Risk factors for \textit{Staphylococcus aureus} nasal colonization in Danish middle-aged and elderly twins

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Abstract \textit{Staphylococcus aureus} is a human commensal bacterium found in the nasal cavity and other body sites. Identifying risk factors for \textit{S. aureus} nasal carriage is of interest, as nasal carriage is a risk factor for subsequent invasive infection. We recently investigated the influence of host genetics on \textit{S. aureus} carriage in Danish middle-aged and elderly twins, which indicated no significant heritability that could account for the observed \textit{S. aureus} carriage. In the present study, we performed a questionnaire-based study of \textit{S. aureus} colonization on the same cohort of 2,196 Danish middle-aged and elderly twins to identify specific risk factors for \textit{S. aureus} nasal colonization, including analyzing the paired twins (\( n=478 \)) that were discordant for \textit{S. aureus} colonization. We found associations between risk factors and \textit{S. aureus} nasal colonization among middle-aged and elderly twins, including age, male gender, psoriasis, and atopic diseases. Also, present living on a farm is clearly associated with \textit{S. aureus} colonization, while smoking had a borderline statistically significant protective effect.

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Introduction

\textit{Staphylococcus aureus} is an opportunistic pathogen that normally is found as a commensal of the human skin, as well as in the nasal cavity. In cross-sectional studies, about 20–40 % of the adult population is colonized with \textit{S. aureus} [1–5]. Three types of carriage have been described: permanent carriage, i.e., carriage over a prolonged period of time and most often with the same strain (approximately 20 %), intermittent carriage (approximately 30 %), and non-carriage (50 %) [2, 6]. These may be classified as only two groups, carriers and non-carriers [7], as the intermittent carriage and non-carriage groups are similar in respect to the risk of acquiring \textit{S. aureus} infection. We recently reported that the \textit{S. aureus} carriage rate in a large cohort of elderly Danish twins was 24.7 % and was not primarily due to genetic factors [8]. However, identifying risk factors for \textit{S. aureus} carriage is of major concern, as nasal carriage is a significant risk factor for subsequent invasive infection [6, 9].

In the present study, we used a large Danish twins cohort to examine potential associations between putative risk factors and \textit{S. aureus} nasal colonization. We investigate the cohort both as individuals and also by pairing the twins, taking into account that their upbringings have been similar.

Materials and methods

Study population

The Danish Twin Registry is a nationwide, population-based registry established in 1954 containing data of >85,000 twin pairs born between 1870 and 2009. In the period 2008–2011, twins born in Denmark between 1931 and 1969 were invited to a clinical investigation in one of five centers in Denmark. Nasal swabs for the determination of the presence of \textit{S. aureus} in the nasal bacterial flora were collected, resulting in a total of
2,213 nasal swabs. The 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 [8, 10].

Determination of *S. aureus* colonization in the anterior nares

Colonization was determined by isolating *S. aureus* from nasal swabs, as previously described [8]. Briefly, the anterior nares were swabbed using E-swabs (Copan, Italy) and transferred directly to carrier medium. *S. aureus*-positive swabs were determined by culturing on *S. aureus*-selective plates (chromID, bioMérieux, France) for 48 h and positive colonies were identified by colony morphology and catalase test (SSI Diagnostika, Denmark).

Epidemiological data

The twins in the study were asked to fill out two questionnaires. One concerned diseases, infections, environment, and contact with animals which were variables expected to be associated with the carriage of *S. aureus*. The other questionnaire was about general health. Based on these questionnaires, the preselected covariates for the analyses included psoriasis, atopic dermatitis, asthma, allergy, (repeated) staphylococcal infection, hospitalization within 3 months, smoking, urban/rural living at the age of 6 years, animals in the household (animals), and present living or working at a farm. All covariates were binary. Gender and age (below and above the median age of subjects) were also covariates in this study. All covariates were tested for pairwise correlation in order to avoid collinear covariates. To obtain sufficiently high numbers, the answers to questions regarding atopic dermatitis, asthma, and allergy were merged into “atopic disease”. The study included 2,213 twins, among which 2,196 (99.2 %) had filled out the questionnaires. The frequency of replies missing was, on average, 1.4 % and, for all covariates, less than 4.6 %. The 2,196 twins comprised 627 intact twin pairs (1,254 twins who both participated) and 942 broken pairs (where only one twin from the pair participated). Dismissing twins with unknown zygosity, a separate twin analysis was made based on the 239 discordant twin pairs in which one of the twins in the twin pair was a carrier of *S. aureus*. This sub-analysis comprised 40 monozygotic (MZ), 112 same-sex dizygotic (SS–DZ), and 87 opposite-sex dizygotic (OS–DZ) twin pairs. The twins (N=2,196) had a mean age of 62.6 (±7.5) years and consisted of 51.2 % males. The age distributions among men and women were similar, with 49.5 % of the men aged <63 years and 47.7 % of the women aged <63 years. Among the discordant twin pairs, 50 % were males. The age distribution was more skewed than the overall sample, with 33.1 % of the men aged <63 years and 40.2 % of the women aged <63 years.

