Hormonal Contraceptive Use and Persistent Staphylococcus aureus Nasal Carriage

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Background. Human nares colonized with Staphylococcus aureus are the most important reservoir for this pathogen. We studied the influence of sex and hormonal contraceptive use on persistent S. aureus nasal carriage.

Methods. We conducted a cohort study in healthy volunteers and determined carriage status at baseline and again at follow-up by using the results of 2 swab samples at each time point. We applied logistic regression to analyze associations of interest.

Results. At baseline, 266 of 1180 volunteers (22.5%) were classified as persistent nasal carriers. Compared with women not using hormonal contraceptives, women taking reproductive hormones (odds ratio [OR], 1.88; 95% confidence interval [CI], 1.29–2.75; P = .001) and men (OR, 1.57; 95% CI, 1.08–2.28; P = .02) were more likely to be persistent carriers. These associations remained stable after adjusting for known risk factors of nasal carriage. Women taking hormonal contraceptives and being persistent carriers at baseline were more likely to remain carriers after a median follow-up time of 70 days than women not using such medication (OR, 3.25; 95% CI, 1.44–7.34; P = .005). No patterns of association could be observed between persistent carriage among women and type of progestin or dose of estrogen used. Assuming causality and using estimates from multivariable logistic regression, we approximated that 20% (95% CI, 2.4%–34.9%) of persistent nasal carriage among women represented by our sample is attributable to hormonal contraception (population-attributable fraction).

Conclusions. The widespread use of hormonal contraception may substantially increase the human S. aureus reservoir with potential impact on S. aureus infection and transmission.

Colonized human skin, nares, and mucosal surfaces are major reservoirs for Staphylococcus aureus and were found to have important implications with regard to pathogen spread and infection. The high S. aureus burden found in persistently colonized nares is of particular public health relevance [1–4] and thus at the center of translational research [5].

Epidemiological studies have identified a variety of risk factors for S. aureus nasal carriage in healthy individuals. An association of lower carriage with older age [6–8], female sex [6, 9, 10], and smoking [6, 10, 11] could be consistently reproduced in different populations and settings. Although the biological mechanisms underlying the effect of smoking on nasal carriage remain enigmatic, its association with age and sex suggest a role of reproductive hormones on colonization. In fact, one study found an association of S. aureus nasal carriage with hormonal status in women as determined by the karyopyknotic index of vaginal smears [12]. S. aureus cervical colonization was also reported to be more likely during the estrogen peak at the mid of the menstrual cycle [13]. Furthermore, studies have found increased staphylococcal binding to cultured HeLa cells after stimulation with estrogens [14]. Finally, there seems to be an association of nasal carriage with glucocorticoid receptor gene polymorphisms [15] and 25-hydroxyvitamin D serum levels [10], allowing hypotheses about a role of reproductive steroid hormones in nasal carriage as well.

Large epidemiological studies investigating the role of reproductive hormones on S. aureus colonization, however, are missing. Of particular interest is whether hormone intake influences the risk of persistent nasal carriage. If so, the widespread use of hormonal
contraceptives could have major impact on the overall *S. aureus* burden in a population. To this end, we analyzed data on sex and hormonal contraceptive use among volunteers enrolled in a cohort that was designed to investigate various determinants of persistent *S. aureus* nasal carriage.

**MATERIALS AND METHOD**

**Study Population**

This cohort study was designed to investigate a potential influence of sociodemographic and behavioral characteristics on *S. aureus* nasal carriage and skin infections, including current use of hormonal contraceptives, current smoking, regular animal contact, medical profession with patient contact, inpatient treatment, antibiotic usage, allergy, history of purulent skin infection, and international travel. Beginning in May 2009, we recruited volunteers from 2 sources: (1) through public advertising and (2) from individuals seeking pretravel advice. Enrollment and follow-up visits took place at the University of Tübingen travel clinic. The presented work uses data of all subjects enrolled until March 2011.

