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# Intermittent nasal carriage with *Staphylococcus aureus* within a menstrual cycle

## Results from a prospective cohort of healthy carriers

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**Abstract**

Female sex hormones have been related to nasal *Staphylococcus aureus* carriage in healthy individuals; however, whether nasal staphylococcal carriage varies by menstrual cycle phase remains unknown.

We sampled anterior nares of female healthcare workers twice per week for 6 consecutive menstrual cycles. We used mixed-effects Poisson regression models to determine whether intermittent carriage was associated with cycle phases in a given individual. We also performed recurrent event survival analysis to identify host factors linked to incident carriage status.

Overall, we collected 754 nasal swabs over 89 consecutive person-cycles from 14 intermittent carriers. In 84 ovulation-defined menstrual cycles (715 swabs), the period prevalence of staphylococcal carriage was 58.7%, 63.1%, and 64.9% in the follicular, periovulatory, and luteal phases, respectively; these differences were not statistically significant after multivariable adjustment and correction for within-person correlation (adjusted relative risk [RR]—periovulatory 0.92, *P*: 0.30; luteal 1.00, *P*: 0.98).

Using survival analysis, we identified several host factors that were associated with incident loss, gain of colonization, or both. For example, as compared to women aged 20 to 30 years, those aged 30 to 40 years were less likely to losing carriage (hazard ratio [HR]: 0.26, 95% confidence interval [CI]: 0.09, 0.80) but were as likely to regaining carriage (HR: 0.53, 95% CI: 0.21, 1.34). In comparison, being underweight (body mass index [BMI] <18.5) was significantly associated with a higher risk for regaining (HR: 1.95, 95% CI: 1.34, 1.51) and losing (HR: 1.57, 95% CI: 1.16, 2.12) colonization, indicating the alternating tendency for status changes. Personal hygiene behaviors, such as nostril cleansing habit and methods, differentially affected carriers' risk for losing or regaining staphylococcal colonization.

Using an intensive sampling scheme, we found that nasal staphylococcal carriage could vary substantially over time in healthy carriers. Yet, such dynamic intraperson changes in carriage status did not depend on menstrual cycle phases but were associated with host age, BMI, and personal hygiene behavior.

**Abbreviations:** BMI = body mass index, CI = confidence interval, E2 = estradiol, HR = hazard ratio, IQR = interquartile range, IR = incidence rate, LH = luteinizing hormone, MICU = adult intensive care unit, MRSA = methicillin-resistant *Staphylococcus aureus*, OR = odds ratio, P4 = progesterone, PFGE = pulse-field gel electrophoresis, RR = relative risk.

**Keywords:** healthcare workers, menstrual cycle phase, mixed-effects Poisson regression, nasal carriage, recurrent event survival analysis, repeated measures, *Staphylococcus aureus*

## 1. Introduction

Understanding host factors that influence the nasal carriage status with *Staphylococcus aureus* is critical in targeted screening, intervention, and prevention programs. Well-known host characteristics associated with nasal *S aureus* carriage include young age, male sex, being non-Hispanic white, a large size of household, past history of antibiotic usage, prior *S aureus* skin infections, and major medical comorbidities.[1,2] Healthcare professionals are also known for their high likelihood for carrying or transmitting *S aureus* due to their frequent and close contact with high-risk patients.[3–6] Despite the predominant presence of intermittent carriage,[2] the above-mentioned host factors are time-invariant for observational studies and thus could not explain for the temporal dynamics in nasal carriage rates.

Over the past 2 decades, a growing body of literature has suggested that fluctuating female sex hormones, particularly estrogens, have a potent immunomodulaitng effect, capable of modifying host innate and adaptive immune responses to viral and bacterial infections even in immunocompetent populations.[7,8] Estrogen can exert both anti-inflammatory and proinflammatory effects but at different physiological levels.[9,10] Winkler et al first reported an epidemiological link between women's changing hormonal status and nasal carriage with

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*S aureus* in 1990.[11] Among 479 women attending a gynecology clinic, Winkler et al found that, in premenopausal women, prevalence of *S aureus* carriage was lower (14.0%) in the first one-third of a cycle than that in the middle (30.8%) or the last third (34.9%, *P*: 0.008) of a cycle. Recently, Zanger et al also showed that women taking combined oral contraceptives were, like men (odds ratio [OR], 1.57; *P:* 0.02), more likely to have persistent nasal carriage than women not using hormonal contraceptives (OR, 1.88; *P*: 0.001).[12]

However, the analytic approach in previous work was mostly cross-sectional in nature, focusing on single-time swab results[13,14] or a summarizing "persistent" carriage status based on multiple swab cultures spanning over the whole study period (and often including the baseline swab).[15–17] These investigations did not explore the temporal pattern or the driving forces for a changing carriage status. As endogenous estrogen has been shown to be at a relatively low level among women taking oral contraceptives,[18,19] it remains unexplored how cyclic changes of estrogen or progesterone (P4) might influence *S aureus* nasal carriage within a menstrual cycle. Therefore, we sought to test the hypothesis that whether nasal *S aureus* carriage rates varied by the menstrual cycle phase. Specifically, we hypothesized that, for a given female carrier, staphylococcal carriage would be higher in the periovulatory phase than in the follicular (menstrual) phase. Assuming no or negligible sampling errors, nasal carriage status was based on single nasal sample (swabbed bilaterally) and we also attempted to identify host characteristics associated with temporal patterns of alternating carriage status. In a subgroup of women, we additionally assessed how nasal carriage with *S aureus* varied with serum concentrations of estrogen and P4 within a menstrual cycle.

