MAJOR ARTICLE

# Influence of Host Genetics and Environment on Nasal Carriage of *Staphylococcus aureus* in Danish Middle-Aged and Elderly Twins

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***Background.*** Nasal carriage is a major risk factor for *Staphylococcus aureus* infection. Approximately, one-quarter of adults carry *S. aureus*. However, the role of host genetics on *S. aureus* nasal carriage is unknown.

***Methods.*** Nasal swabs were obtained from a national cohort of middle-aged and elderly Danish twins. Subjects colonized with *S. aureus* were identified by growth on selective plates and *spa* typing. A second sample was obtained from twins initially concordant for carriage. Twins found to again be colonized with *S. aureus* were defined as persistent carriers.

***Results.*** The prevalence of *S. aureus* carriage among 617 twin pairs (monozygotic/dizygotic pairs: 112/505) was 26.3% (95% confidence interval [CI], 24.0%–28.9%). The concordance rate for carriage did not differ significantly between pairs of monozygotic (37.5%; 95% CI, 22.3%–53.8%) twins and same sex (24.2%; 95% CI, 15.4%–34.5%), and opposite sex (21.4%; 95% CI, 12.0%–33.4%) dizygotic twins. Despite shared childhoods, only 1 of 617 pairs was concordant with respect to lineage. Although heritability increased for *S. aureus* and lineage persistency, no significant heritability was detected.

***Conclusion.*** In this study, host genetic factors exhibited only a modest influence on the *S. aureus* carrier state of middle-aged and elderly individuals.

*Staphylococcus aureus* is a leading cause of life-threatening infections [1]. Paradoxically, it also asymptomatically colonizes approximately 20%–30% of healthy adults [2]. Nasal carriage of *S. aureus* has been shown to be a major risk factor for subsequent *S. aureus* infection [3, 4].

The host genetic contribution to nasal carriage of *S. aureus* is incompletely understood. Only 2 small twin studies, both published >30 years ago and collectively totaling <100 pairs, have been conducted, with conflicting results [5, 6]. Both studies severely lacked statistical power, and the general problem has been that there has not been a sufficiently large and phenotypically well-defined study population available.

In the present study we used a unique resource of a large Danish twin registry to examine potential associations between host genotype and *S. aureus* nasal carriage. We hypothesized that *S. aureus* carriage would be higher in monozygotic (MZ) twins than in dizygotic (DZ) twins, and we tested this hypothesis in a large, national cohort of middle-aged and elderly twins.

## MATERIALS AND METHODS

### Study Population

#### *The Danish Twin Registry*

The Danish Twin Registry is a nationwide, population-based registry established in 1954. It includes data from >75 000 twin pairs born between 1870 and 2004 [7]. Zygosity of the twin couples was established through a questionnaire on the degree of similarity

Received 27 March 2012; accepted 4 May 2012; electronically published 7 August 2012.

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**The Journal of Infectious Diseases 2012;206:1178–84**



DOI: 10.1093/infdis/jis491

between twins in a pair. The zygosity classification in the registry has been evaluated by comparison with genetic markers; the proportion of misclassifications was <5% [8]. Since the 1960s, selected cohorts from the registry have participated in questionnaire and survey studies.

In the period 2008–2011, twins born in Denmark between 1931 and 1969 were invited to a clinical investigation in 1 of 5 centers in Denmark. Nasal swabs for determination of the presence of *S. aureus* in the nasal bacterial flora were part of the primary examination from 1 April 2008 to 1 April 2010. Secondary self-administered swabbing was obtained from the subgroup of initially concordant *S. aureus* colonizers from 1 April 2011 to 1 July 2011, resulting in a total of 2231 nasal swabs. The study was approved by the Science Ethics Committee of Southern Denmark (project number S-VF-19980072, addendum nos. 8 and 9) with appropriate informed consent following the guidelines of the approved protocol.

## Laboratory Methods

### *Determination of S. aureus Colonization in the Anterior Nares*

Nasal swabs were taken using Copan nasal E-Swabs (Copan, Italy) and bacterial transport medium. The swabs were plated on *S. aureus* chromID selective plates (bioMérieux) no more than 72 hours after the sample was taken and incubated for 48 hours. Identification of *S. aureus* was based on colony morphology and catalase (SSI Diagnostika) and latex-agglutination (Slidex Staph Plus; bioMérieux) tests. For concordant twins colonized with *S. aureus* in the initial sampling, a second sample was requested a year after the first sampling in order to investigate heredity versus persistent carriage. Persistent carriage was determined as colonization at both samplings [9].