For inclusion, individuals had to reside within 15 kilometers of the clinic, be ≥18 years of age, and provide ≥2 nasal swab samples at baseline. Subjects with human immunodeficiency virus infection, diabetes mellitus, immunosuppressive medication, active malignancy, renal insufficiency, or other forms of chronic immunosuppression were not enrolled. At the first visit, information on the exposures of interest was collected using a standardized questionnaire. A picture board with depictions of folliculitis, abscesses and impetigo was also used to inquire about a history of purulent skin infection.

*S. aureus* nasal carriage status was determined at 2 time points: at baseline and at follow-up. All subjects were asked to provide 4 nasal swab samples—2 at enrollment and 2 after traveling or, in the case of nontravelers, after a default follow-up period of ≥28 days. There was ≥1 week between the 2 samples at baseline and follow-up, respectively. At follow-up, all participants were asked about the use of antibiotics since enrollment. In March 2011, we extracted data from all participants enrolled so far and collected information on brand name and dosage from all women who had indicated use of hormonal contraceptives at baseline.

**Laboratory Methods**

Nasal swab samples were collected by circulating a cotton-tipped swab 4 times with gentle pressure against the inner wall of both anterior nares. Disposable rubber gloves were used to prevent cross-contamination. Specimens were stored in Amies transport media at 4°–6°C for <24 hours. Swab samples were streaked on mannitol salt agar (Oxoid), incubated for 48 hours at 37°C and inspected for growth of mannitol-fermenting colonies. Plates that did not show mannitol fermentation were further incubated at room temperature for 24 hours, and then analyzed for growth of mannitol-fermenting colonies. Mannitol-fermenting colonies were subcultured on 5% sheep blood agar (Oxoid) and *S. aureus* is identified based on latex coagulation for the presence of protein A (clumping factor) and/or capsular 5 or 8 antigens (Staph Plus; DiaMond-iaL). In case of a negative result, additional testing for free coagulase using the tube coagulation test (BBL Coagulate Plasma; Becton Dickinson) was performed. Only bacteria negative for both tests were classified as coagulase-negative staphylococci, whereas 1 positive test was assumed to be indicative for *S. aureus*. Both participants and team members collecting swab samples and issuing questionnaires were kept blinded to the laboratory results.

**Data Management**

Data from questionnaires and laboratory sheets collected until March 2011 were double entered and then checked for plausibility and inconsistencies using EpiData software (version 3; EpiData Association). After importation into the Stata statistical software package (version 11; Stata) each variable was checked for missingness, and missing values were confirmed or corrected by reviewing the source document. This revealed that, owing to a production error in a subset of questionnaires, the items on smoking, history of allergy, and hormonal contraception were missing at baseline. This information could be obtained later by email contact in all but 46 subjects, of whom 26 were women. Four other subjects had missing information owing to single or multiple unanswered or ambiguously answered questions that could not be clarified later.

**Statistical Analysis**

Based on a validated algorithm [16], subjects with 2 nasal swab samples positive for *S. aureus* were defined as persistently colonized at either time point (ie, baseline or follow-up); all remaining combinations were interpreted as other forms of carriage (ie, noncarrier or intermittent *S. aureus* nasal carriage). At baseline, risk factors for persistent nasal carriage (outcome) were analyzed by comparing the proportion exposed to a certain risk factor between persistent and other carriage using univariable logistic regression. All variables that showed at least weak evidence (*P* ≤ .1) for an association with the outcome were used to adjust a multivariable logistic regression model describing the association of sex and hormonal contraceptive use (main exposures) with persistent nasal carriage (outcome). This was done in restricted data sets and, to increase power and to give estimates for the overall effect of either sex or hormonal contraceptive use while adjusting for the other factor, by fitting a model that contained a categorical variable that combined information on sex and hormonal
contraceptive use. This categorical variable consisted of 3 strata: (1) women not using hormonal contraceptives (baseline), (2) men, and (3) women using hormonal contraceptives. Progestins were grouped according to their activity profile, as suggested elsewhere [17], to analyze their effect on nasal carriage. Follow-up data on women classified as persistent carriers at baseline were used to assess the association of hormonal contraceptive use with a subsequent change in persistent nasal carriage status. The attributable fraction of hormonal contraceptive use among women was calculated using the alogit command [18] after having fitted the adjusted logistic regression model to the data set containing observations in women only. At baseline, 4% of study subjects (50 of 1180) had missing information on ≥1 exposure variable. In the univariable analysis (Table 1), these subjects were excluded from respective denominators. Multivariable logistic regression (Table 2) was performed using a data set restricted to subjects with complete information on all covariates. All statistical analyses were performed with the software package Stata 11 (Stata Corp).