Statistical methods

The analyses were performed in two steps: first for all participants disregarding that the participants were twins and second for discordant twin pairs alone, where one of the twins in a pair was colonized with *S. aureus* and the other was not. All analyses were performed using the statistical program Stata 11.2.

In the univariate analyses of covariates, logistic regression was used, including the analyses when controlling for age and gender. The correlation between covariates was assessed using tetrachoric correlations.

For the discordant twin pairs, a conditional multivariate logistic regression was made, modeling for fixed effects by grouping the twins pairwise. If one twin did not answer a question, the answer from the co-twin was removed as well. Conditional logistic regression was made for modeling fixed effects, and the twins were grouped by their pair number. The same covariates were included, except for age, which was matched for each twin pair by definition.

Results

Prevalence by age group and gender

Based on the overall sample (N=2,196) the overall prevalence of *S. aureus* nasal colonization was 24.7 % [95 % confidence interval (CI): 22.9 %–26.5 %]. Males were significantly more likely to be colonizers than females [males: 27.6 % (95 % CI: 25.0 %–30.3 %) vs. females: 21.6 % (95 % CI: 19.1 %–24.0 %), p<0.01]. Also, the prevalence of *S. aureus* nasal colonization in the younger half of the individuals did not differ significantly from that in the older half (26.0 % vs. 23.3 %; p=0.15). Among discordant twin pairs, males were also significantly more likely to be colonized than females [56.1 % (95 % CI: 49.7 %–62.4 %) vs. 43.9 % (95 % CI: 37.6 %–50.3 %), p<0.01]. Here, the prevalence by age was almost the same, the youngest being 49.8 % (95 % CI: 44.2 %–55.5 %), and the oldest being 50.3 % (95 % CI: 42.8 %–57.8 %).

Univariate analyses

Colonization of *S. aureus* was tested in univariate analyses for all the selected risk factors, including an analysis adjusting for age and gender. Psoriasis, atopic disease, living/working at a farm, and animals in the household were significant. Living/working at a farm doubles the risk (odds ratio (OR) 2.05; 95 % CI:1.35–3.11), while the risk
increased by 73% for those having psoriasis (OR 1.73; 95% CI: 1.16–2.58) and having atopic disease increased the risk by 36% (OR 1.36; 95% CI: 1.11–1.68) of S. aureus colonization. Adjusting for age and gender made “animals in the household” not significant (OR 1.20; 95% CI: 0.97–1.48), while being a current smoker was borderline significant (OR 0.78; 95% CI: 0.60–1.01). Generally, adjusting for age and gender did not change the OR notably (Table 1).

When analyzing the discordant twin pairs, only gender, psoriasis, and living at a farm were statistically significant, the last two with large confidence limits (Supplementary Table 1).

Multivariate logistic regression analyses

For the overall cohort, a multivariate logistic regression analysis was made including all risk factors listed in Table 1, except “ever smoked” and repeated S. aureus infection, since they were highly correlated with, respectively, “current smoker” and S. aureus infection, leaving N=2,033 for analyses (Table 2). In the multivariate analyses, gender, psoriasis, atopic disease, current smoker, and living/working at a farm were statistically significant, while age, S. aureus infection, urban/rural living at the age of 6 years, animals in the household, and hospitalization within the last 3 months were not.

Omitting the statistically non-significant risk factors, except for age, from the analysis made smoking borderline statistically significant (p=0.05) (OR 0.76; 95% CI: 0.58–1.00) (Table 3). The largest effects were seen for living/working on a farm (OR 2.09; 95% CI: 1.36–3.21) and psoriasis (OR=1.82; 95% CI: 1.21–2.74).

Conditional logistic regression for discordant twin pairs

The same selected risk factors (shown in Table 3) were included in the analysis for the discordant twin pairs, except for age, which was perfectly matched for each twin pair (N=436) (Table 4). In this sub-analysis, gender, psoriasis (OR 5.14; 95% CI: 1.81–14.59) and living/working at a farm (OR 4.82; 95% CI: 1.40–16.60) were significant, but with large confidence limits, while current smoker was borderline significant and atopic disease was no longer significant.

This analysis was also performed for same-sexed discordant twin pairs alone (N=280), where the covariate gender also was omitted. This analysis showed that psoriasis, atopic diseases, and living/working at a farm were significant, while “current smoker” was not significant (table not shown).