Confirmation of *S. aureus* colonization and identification of clonal lineages were established by performing *spa* typing of the *S. aureus* isolates by DNA sequencing. Clonal lineage associations were determined by multilocus sequence typing (MLST) mapping using Ridom Spa Server (http://www.spaserver.ridom.de) or by comparison with similar *spa* types or *spa* repeat compositions to MLST typed isolates at the Danish National Staphylococcal Reference Laboratory at Statens Serum Institut, with subsequent grouping using eBURST (http://saureus.mlst.net/eburst).

## Statistical Analysis

### *Analyses of Twin Similarity*

We assessed the similarity of MZ and DZ twins using proband-wise concordance rates and tetrachoric correlations for nasal carriage of *S. aureus*. The classic twin study methodology is based on the assumption that MZ twins have identical genotypes, whereas DZ twins share, on average, half of their segregating genes and thus are no more genetically related than biological siblings [10]. A greater phenotypic similarity in MZ twins than in DZ twins is expected if there is a substantial genetic component in the etiology of the condition.

### *Concordance Rates*

The proband-wise concordance rate is defined as the proportion of affected twin partners of probands. It reflects the probability that 1 twin will be a carrier of *S. aureus*, given that the twin partner was a carrier. Thus, it is directly comparable with risk rates reported for other relatives [11].

### *Correlation*

The correlations between twin and co-twin attributable to the dichotomous outcome (ie, colonization of *S. aureus*) were investigated by assuming an underlying bivariate normally distributed liability (susceptibility) to a condition (nasal colonization of *S. aureus*) because of genetic and environmental factors. The manifestation of a condition is established when an individual exceeds the threshold of affliction on the liability distribution, and the impact of genetic and environmental effects is reflected in the similarity of the other twin's liability to the condition [10]. We estimated the correlations in liability by using a threshold model [12] and the Mx software package [13]. Likelihood-based confidence intervals were estimated by structural equation modeling, as described in detail elsewhere [10].

### *Heritability*

According to standard biometric practice, when estimating heritability as the proportion of total phenotypic variance attributable to genetic variance in the population, we assumed no epistasis (gene–gene interaction), no gene–environment interaction or correlation, and no assortative mating with respect to loci affecting the risk of S. *aureus* carriage. The phenotypic variance can then be separated into four variance components: variance attributable to additive genetic effects (A), genetic dominance (D), shared environment (C), and nonshared (individual-specific) environment (E) [10]. Only nonshared environments contribute to dissimilarity within MZ twin pairs because of their presumed genetic identity, whereas the effects of additive genetic factors and genetic dominance may also contribute to dissimilarity within DZ pairs, who share, on average, half of the additive and one-quarter of the dominant genetic factors. The method for selecting the best model followed standard procedures (structural-equation analyses) [10]. Because the effects of genetic dominance (D) and shared environment (C) are completely confounded in the classic study of twins reared together, it is not possible to estimate all parameters simultaneously in a single model [10]. Thus, we fitted 5 models (ACE, ADE, AE, CE, and E) to the data. The best model is considered one that fits the data well (by a $\chi^2$ goodness-of-fit test based on the log-likelihood difference of nested models) and is the most

parsimonious (ie, none of the parameters in the model can be deleted without a substantial increase in the $\chi^2$ value). To compare non-nested models, we used the Akaike information criterion (AIC; $-2 \times$ log-likelihood – twice the degrees of freedom). The model with the lowest AIC represents the best balance of goodness of fit and parsimony [14]. To compare nested models, we used the $\chi^2$ difference test (ie, $\Delta\chi^2 = 2 \times \Delta$log-likelihood of the nested models). The $\chi^2$ difference of the models is distributed as a $\chi^2$ statistic, with the degrees of freedom equal to the difference in the degrees of freedom of the models being compared.