**Ethics**
All participants gave written informed consent. The protocol for this study was approved by the ethics committee at the University of Tübingen Medical School (Approval No. 145/2009BO2).

**RESULTS**
Of 1367 individuals that had consented to participate, 1180 (86%) provided 2 swab samples at baseline; 22.5% of these (266 of 1180) were classified as persistent nasal carriers of *S. aureus*. Table 1 shows the distribution of putative risk factors and *S. aureus* nasal carriage types.

Persistent nasal carriage was more common in nonsmokers and in individuals reporting regular animal contact. There was also weak statistical evidence for a positive association with younger age, intention to travel, and shorter periods of time between the 2 baseline swab samples (Table 1).

When the analysis was restricted to women, there was strong evidence that women using hormones were more

### Table 1. Baseline Characteristics and *Staphylococcus aureus* Nasal Carriage in 1180 Healthy Volunteers

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Nasal Carriage of <em>S. aureus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Persistent (n = 266)</td>
</tr>
<tr>
<td>Age, median (IQR), years</td>
<td>24 (22–27)</td>
</tr>
<tr>
<td>Male sex</td>
<td>107 (40.2)</td>
</tr>
<tr>
<td>Weight, mean (SD), kg</td>
<td>68.9 (14.2)</td>
</tr>
<tr>
<td>Height, mean (SD), cm</td>
<td>173.5 (9.3)</td>
</tr>
<tr>
<td>Current smoking</td>
<td>42 (16.3)(d)</td>
</tr>
<tr>
<td>Allergy</td>
<td>99 (38.5)(d)</td>
</tr>
<tr>
<td>Hormonal contraception(g)</td>
<td>102 (66.2)(h)</td>
</tr>
<tr>
<td>Regular animal contact</td>
<td>111 (41.7)</td>
</tr>
<tr>
<td>History of purulent skin infection</td>
<td>40 (15.1)(j)</td>
</tr>
<tr>
<td>Medical profession</td>
<td>63 (23.7)</td>
</tr>
<tr>
<td>Intention to travel</td>
<td>154 (57.9)</td>
</tr>
<tr>
<td>Inpatient treatment within last 3 months</td>
<td>15 (5.6)</td>
</tr>
<tr>
<td>Antibiotic usage within last 3 months</td>
<td>77 (28.9)</td>
</tr>
<tr>
<td>Interval between swab samples, median (IQR), days</td>
<td>8 (7–14)</td>
</tr>
</tbody>
</table>

**Abbreviations:** CI, confidence interval; IQR, interquartile range; OR, odds ratio; SD, standard deviation. Unless otherwise indicated, data represent No. of subjects (%) and results from univariable logistic regression.

\(a\) Per 10-year increase in age.

\(b\) Per 10-kg increase in body weight.

\(c\) Per 10-cm increase in height.

\(d\) n = 257.

\(e\) n = 876.

\(f\) n = 873.

\(g\) Data set restricted to women.

\(h\) n = 154.

\(i\) n = 540.

\(j\) Information missing for 1 subject.

\(k\) Information missing for 2 subjects.