Discussion

As genetic factors were recently shown to be of limited importance for S. aureus nasal colonization [8], here, we aimed to identify specific risk factors that could improve our

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**Table 1** Univariate analyses of risk factors in the overall cohort

| Risk factor                  | Exposure | N | Colonized, n (%) | Not colonized, n (%) | ORb 95% CI | p-Valuec | OR adjustedb 95% CI | p-Valuec |
|------------------------------|----------|---|------------------|---------------------|------------|----------|---------------------|----------|
| Total number                 |          | 2,196 | 542 (24.7)       | 1,654 (75.3)        |            |          |                     |          |
| Age group ≥63                |          | 2,196 | 249 (45.9)       | 819 (49.5)          | 0.87 (0.71–1.05) | 0.1      |                     |          |
| Gender Females               |          | 2,196 | 231 (42.6)       | 840 (50.8)          | 0.72 (0.59–0.88) | <0.01    |                     |          |
| Psoriasis                    | Yes      | 2,097 | 72 (4.6)         | 431 (26.1)          | 1.36 (1.11–1.68) | <0.01    | 1.42 (1.15–1.76)    | <0.01    |
| S. aureus infection Yes      | 2,175    | 86 (4.5) | 1.38 (1.14–1.68) |                     |            |          |                     |          |
| Current smoker               | Yes      | 2,183 | 89 (4.5)         | 72 (39.8)           | 0.8 (0.62–1.03) | 0.1      | 0.78 (0.60–1.10)    | <0.06    |
| Ever smoked                  | Yes      | 2,183 | 310 (57.8)       | 971 (59.0)          | 0.95 (0.78–1.16) | 0.6      | 0.94 (0.77–1.14)    | 0.5      |
| Childhood, 6 years Urban     |          | 2,186 | 155 (28.7)       | 468 (82.5)          | 1.01 (0.81–1.25) | 0.9      | 1.01 (0.81–1.25)    | 1.0      |
| Farm living/working Yes      |          | 2,147 | 60 (3.7)         | 2.05 (1.35–3.11)    | <0.01      | 1.97 (1.30–3.00)    | <0.01    |
| Animals Yes                  |          | 2,160 | 516 (31.7)       | 1.25 (1.02–1.54)    | <0.04      | 1.20 (0.97–1.48)    | <0.09    |
| Hospitalization Yes          |          | 2,174 | 63 (3.9)         | 0.81 (0.47–1.40)    | 0.5        | 0.80 (0.46–1.38)    | 0.4      |

a Varying numbers due to missing values
b OR and p-values are shown with and without adjusting for age and gender
c Logistic regression
d Includes asthma, atopic dermatitis, and allergy
e Urban/rural living when 6 years old
f In eight cases, there were pigs on the farm resulting in 1.5% for the colonized and 0.5% for the non-colonized (p<0.05)
g Hospitalization within the last 3 months
understanding of factors associated with S. aureus nasal colonization using a large twin population.

We have investigated the cohort both as individuals and as twin pairs. We showed consistently that living on a farm doubles the risk for S. aureus nasal colonization, which has not previously been demonstrated. Also, we saw a similar correlation between psoriasis and colonization. This is in agreement with two studies that found an association between psoriasis and S. aureus infection and nasal colonization [11, 12]. The less apparent, although significant, association to atopic disease is in agreement with previous studies, as atopic dermatitis patients have a high incidence of S. aureus infections and increased rates of nasal carriage [13]. Smoking appears to be antagonistic to S. aureus nasal colonization, which is in agreement with previous studies [14–16]. Recent hospitalization was not associated with S. aureus colonization, in contrast to a recent study [1]; however, the sample size used was not large enough to rule out a possible association.

Table 2 Multivariate analyses of risk factors in the overall cohort (N=2,033)\(^a\)

| Risk factor | Exposure | OR  | 95% CI       | p-Value |
|-------------|----------|-----|--------------|---------|
| Age group   | ≥63      | 0.87| (0.71–1.08)  | 0.21    |
| Gender      | Female   | 0.67| (0.54–0.82)  | <0.01   |
| Psoriasis   | Yes      | 1.81| (1.20–2.73)  | <0.01   |
| Atopic disease\(^b\) | Yes | 1.45| (1.16–1.82)  | <0.01   |
| S. aureus infection | Yes | 0.86| (0.53–1.41)  | 0.56    |
| Current smoker | Yes | 0.75| (0.57–0.99)  | <0.05   |
| Childhood, 6 years\(^c\) | Urban | 1.02| (0.81–1.28)  | 0.89    |
| Farm living/working | Yes | 1.90| (1.20–3.00)  | <0.01   |
| Animals     | Yes      | 1.14| (0.91–1.44)  | 0.26    |
| Hospitalization\(^d\) | Yes | 0.82| (0.46–1.45)  | 0.50    |