## RESULTS

During the 2-year study period, a total of 2213 individuals were included, comprising 1264 twins from "intact pairs" (both twins participated) and 949 twins from "broken pairs" (only 1 twin from the pair participated). Dismissing twins with their co-twin not in the sample and twins with unknown or uncertain zygosity as well as triplets resulted in 617 twin pairs (112 MZ twin pairs, 262 same sex (SS) DZ twin pairs and 243 opposite sex (OS) DZ twin pairs) were enrolled in the study. The age distributions among men and women were similar, with 55.2% of the men aged <63 years and 53.3% of the women aged <63 years. Monozygotic twins were somewhat younger than the SS DZ and OS DZ twins, with 70.1% of the MZ twins aged <63 years compared with 51.1% of the SS DZ twins and 50.2% of the OS DZ twins. The distribution of gender was 1-to-1 for MZ, SS DZ, and OS DZ twins.

### Prevalence of *S. aureus* Nasal Carriage

The overall prevalence of *S. aureus* nasal carriage was 26.3% (95% confidence interval [CI], 24.0%–28.9%). Males were significantly more likely to be carriers than females (males, 30.1%; 95% CI, 26.6%–33.9% vs females, 22.6%; 95% CI, 19.6%–26.1%; $P = .003$). Also, the prevalence in the younger half of the individuals (29.2%; 95% CI, 26.0%–32.5%) was higher than that in the older half (23.0%; 95% CI, 19.5%–26.8%).

The patterns of concordant and discordant pairs among MZ, SS DZ, and OS DZ twins are shown in Table 1. The overall prevalence of *S. aureus* in the twin sample was 26.3% (95% CI, 24.0%–28.9%). The prevalence did not differ among MZ twins (28.6%; 95% CI, 22.7%–35.3%), SS DZ twins (28.4%; 95% CI, 24.8%–32.3%), and OS DZ twins (23.0%; 95% CI, 19.5%–27.0%). Comparing concordance rates across zygosity, similar concordance rates were observed among SS DZ (24.2%; 95% CI, 15.4%–34.5%) and OS DZ twins (21.4%; 95% CI, 12.0%–33.4%), whereas the concordance rate among MZ twins (37.5%; 95% CI, 22.3%–53.8%) was somewhat higher, although the differences were not statistically significant (Table 1). Only 2 dizygotic twin pairs were cohabitating;

**Table 1. Prevalence and Concordance Rates of *Staphylococcus aureus* Nasal Carriage in Twins**

| Categories | Pairs | Concordant | Discordant | Prevalence (%) | 95% CI (%) | Concordance Rate (%) | 95% CI (%) |
|---|---|---|---|---|---|---|---|
| All | | | | | | | |
| | 617 | 42 | 241 | 26.3 | 24.0–28.9 | 25.8 | 19.7–32.7 |
| Zygosity | | | | | | | |
| MZ | 112 | 12 | 40 | 28.6 | 22.7–35.3 | 37.5 | 22.3–53.8 |
| SS DZ | 262 | 18 | 113 | 28.4 | 24.8–32.3 | 24.2 | 15.4–34.5 |
| OS DZ | 243 | 12 | 88 | 23.0 | 19.5–27.0 | 21.4 | 12.0–33.4 |
| Zygosity × Gender | | | | | | | |
| MZ × Male | 53 | 6 | 21 | 31.1 | 22.7–41.0 | 36.4 | 15.9–59.4 |
| MZ × Female | 59 | 6 | 19 | 26.3 | 18.6–35.8 | 38.7 | 17.1–62.2 |
| SS DZ × Male | 129 | 12 | 56 | 31.0 | 25.7–36.9 | 30.0 | 17.3–44.7 |
| SS DZ × Female | 133 | 6 | 57 | 25.9 | 21.3–31.2 | 17.4 | 6.9–32.8 |
| OS DZ × Male | | | | 28.8 | 23.5–34.8 | | |
| OS DZ × Female | | | | 17.3 | 13.0–22.6 | | |
| Zygosity × Age | | | | | | | |
| MZ × 44–62 | 79 | 7 | 28 | 26.6 | 20.0–34.4 | 33.3 | 15.6–53.9 |
| MZ × 62–79 | 33 | 5 | 12 | 33.3 | 22.1–46.8 | 45.5 | 18.7–71.8 |
| SS DZ × 44–62 | 135 | 11 | 73 | 35.2 | 30.2–40.5 | 23.2 | 12.6–36.4 |
| SS DZ × 63–79 | 127 | 7 | 40 | 21.3 | 16.5–29.9 | 25.9 | 11.7–44.1 |
| OS DZ × 44–62 | 122 | 4 | 51 | 24.2 | 19.5–29.5 | 13.6 | 4.0–29.9 |
| OS DZ × 63–79 | 121 | 8 | 37 | 21.9 | 16.9–27.9 | 30.2 | 14.8–48.5 |