\(l\) Variable log-transformed to achieve approximately linear effect on the log odds of outcome.
likely to be persistently colonized with *S. aureus* than women not using these (Table 1). This association became weaker but remained statistically significant after adjusting for age, animal contact, current smoking, history of skin infection, intention to travel, and time that had elapsed between the 2 swab samples at baseline (odds ratio [OR], 1.62; 95% confidence interval [CI], 1.09–2.41; \( P = .02 \)).

Sex alone was not associated with carriage when the complete data set was analyzed (OR, 1.19; 95% CI, 0.89–1.59; \( P = .3 \)). However, after exclusion of women using hormonal contraceptives, there was evidence suggesting that persistent nasal carriage was more likely in men than in women not using such medication (OR, 1.64; 95% CI, 1.12–2.39; \( P = .01 \)).

When using one categorical variable for sex and hormonal contraceptive use, we found strong statistical evidence for an independent and positive association of both hormonal contraceptive use and male sex with persistent nasal carriage (Table 2, left column). Adjustment for other risk factors did not change these associations substantially (Table 2).

Of 694 women who had provided information on hormonal contraceptive intake in the questionnaire, 54.8% (380 of 694) reported they were using such medication and 73% of these (276 of 380) also provided information on the type of hormonal contraceptive used. No differences in the proportion of persistent nasal carriage could be found after stratification by type, dosage, or marketed combination (Table 3; data for marketed combinations not shown).

After a median follow-up time of 70 days, 127 of 154 women (82%) who had provided information on hormonal contraception and had been classified as persistent carriers at baseline provided a second set of 2 swab samples. Ninety-one of these 127 (72%) were still persistent carriers at follow-up (Table 4). There was strong evidence that preserved persistent carrier status at follow-up was more likely in women using hormonal contraceptives (crude OR, 3.25; 95% CI, 1.44–7.34; \( P = .005 \)). Adjusting this association for differences in antibiotic intake and travel during follow-up marginally strengthened this association (adjusted OR, 3.49; 95% CI, 1.47–8.27; \( P = .004 \)).

A conservative estimate of the population-attributable fraction was calculated by using the least extreme effect estimate obtained from the adjusted regression analysis in observations from women only (OR, 1.62; 95% CI, 1.09–2.41; \( P = .02 \)); this showed that 20.4% (95% CI, 2.4%–34.9%) of cases of persistent nasal carriage in women represented by the study sample are attributable to hormonal contraceptive use, if we can assume causality.

**DISCUSSION**

This study provides strong evidence for an association of hormonal contraceptive use and persistent *S. aureus* nasal carriage. We show that women taking hormonal contraceptives are more likely to be persistent nasal carriers of *S. aureus* and that reproductive hormone intake is associated with persistence of nasal colonization of *S. aureus* over time. Our findings allow hypothesizing that hormonal contraceptive use has major impact on the human *S. aureus* reservoir.

Two studies have previously reported an association of reproductive hormones and *S. aureus* nasal carriage. The first study included 479 women at a colpocytologic clinic and found *S. aureus* to be more often present in the nares of

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**Table 2. Association of Hormonal Contraceptive Use and Sex With Persistent Nasal Carriage of *Staphylococcus aureus***

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Crude OR (95% CI)a</th>
<th>Intention to Travel</th>
<th>Current Smoking</th>
<th>Animal Contact</th>
<th>Multiple Exposuresb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female, without hormonal contraception</td>
<td>1.0 (1.08–2.28)</td>
<td>.02</td>
<td>1.0 (1.09–2.29)</td>
<td>1.0 (1.09–2.30)</td>
<td>1.0 (1.10–2.32)</td>
</tr>
<tr>
<td>Male</td>
<td>1.58 (1.29–2.73)</td>
<td>.001</td>
<td>1.88 (1.29–2.73)</td>
<td>1.85 (1.27–2.70)</td>
<td>1.82 (1.25–2.65)</td>
</tr>
<tr>
<td>Female, using hormonal contraception</td>
<td>1.88 (1.29–2.75)</td>
<td>.001</td>
<td>1.88 (1.29–2.73)</td>
<td>1.85 (1.27–2.70)</td>
<td>1.82 (1.25–2.65)</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; OR, odds ratio.