\(^a\)2,033 twins have answered all the selected questions
\(^b\)Includes asthma, atopic dermatitis, and allergy
\(^c\)Urban/rural living when 6 years old
\(^d\)Hospitalization within the last 3 months

Rural living as a child was not associated with S. aureus colonization but current living/working on a farm was associated with S. aureus nasal colonization, suggesting that these individuals may be colonized or transiently contaminated from the animals on the farm, as previously described [17]. This may be part of the reason why we find that two of the S. aureus colonizers were positive for CC398 (data not shown), a lineage normally associated with pigs [18], which is in agreement with the finding that, among the S. aureus colonizers and those not colonized with S. aureus, there were eight individuals in each group that worked or lived on a farm with pigs. Further samplings of these individuals living/working on farms could clarify this observation despite the non-colonized group being approximately three times bigger (P<0.05).

Furthermore, we also address the same questions taking into consideration that it is a twin study and we can, therefore, adjust for common childhood environment and common genes. Although the numbers are greatly reduced, the intra-pair analysis confirmed the findings. Thus, the significant association between nasal colonization and living on a farm as well as psoriasis are retained. With this further matching, smoking and atopic disease association became significant.

The current study only investigates the association between specific risk factors and a single time point of S. aureus nasal colonization. Therefore, this study does not address the issue of association between environmental conditions and persistent S. aureus or non-colonization, where the association may be different. However, certain factors indicate that such a grouping may only strengthen the observed associations. A recent study of S. aureus nasal colonization among Norwegian adults where there had been more than one sampling of the studied individuals performed clearly showed that smoking was a protective factor in relation to S. aureus nasal carriage [14]. In our study, we observed a borderline association. No other associations of risk factors and S. aureus nasal colonization coincide in the two studies. Olsen and coworkers showed that an inverse correlation exists between vitamin D levels and S. aureus colonization [14].

Table 3 Multivariate analyses of selected risk factors in the overall cohort (N=2,073)\(^a\)

| Risk factor     | Exposure | OR  | 95% CI       | p-Value |
|-----------------|----------|-----|--------------|---------|
| Age group       | ≥63      | 0.86| (0.70–1.05)  | 0.15    |
| Gender          | Female   | 0.68| (0.56–0.84)  | <0.01   |
| Psoriasis       | Yes      | 1.82| (1.21–2.74)  | <0.01   |
| Atopic disease\(^b\) | Yes | 1.43| (1.15–1.79)  | <0.01   |
| Current smoker  | Yes      | 0.76| (0.58–1.00)  | 0.05    |
| Farm living/working | Yes | 2.09| (1.36–3.21)  | <0.01   |

\(^a\)2,073 twins have answered all the selected questions
\(^b\)Includes asthma, atopic dermatitis, and allergy

Table 4 Multivariate analysis of selected risk factors among twins discordant for Staphylococcus aureus colonization (N=436)\(^a\)

| Risk factor        | Exposure | OR  | 95% CI       | p-Value |
|--------------------|----------|-----|--------------|---------|
| Gender             | Female   | 0.51| (0.31–0.86)  | 0.01    |
| Psoriasis          | Yes      | 5.14| (1.81–14.59)| <0.01   |
| Atopic disease\(^b\) | Yes | 1.52| (0.95–2.46)  | <0.09   |
| Current smoker     | Yes      | 0.59| (0.34–1.02)  | 0.06    |
| Farm living/working | Yes | 4.82| (1.40–16.60)| <0.02   |

\(^a\)436 discordant twin pairs have answered all the selected questions
\(^b\)Includes asthma, atopic dermatitis, and allergy
Atopic disease, including atopnic dermatitis, allergies, and asthma, was also weakly associated with S. aureus nasal colonization. The need to converge all three questions will certainly reduce the power of any specific association, as we would expect there to be an association with atopnic dermatitis but not necessarily asthma or the less well defined condition of allergies. The sample size was too small to investigate the association between atopnic dermatitis and S. aureus colonization.

The questionnaire has some limitations due to the types of questions it contained. We did not clearly distinguish between farm animals and pets and, therefore, had to group them as one. The disease questions are also, to a certain extent, difficult to answer, as some people may not know the correct terminology. This is the case for atopnic dermatitis, asthma, and allergies and, to a lesser degree, psoriasis. This misclassification is unlikely to be associated with S. aureus nasal colonization and, therefore, the misclassification will tend to underestimate the association between these risk factors and S. aureus colonization.

Conclusion

In conclusion, the strongest risk factors of Staphylococcus aureus colonization were male gender, living on a farm, psoriasis, and any atopnic disease, while smoking had a borderline protective effect. Future studies involving the closer environment of S. aureus, the microbial community of the nares, may give further clues as to the selective colonization of S. aureus in only a fraction of all individuals.

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Conflict of interest The authors declare that they have no conflict of interest.

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