Abbreviations: CI, confidence interval; DZ, dizygotic; MZ, monozygotic; OS, opposite sex; SS, same sex

**Table 2. Estimates of the Within Twin Pair Tetrachoric Correlations Under the Conditions of Same Prevalence for Twins in a Twin Pair and for Monozygotic and Dizygotic Twins**

| Zygosity | Number of Twin Pairs | Tetrachoric Correlation | 95% CI |
|---|---|---|---|
| All | 617 | −0.01 | −.15 to .13 |
| Monozygotic | 112 | 0.21 | −.10 to .50 |
| Dizygotic | 505 | −0.07 | −.23 to .09 |
| Monozygotic | 112 | 0.21 | −.10 to .50 |
| SS Dizygotic | 262 | −0.10 | −.30 to .11 |
| OS Dizygotic | 243 | −0.03 | −.28 to .22 |

Abbreviations: OS, opposite sex; SS, same sex.

one SS pair was discordant for *S. aureus* carriage, and one OS pair was concordant for noncarriage. The concordance rate in the MZ sample was slightly greater than the overall prevalence, suggesting a possible genetic influence on the risk of being a colonizer of *S. aureus*, but this was not the case for the DZ sample. Similarly, tetrachoric correlations were essentially 0, both among SS DZ twins (ρ = −0.10; 95% CI, −.30 to .11) and among OS DZ twins (ρ = −0.03; 95% CI, −.28 to .22), and although there was a tendency toward a positive correlation among MZ twins (ρ = 0.21; 95% CI: −.10 to .50), this was not significant (Table 2).

In agreement with this finding, the structural-equation analyses showed that the model with nonshared effects (E) as the only term was the best-fitting model (−2 log-likelihood = 1422.934; parameters = 2) (Table 3). Consequently, the biometric modeling did not provide evidence for a genetic influence on the susceptibility to be a carrier of *S. aureus*. Also in agreement, the ADE model showed that an estimated 12% (95% CI, 0%–39%) of the phenotypic variation could be ascribed to genetic factors, but as expected, the heritability estimate in this model was not statistically significant from 0. The ADE model ascribed 88% (95% CI, 61%–100%) of the variation in liability to nonshared effects. Adjusting for age and gender differences in prevalence left estimates virtually unchanged and did not change conclusions on heritability. A comparison of tetrachoric correlations between MZ, SS DZ, and OS DZ twins stratified on gender and age was also performed and showed no statistically significant differences, neither between different zygosities within strata nor between different strata (data not shown). It should, however, be noted that in order to justify a comparison of tetrachoric correlations across zygosities, the prevalence should be the same for the different zygosities. In the comparison of prevalence in the stratified analysis, no differences between MZ and SS DZ twins was observed, but the OS DZ twins had a statistically significantly lower prevalence ($P = .02$). We could find no biological explanation for such a difference, and interpreting it as a spurious result, the stratified comparison of tetrachoric correlations among MZ, SS DZ, and OS DZ twins was performed under the assumption of equal prevalence within each stratum.

**Table 3. Biometric Models for Heritability Analysis of *Staphylococcus aureus* Nasal Carriage in 617 Twin Pairs**

| Model[a] | − 2 Log-Likelihood | Degrees of Freedom (df) | $\Delta\chi^{2}$ [b] | Δdf | *P* Value |
|---|---|---|---|---|---|
| ACE | 1422.816 | 1231 | | | |
| AE | 1422.816 | 1232 | 0.000 | 1 | 1.00 |
| CE | 1422.934 | 1232 | 0.118 | 1 | .73 |
| E | 1422.934 | 1233 | 0.118 | 2 | .94 |
| ADE | 1422.274 | 1231 | | | |
| AE | 1422.816 | 1232 | 0.542 | 1 | .46 |
| E | 1422.934 | 1233 | 0.660 | 2 | .72 |

[a]A, additive genetic component; C, shared environmental component; D, dominant genetic component; E, unique environmental component.

[b]Difference between −2 log-likelihood of the model and that of the ACE model for the first 4 rows and that of the ADE-model for the last 3 rows.