Data are ORs (95% CIs) from logistic regression describing the effect of sex and hormonal contraceptive use on persistent nasal carriage of *S. aureus*, after restriction of the data set to 1130 observations, with information on all characteristics adjusted for in either of the models.

a ORs for each stratum of a single variable that combines information on hormonal contraceptive use and sex and uses the odds of persistent nasal carriage in women not using hormonal contraception as baseline.
b Adjustment for age, current smoking, animal contact, history of skin infection, intention to travel, and time between first and second swab samples (after logarithmic transformation).
women with high estrogen levels [12]. These findings strongly support what we report here. However, the authors used single swab samples to define nasal carriage status and thus inferences on an association with persistent S. aureus nasal colonization could not be readily drawn. The second study investigated multiple risk factors of S. aureus nasal carriage in

Table 3. Characteristics of Hormonal Contraceptive Usage and Their Association With Persistent Nasal Carriage of Staphylococcus aureus

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Subjects, No.</th>
<th>Persistent</th>
<th>Other</th>
<th>P^a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hormonal contraceptive usage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>380</td>
<td>102 (26.8)</td>
<td>278 (73.2)</td>
<td>.001</td>
</tr>
<tr>
<td>No</td>
<td>314</td>
<td>52 (16.6)</td>
<td>262 (83.4)</td>
<td></td>
</tr>
<tr>
<td>Type of hormonal contraceptive</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Combination, oral</td>
<td>243</td>
<td>64 (26.3)</td>
<td>179 (73.6)</td>
<td></td>
</tr>
<tr>
<td>Combination, vaginal or dermal</td>
<td>23</td>
<td>6 (26.1)</td>
<td>17 (73.9)</td>
<td></td>
</tr>
<tr>
<td>Progestin only</td>
<td>10</td>
<td>2 (20.0)</td>
<td>8 (80.0)</td>
<td>.9</td>
</tr>
<tr>
<td>Ethynyl estradiol dosage^b</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High^c</td>
<td>50</td>
<td>11 (22.0)</td>
<td>39 (78.0)</td>
<td></td>
</tr>
<tr>
<td>Medium^c</td>
<td>140</td>
<td>37 (26.4)</td>
<td>103 (73.6)</td>
<td></td>
</tr>
<tr>
<td>Low^c</td>
<td>73</td>
<td>21 (28.8)</td>
<td>52 (71.2)</td>
<td>.7</td>
</tr>
<tr>
<td>Type of progestin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antiandrogenic effect^d</td>
<td>160</td>
<td>41 (25.6)</td>
<td>119 (74.4)</td>
<td></td>
</tr>
<tr>
<td>No antiandrogenic effect^d</td>
<td>116</td>
<td>31 (26.7)</td>
<td>85 (73.3)</td>
<td>.8</td>
</tr>
<tr>
<td>Glucocorticoid effect^e</td>
<td>97</td>
<td>22 (22.7)</td>
<td>75 (77.3)</td>
<td></td>
</tr>
<tr>
<td>No glucocorticoid effect^e</td>
<td>174</td>
<td>49 (28.2)</td>
<td>125 (71.8)</td>
<td>.3</td>
</tr>
</tbody>
</table>

^a χ^2 test.

^b Excluding 10 women using progestins only and 3 using combinations containing estradiol valerate.

^c Low dosage was defined as 15–29.9 µg/dose; medium, 30–34.9 µg/dose; high, ≥35 µg/dose.

^d Comparing observations from subjects using chlormadin acetate, cyproteron acetate, Dienogest, or drospirenone vs the rest; classification according to [17].

