propensity for high-density nasal CSTs. We found no significant sex difference in nasal CST distribution (\( \chi^2 = 7.8, df = 6, P = 0.25 \)) (table S2). Overall, men had higher nasal bacterial density than women, irrespective of nasal CSTs (\( P < 0.001 \)) (table S6).

**Can the nasal microbiota predict \( S. \text{ aureus} \) nasal colonization?**

The rates and absolute abundance of \( S. \text{ aureus} \) differed among nasal CSTs (fig. S2, A and B). Some taxa predict the presence or absence of \( S. \text{ aureus} \), whereas others predict \( S. \text{ aureus} \) absolute abundance in a threshold-dependent fashion (Fig. 3A). Dolosigranulum spp. was the most informative predictor of the presence or absence of \( S. \text{ aureus} \). Specifically, the rate of \( S. \text{ aureus} \) nasal colonization among individuals at or above the Dolosigranulum threshold was 16.0\% (\( n = 4/25 \)), as compared with 56.0\% among the simulated population (\( n = 56/100 \)). Likewise, we observed threshold effects for nasal taxa such as Propionibacterium granulosum and \( S. \text{ epidermidis} \); however, \( P. \text{ granulosum} \) was negatively correlated with the presence of \( S. \text{ aureus} \), but \( S. \text{ epidermidis} \) was positively correlated (\( P. \text{ granulosum node } n = 4/34, 11.8\%; S. \text{ epidermidis node } n = 13/14, 92.9\% \) (Fig. 3A).

The \( S. \text{ aureus} \) absolute abundance model indicated that having low Corynebacterium abundance predicts high \( S. \text{ aureus} \) absolute abundance, that is, category 5, which comprised 10\,6 to 10\,7 (\( n = 23 \)) as compared to or above the Dolosigranulum threshold was 16.0\% (\( n = 4/25 \), as compared with 56.0\% among the simulated population (\( n = 56/100 \)). Likewise, we observed threshold effects for nasal taxa such as Propionibacterium granulosum and \( S. \text{ epidermidis} \); however, \( P. \text{ granulosum} \) was negatively correlated with the presence of \( S. \text{ aureus} \), but \( S. \text{ epidermidis} \) was positively correlated (\( P. \text{ granulosum node } n = 4/34, 11.8\%; S. \text{ epidermidis node } n = 13/14, 92.9\% \) (Fig. 3A).

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**Culture-negative \( S. \text{ aureus} \) nasal colonization**