### Bacterial Genotyping

Nasal strains from all *S. aureus* colonized individuals included in the study were classified into lineages based on *spa* typing, and only one twin pair was concordant in respect to *spa* type. The majority of isolates (n = 462) were grouped into 18 clonal lineages, and 10 were not assigned any clonal relation. A total of 146 different *spa* types were found. Overall, we found the majority of isolates to belong to CC45 (n = 109), CC30 (n = 107), CC15 (n = 46), CC1 (n = 28), and CC5 (n = 27), with the remaining 155 belong to at least 13 other genetic lineages.

### Analysis of Persistent Carriers

A second swab was taken 1 year after the initial swab from the 42 twin pairs concordant for *S. aureus* carriage. A total of 74 twins (88%) responded with a second sample, resulting in 11 MZ and 21 DZ twin pairs for whom both twins had provided a second swab. A total of 60 of the 74 individuals (81%) were persistent carriers. Among the MZ twins, 7 of 11 pairs (64%) were still concordant, whereas among the DZ twins, 18 of 21 pairs (86%) were still concordant with respect to *S. aureus* carriage. Assuming the same 81% recurrence of carriage of *S aureus* among carriers from discordant twin pairs, concordance rates, tetrachoric correlations, and heritability were estimated. All estimates were similar to those found in the analyses based on a single culture, as were the statistical conclusions. In particular, there was no statistically significant genetic effect on carrier status. In the ADE model, which was the best fitting nonparsimonious model, the heritability was estimated to be 20.5% (95% CI, 0%–49.5%).

Assuming that discordance with respect to lineage could imply intermittent carriage, lineage concordance with respect to *spa* type was investigated. Overall, 41 of 74 (55%) second-time *S. aureus* carriers were persistent with respect to lineage. Four of 11 MZ pairs (36%) and 11 of 21 DZ pairs (52%) were persistent with respect to lineage (*spa* type) at the 2 samplings. There were no significant differences in concordance rates between MZ and DZ twins. By assuming the 55% recurrence of lineage carriage found among the first-time carrier concordant pairs to also be representative for recurrence among the carrier twins from the first-time discordant pairs, there was no statistically significant genetic effect on carrier status. In the best-fitting nonparsimonious model (ADE), we found a heritability estimate of 29.6% (95% CI, 0%–62.8%).

## DISCUSSION

Nasal carriage of *S. aureus*, the major risk factor for invasive *S. aureus* serious infections with high mortality [3, 4], is a complex condition determined by both bacterial and host genetics as well as environmental factors. We have collected the largest reported cohort of twin nasal samples and investigated the heredity of *S. aureus* nasal carriage in a sample size >6 times the size of all previous twin studies performed [5, 6]. We furthermore performed a long-term follow-up on concordant twin pairs to evaluate the role of host genetics for long-term carriers, which has not been performed in earlier studies. The size of the study and the use of robust twin statistical methods make the results and conclusions of this study very strong.

Our study clearly demonstrates that the host genetic contribution to nasal colonization with *S. aureus* is, at most, very limited. This conclusion is consistent with other recent studies among Amish families [15] and among a large Dutch genome-wide association study on Dutch nasal carriers (A. van Belkum, personal communication). Early cross-sectional concordance studies of *S. aureus* nasal carriage were either too small to clearly determine heritability [5, 6] or contained cohabitating individuals [16] that might have increased carriage prevalence. The large number of both MZ and DZ twins in this study allowed for a more thorough estimation of heritability for *S. aureus* colonization and carriage.

A number of studies estimate the prevalence of *S. aureus* colonization in the general population to be approximately 20%–32% [2, 16, 17]. In the present study, we found a prevalence of 325 carriers in 1234 individuals, corresponding to 26.3%, which shows good accordance with these general findings as well as with the average prevalence estimate of 27% [18]. Moreover, it is estimated that approximately 20% of the general population are persistent carriers [4]. Therefore, in a cross-sectional sample such as ours, approximately 20% of the 27% should also be carriers at a second culture, corresponding to a recurrence percentage of about 20 of 27 (74.1%), which is very similar to the 60 of 74 (81.1%) we found in our sample of first-time carrier-concordant twins. Because this sample does not differ with respect to recurrence when compared with the general population, it is not likely that it should differ from the recurrence in the sample of first-time carrier-discordant twins either.