^e Comparing observations from subjects using chlormadin acetate, cyproteron acetate, Desogestrel, or Etonogestrel vs the rest, after exclusion of 4 subjects using norgestim and 1 using norelgestromin because of their unknown glucocorticoid effect; classification according to [17].

Table 4. Hormonal Contraceptive Use and Persistence of Staphylococcus aureus Nasal Carriage Over Time

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>S. aureus Carriage at Follow-up in Women Classified as Persistent Carriers at Baseline (n = 127)</th>
<th>OR (95% CI)^b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Still Persistent (n = 91) Change to Other (n = 36)</td>
<td>P^a</td>
</tr>
<tr>
<td>Hormonal contraception</td>
<td>66 (73) Change to Other (n = 47)</td>
<td>.007</td>
</tr>
<tr>
<td>Age, mean (SD), years</td>
<td>25.3 (5.6) Change to Other (n = 25.1 (9.0)</td>
<td>.9</td>
</tr>
<tr>
<td>Current smoking at baseline</td>
<td>9 (10) Change to Other (n = 3)</td>
<td>.8</td>
</tr>
<tr>
<td>Animal contact at baseline</td>
<td>42 (46) Change to Other (n = 14)</td>
<td>.5</td>
</tr>
<tr>
<td>Travel during follow-up</td>
<td>30 (33) Change to Other (n = 21)</td>
<td>.009</td>
</tr>
<tr>
<td>Antibiotic use during follow-up</td>
<td>6 (7)^d Change to Other (n = 9)</td>
<td>.006</td>
</tr>
<tr>
<td>Follow-up time, mean (SD) as natural log, days</td>
<td>4.3 (0.7) Change to Other (n = 4.5 (0.5)</td>
<td>.2</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; OR, odds ratio; SD, standard deviation.

Unless otherwise indicated, data represent No. (%) of subjects and ORs for preserved carriage at follow-up. ORs are given for those characteristics that were included in the multivariable analysis.

^a From χ^2 or t test as appropriate.

^b From logistic regression restricted to 122 observations with information on all exposure variables and comparing the odds of exposure among women who still have persistent carriage after follow-up vs those showing a change to other carriage types.

^c Adjusted for travel and antibiotic use during follow-up.

^d n = 86 owing to missing data.
healthy Malaysian adults and found that the intake of hormonal contraceptives was associated with *S. aureus* nasal carriage [19]. Although this is in line with our findings, the study included only 9 hormonal contraceptive users overall and did not exclude men from the denominators when analyzing the effect of hormone intake, thus raising questions about its validity.

Various findings from published research render a causal association of hormone intake and colonization with *staphylococci* biologically plausible. Nasal carriage is characterized by a subclinical inflammatory response that is insufficient to remove *S. aureus* from the nares [20, 21]. Consequently, both host immunity [7, 22–27] and mechanisms of *S. aureus* immune evasion [21, 28–30] have been proposed as determinants of *S. aureus* nasal carriage. Hence, the known immunomodulatory effects of reproductive hormones [31–33] may be the causal link between hormone intake and *S. aureus* carriage. Along this line, it has been reported that “high estrogen states make mucosal surfaces more hospitable to a variety of pathogens” [33] which can be demonstrated by increased binding of *S. aureus* to HeLa cells after pretreatment with estrogens [14]. This finding from in vitro studies may also explain a markedly higher proportion of *S. aureus* nasal carriage in the middle and late third of the menstrual cycle (14% during the early vs 31% during the middle and 35% during the late menstrual cycle) [12] when endogenous estrogen levels are high. Moreover, various studies reported hormonal contraceptive use as a risk factor for symptomatic infection [34–39] further suggesting an immunomodulatory effect of this medication.