In the current study, men and women did not differ in \( S. \text{ aureus} \) nasal colonization rates by DNA sequencing (women 52.9\%; men 52.6\%). This contradicted previous culture-based studies that have shown that men are more likely to be colonized by \( S. \text{ aureus} \) (2, 11–13). However, this discrepancy could be explained by the higher absolute abundance of \( S. \text{ aureus} \) in men and its influence on culture outcomes. Specifically, except in CST1, women frequently had 10- to 100-fold lower \( S. \text{ aureus} \) absolute abundance than men (Fig. 2B). At the same time, \( S. \text{ aureus} \) absolute abundance had a strong positive link to culture outcome. Each 10-fold increase in \( S. \text{ aureus} \) increased the probability of a positive culture by 30.0\% (\( r^2 = 0.33, P < 0.001 \)) (Fig. 2C). After adjusting for sex and other host factors, \( S. \text{ aureus} \) absolute abundance was the key determinant of culture-positive \( S. \text{ aureus} \) nasal colonization (\( r^2 = 0.33, P < 0.05 \)). This suggests that culture-based methods fail to identify a substantial proportion of \( S. \text{ aureus} \) carriers.
Fig. 1. The seven nasal CSTs and their respective bacterial densities shown in boxplots and composition shown in heatmap visualization and non-metric multidimensional scaling (nMDS) ordination plot. (A) In the boxplots, the box of each boxplot denotes the IQR (Q2-Q3) and the corresponding median, whereas the whiskers signify the upper and lower 1.5 × IQR, and the open circles denote outliers beyond the whiskers. The difference in bacterial density was significantly greater across than within CSTs [analysis of variance (ANOVA), P < 0.001]. In particular, CST3 had significantly lower bacterial density than all other CSTs except CST4, and CST2 had significantly higher bacterial density than all other CSTs except CST6 (two-tailed Wilcoxon rank sum, P < 0.05) (A). (B) In the heatmap visualization, each participant’s nasal microbiota is represented in a single column, and proportional abundance of each nasal bacterial taxon is shown by row according to the color key to the left. The nasal microbiota is grouped by CSTs, as indicated by the CST color bar above. The S. aureus culture result of each participant is noted by the green/black color bar above. (C) In the nMDS ordination plot, each participant’s nasal microbiota (in proportional abundance) is represented by a single data point, and data points that are closer have a more similar composition than those that are farther apart. The centroids and 95% confidence ellipse for each CST are shown.
trumps nasal bacterial density in determining nasal colonization. On the basis of the limited influence of host genetics on nasal bacterial density, in addition, although early environment had no significant influence in our cohort, which was middle age or older, it could play a role in a younger cohort. In this study, absolute abundance emerged as a critical factor in nasal bacterial density. In particular, the negative interactions between nasal taxa and S. aureus depended on absolute abundance thresholds, consistent with the ecological notion that absolute abundances, not relative abundances, reveal the importance of ecological interactions such as competition. Thus, the utility of nasal probiotics will rely on whether nasal microbiota composition trumps nasal bacterial density in determining S. aureus nasal colonization. On the basis of the limited influence of host genetics on S. aureus nasal colonization, we predict that the answer will be “yes.”

Fig. 2. Nasal bacterial density and S. aureus absolute abundance by sex and the relationship between S. aureus absolute abundance and S. aureus culture. (A) The scatterplot shows the higher nasal bacterial density in men than in women. Individuals (non-CST1) with detectable S. aureus nasal colonization could be divided on the basis of S. aureus absolute abundance into four categories. (B) Women were more likely to have the two lowest categories of S. aureus absolute abundance (that is, <10⁴ and 10⁴-10⁵), whereas men are more likely to have the middle two categories (that is, 10⁵-10⁶ and 10⁶-10⁷). (C) Culture outcome was strongly linked to S. aureus absolute abundance, and each 10-fold increase in S. aureus absolute abundance increases the probability of positive S. aureus culture by 30%, which suggests that the sex difference in S. aureus absolute abundance might explain the lower S. aureus culture rates in women than in men.

**MATERIALS AND METHODS**

This study was approved by the Science Ethics Committee for Southern Denmark (project number S-VF-19980072, addendum nos. 8 and 9) with appropriate informed consent following the guidelines of the approved protocol. At the Translational Genomics Research Institute, this study was approved as a study using coded specimens based on the Department of Health and Human Services Office of Human Research Protections Guidance on Coded Private Information of Biological Specimens.