Our study confirms the gender bias of nasal carriage, with men more prevalent carriers than women [19]. Only 1 twin pair of 617 twin pairs carried *S. aureus* isolates of the same clonal lineage, indicating that twins are not generally colonized with the same isolate and that the nasal *S. aureus* is not transferred from one twin to the other. Thus, our study clearly shows that despite common environment and, particularly for the MZ twins, closer relationships than most siblings, the childhood environment does not determine the long-term *S. aureus* carried in the nose. In early childhood, there is a high prevalence of *S. aureus* colonization [20, 21], which decreases to adult levels in the teens [4]. To our knowledge, no study has addressed the long-term carriage from childhood into adulthood.

Our results from 2 samplings are also in agreement with previous findings for which different approaches were followed to determine persistent carriage [9, 22–24] because we found 21% persistent carriers overall. Persistent carriage of *S. aureus* as determined from 2 nasal swabs is also not a strongly heritable trait; we could not find an overrepresentation of concordant persistent *S. aureus* carriers among the MZ twins. Assuming that 81% of the first carriers among the first carrier discordant twins are *S. aureus* persistent carriers, we find a heritability estimate of 20.5%, (assuming an ADE model, which is the best fitting nonparsimonious model for these predicted tables). This heritability estimate is not significantly statistically different from 0 and is quite similar to the heritability estimate for *S. aureus* colonization of 12%.

Individuals carry the same strain over time in more than half of cases, as we observed among the first-time concordant twins with a long-term carriage of the same clonal lineage in approximately 55% of the individuals. Other studies have implicated that only carriers of the same clone should be considered truly persistent carrier [24–26] because change of nasal isolate could also implicate intermittent carriage; we therefore also investigated whether persistence of the same clonal isolate over time was influenced by genetic factors. However, assuming a 55% chance of same clonal lineage among first carrier twins of discordant pairs yielded results similar to those seen for first-time carriers and persistent carriers, with no statistically significant genetic component, and only a modest heritability estimate of 29.6% (95% CI, 0%–62.8%), although there was a trend with higher heritability estimates in the analyses of *S. aureus* persistent and lineage carriers.

Previous studies have suggested that host genetics are important determinants of *S. aureus* nasal carriage. Different

results have been obtained from candidate gene case–control studies with both genetic association to glucocorticoid receptor gene [27] and genes encoding interleukin 4, complement factor H, C-reactive protein [28], and HLA [29] and no genetic association to the Vitamin D receptor [30] or beta-defensins [31]. Neither of the former studies has so far been confirmed in other populations. Our present study does not preclude such findings, and there certainly may be genetic factors weakly associated with carriage.

The present study, although the largest so far, is still limited in power to identify modest heritability. The number of samplings required to identify persistent carriers and noncarriers has been debated over time, and the more samplings that are performed, the more accurate the classification [22, 32]. However, due to limited access to test persons, this study relied on only 2 samplings, similar to Olsen et al [9], which has been shown to have a sensitivity of 95.5% and specificity of 88.8% in the detection of persistent carriers [22]. Another limitation of the study may be the age groups investigated because genetic association may be age dependent as carriage in general is. Carriage among elderly people appears to differ from that among younger people [23].

What may determine *S. aureus* carriage if the strains themselves alone cannot determine carriage [19] and there is little heritability? Gene–environment interactions appear to be the most likely determinants, as has recently been shown in a large Norwegian study in which smoking and vitamin D levels were shown to be associated with *S. aureus* nasal carriage [9]. The microbial community may be another important factor. A recent study implicated that nasal carriage of specific strains of *Staphylococcus epidermidis* appeared to exclude carriage of *S. aureus* [33, 34], and another study concluded that nasal microbiomes may be grouped into 12 supergroups, with *S. aureus* present in 2 but absent in others [35]. Thus, limited direct host heritability together with these intriguing recent findings suggests a very complex background for *S. aureus* nasal carriage, and further studies in the area of micro- and macro-environment are clearly warranted.

## Notes

***Acknowledgments.*** We are very grateful to all twin participants for their interest in participating in the study. We are also grateful to all nurses and technicians involved in taking samples and we are particularly grateful to Lotte Corneliussen and Elvira Chapka for their help with characterizing and typing the *S. aureus* isolates.

***Financial support.*** V. G. F. was supported by the National Institutes of Health (R01-AI068804). The Danish Twin Registry is supported by a grant from The National Program for Research Infrastructure 2007 from the Danish Agency for Science Technology and Innovation.

***Potential conflicts of interest.*** All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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