Components of the innate immune system that have received considerable attention in the context of *S. aureus* colonization are skin-derived antimicrobial peptides that are constitutively or inducibly expressed in keratinocytes and exert marked antibacterial activity [22, 40]. Their lower expression was found to be associated with *S. aureus* skin infections and nasal colonization [22, 41, 42]. This is of interest, because there is increasing evidence that reproductive hormones are important modifiers of antimicrobial peptide expression in the genital tract [43–45]. To what extent this influence may explain increased *S. aureus* nasal colonization in hormonal contraceptive users merits further investigation.

In our study population 96% of women used combined hormonal contraceptives consisting of both an estrogen and a progestin component, rendering the prediction of a net immunomodulatory effect complex. A classic approach to study the combined effects of progesterone and estrogen with regard to immunomodulation is to draw inferences from observations in pregnant women. Indeed, pregnancy is generally perceived as a state of general immunosuppression [46]. However, *S. aureus* nasal carrier proportions reported in pregnant women are similar to those found in the general population [23] and range between 20% and 33% [47–52]. Furthermore, one longitudinal study designed to study mother-to-child transmission of *S. aureus* reported that the proportion of mothers colonized in their nares 2 weeks to 24 months after delivery was constant [7], in contrast to the decline in carriage that would be expected under the hypothesis that combined reproductive hormones promote colonization. Unfortunately, studies that investigate carriage during various stages of pregnancy and after delivery in the same cohort of women are missing. In summary, existing knowledge on *S. aureus* nasal carriage during and after pregnancy is limited and neither supports nor refutes the hypothesis put forward here.

We did not find differences in the proportion of carriers after stratifying by marketed combination, ethinylestradiol dosage or by antiandrogen or glucocorticoid effect of the progestin component in combined preparations [17]. This may be due to a noncausal association or a lack of power to conduct this subgroup analysis. Alternatively, an underlying mechanism that is binary and thus has no gradient may explain the absence of a dose-response relationship. For instance, inhibition of the ovulation leading to suppression of the luteal phase and thus of the endogenous progesterone synthesis affects most combined hormonal contraceptive users and is thus independent of the dose and compound used [53]. One possible approach to explore the role of this effect on *S. aureus* colonization would be to compare combined-preparation users with women who use progestin-only contraceptives that do not suppress ovulation. Unfortunately, in the present study, only 10 women used progestin-only contraceptives, rendering our analysis underpowered to meaningfully explore such a difference.

Bias and confounding are important sources of noncausal associations in epidemiological studies. Although we adjusted our analysis for known risk factors of nasal carriage, we cannot fully exclude that residual confounding may account for the presented association. In particular, sexual activity and/or yet-unknown risk factors for persistent *S. aureus* nasal carriage may account for some of the observed effect based on their association with hormonal contraceptive use. Similarly, bias, and in particular differential bias leading to estimates away from the null, has to be considered when interpreting our findings. Recall bias was greatly minimized through collecting most of the information on exposure variables at enrollment (ie before the outcome was ascertained) and using standardized questionnaires and procedures. Another possible source of bias is missing data. Most of our missing values occurred because of a printing error (ie, completely at random) and at a time point when the outcome status was not yet determined. The proportion of subjects with missing information on ≥1 exposure variable was 4% and was equally
distributed between carriers and noncarriers. It thus seems unlikely that bias because of missing data could explain our results. Finally, it is important to note that our population consists mostly of young individuals who were partially selected by their need for pretravel advice and were thus likely to be selected on a variety of socioeconomic variables. Although this does not limit the internal validity of our findings, the presented association should be reproduced in other populations before being generalized to the population as a whole.

In summary, we report strong evidence for an association of persistent S. aureus nasal colonization with sex and with the use of combined hormonal contraceptives in young women. This finding is in line with published observations and is biologically supported by prevailing concepts on the role of reproductive hormones in modulating the interaction of host and pathogen. If further research can establish causality, the strength of the observed effect together with the widespread use of hormonal contraceptives would translate into major impact on the human S. aureus reservoir, with potential implications for S. aureus transmission and infection.

Notes

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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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