We included 46 monozygotic and 43 dizygotic twin pairs (23 same-sex and 20 opposite-sex twin pairs) from an earlier study of The Danish Twin Registry cohort (2). Briefly, each participant’s anterior nares were sampled using Copan E-Swabs (Copan Diagnostics Inc.), which was placed immediately in 1 ml of Amies transport medium and stored at 4°C until S. aureus testing by standard nonselective medium, as previously described (2). The remaining swab eluant was frozen at −80°C until processing. Total DNA was prepared from 100 µl of eluent as previously described (16). Briefly, each aliquot was chemically lysed with RLT lysis buffer (Qiagen), mechanically lysed using the Barocycler (Pressure BioScience Inc.), and purified using Qiagen AllPrep DNA/RNA 96-well kit following the manufacturer’s instructions, with a final elution volume of 100 µl.
Fig. 3. Results from decision tree model derivation and validation showing threshold-dependent relationships between the absolute abundances of nasal commensals and S. aureus presence/absence. (A) Model predicting S. aureus presence/absence was derived using a randomly drawn group of 100; it showed that the most informative split was a threshold of $1.2 \times 10^6$ Dolosigranulum 16S rRNA gene copies per swab. Having above-threshold Dolosigranulum predicts absence of S. aureus ($n = 4/25, 16.0\%$), as compared to S. aureus nasal colonization rate in the overall derivation group ($n = 56/100, 56\%$). Simonsiella had a similarly negative relationship to S. aureus, where, among individuals who had below-threshold abundance of Dolosigranulum, having $\geq 1.1 \times 10^5$ Simonsiella predicts the absence of S. aureus ($n = 1/7, 14.3\%$). (B) Validation testing using 10 randomly drawn groups of 100 supported the threshold-based relationships between Dolosigranulum, Simonsiella, P. granulosum, and S. epidermidis and S. aureus presence/absence.
We measured nasal bacterial density using a broad-coverage quantitative polymerase chain reaction (17) and characterized nasal microbiota composition by 16S rRNA gene-based sequencing and taxonomic classification, as previously described (18), with some modifications including a custom classifier for classification of Staphylococcus species. Using the taxonomically classified sequence data, we calculated the proportional abundance for each nasal bacterial taxon as: (number of sequences assigned to the taxon from the sample)/(total number of sequences from the sample), which we combined with nasal bacterial density to calculate taxon-specific 16S rRNA gene absolute abundance: (proportional abundance of the taxon from the sample) × (nasal bacterial density of the sample) (18). Additional methodological details, including development and validation of the custom Staphylococcus classifier, can be found in the Supplementary Materials.

All ecological and statistical analyses were performed in R version 3.0.1 (19). CSTs were identified using a proportional abundance-based matrix in Euclidean distance by hierarchical clustering with Ward linkage, as previously described (20). Through an iterative process, we determined a parsimonious number of distinct CSTs and the CST of each participant. We visualized the nasal microbiota in each twin type and in individuals with each nasal CST by heatmap and nonmetric multidimensional scaling using the vegan package (R package version 2.1-10) (21) and examined the prevalence of each nasal CST in our study population.

We identified the indicator bacteria for each nasal CST using indicator analysis in the labdsv package (R package version 1.6-1) at α = 0.05, adjusted for false discovery (22). To assess the contribution of host genetics to nasal microbiome composition and bacterial density, we first assessed nasal CST concordance in twin pairs and difference in pairwise ecological distance between twin types. Next, we compared within-pair nasal bacterial density in each twin type by ICCs and its 95% CI based on variance components of a one-way ANOVA and the exact confidence limit using the ICC package (23).

We estimated the relative contribution of genetic and environmental factors to nasal bacterial diversity using a multivariate linear regression model. To determine nasal microbiome constituents that predict nasal bacterial density and nasal microbiome composition, we assessed the influence of nasal CST, sex, history of atopic disease and psoriasis, smoking status. We compared the nasal bacterial density across CSTs by ANOVA. We evaluated host sex, history of atopic disease and psoriasis, and smoking status with nasal bacterial density using a quasi-Poisson model. To determine nasal microbiome constituents that predict S. aureus colonization or 16S rRNA gene absolute abundance, we applied decision tree analysis with recursive partitioning and splitting by information criteria in the rpart package (24) in the derivative and validation stages. We further assessed the correlation between S. aureus 16S rRNA gene absolute abundance and culture outcome using a multivariate linear regression model.

**SUPPLEMENTARY MATERIALS**

Supplementary material for this article is available at http://advances.sciencemag.org/cgi/content/full/1/5/e1400216/DC1

Fig. S1. Correlation of nasal microbiota composition among monzygotic and among same-sex and opposite-sex dizygotic twin pairs in non-metric multidimensional scaling ordination plots. Fig. S2. Rates of S. aureus nasal colonization by sequencing and by culture and S. aureus absolute abundance for the seven nasal CSTs.

**REFERENCES AND NOTES**


RESEARCH ARTICLE


25. R. C. Edgar, Search and clustering orders of magnitude faster than BLAST.


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