## 2. Materials and methods

### 2.1. Study design, subject screening, and enrollment

We screened for *S aureus* nasal carriers among healthy female healthcare workers in a tertiary teaching hospital between November 2013 and June 2014. After obtaining signed informed consent from each volunteer, a research assistant used a structured questionnaire to collect personal information, including demographic and socioeconomic characteristics; past history of known *S aureus* carriage, of contacts with documented carriers or patients with clinically diagnosed *S aureus* infections; lifetime history of tobacco use including exposure to environmental tobacco smoke; history of allergy or other chronic medical conditions; and use of reproductive hormones. At the end of the screening interview, the research assistant collected 1 nasal swab from bilateral anterior nares to determine a woman's nasal carriage status. We also requested a urine pregnancy test from women whose last menstrual period was at least 28 days prior to the screening date.

We included women who were healthy (as determined by the absence of any self-reported chronic medical condition) and nonpregnant (as determined by self-reported last menstrual period); aged between 20 and 45 years and have had a regular menstrual cycle of length 22 to 42 days in the prior 3 months; and who had no intention for a job change in the following 12 months. We excluded women who reported systemic use of antibiotics, steroids (including by nasal route or by inhalation), cytokines, or chemotherapeutic agents within the previous 6 months. Women planning for conception within the next 12 months, breastfeeding women, and women on oral contraceptive pills were also excluded. On receiving culture results for coagulase-positive *Staphylococcus* based on the single nasal swab at screening, the research assistant contacted each eligible carrier to further confirm her willingness to participate.

### 2.2. Follow-up and data collection

We asked each carrier to enter the study on the third day of the very next menstrual cycle and then every 3 to 4 days afterwards for at least 6 consecutive menstrual cycles. At each visit, the research assistant collected a nasal swab from a participant's bilateral anterior nares using a Copan Transystem culture swab. We also asked women to provide information about interval exposures to cigarettes, antibiotics, immune-modulating agents, oral contraceptive pill use, or antiseptic products for personal hygiene, including facial, hand, and nostril cleansing habits (rarely: 1–3/cycle, frequently: ≥4/cycle; dry, wet, or antiseptic products). Besides, each participant could opt for weekly blood sampling in order to quantify serum estradiol (E2) and P4 concentrations during the last cycle of the study.

When we proposed the study in 2013, there was no institutional or national guideline for universal screening, targeted screening, or decolonization for staphylococcal carriage in Taiwan; no nasal preparation of mupirocin ointment was available at the local pharmacy either. All participants enrolled and followed were clearly aware of their initial carriage status but not of their visit-to-visit colonization status before the study ended. Also, we did not disclose methicillin resistance results until the observation ended, at which time we provided individual consultation regarding their methicillin-resistant *S aureus* (MRSA) carriage status during the study period, the associated risk for overt clinical diseases, and the potential risk for transmitting to their patients based on the current literature at that time.[20] The Institutional Review Board reviewed and approved the study protocol and the consent form, which was read, agreed upon, and signed by each participant enrolled.

### 2.3. Laboratory procedures

**2.3.1. Microbial study and molecular characterization.** We transported nasal swabs to a certified, Biosafety Level 2 research lab for subsequent identification of *S aureus* according to previously described methods.[21,22] Briefly, we plated each swab on a Baird–Parker agar plate within 48 hours of collection, incubated for 48 hours at 37°C, and then manually isolated 1 to 3 dominant colonies for subculture on a rabbit blood agar plate. A positive coagulase test was indicative for *S aureus*, which was later confirmed by molecular typing methods described in the following. For all *S aureus* isolates, we used the disk diffusion method to determine the in vitro antibiotic susceptibility for cefoxitin (30 μg).[21,22]

We extracted and purified staphylococcal DNA from all *S aureus* isolates according to manufacturers' instructions before storage at −80°C. For each first isolate collected in the first cycle, we used pulse-field gel electrophoresis (PFGE) for strain determination.[23] We also examined for the presence of Panton–Valentine leukocidin[24] and staphylococcal protein A gene[25,26] via targeted polymerase chain reaction. We further genotyped for staphylococcal cassette complex *mecA* gene and its variants among MRSA isolates.[27,28] Lastly, we applied multilocus sequence typing methods for additional genotyping using the web-based algorithm on saureus.mlst.net.[29,30]

**2.3.2. Urinary ovulation test.** At follow-up, the study participants self-collected 1 urine sample per day between the 6th and

the 18th day of each cycle to detect urinary luteinizing hormone (LH) using a commercial kit to detect urinary concentration of LH ≥40 mIU/mL.[31] The day on which the study subject obtained a positive urinary LH test was determined as the ovulation day of the cycle. When the ovulation day of a cycle was not identified via the urinary test, we asked the participant for extended observation of 1 or 2 consecutive cycles.

*2.3.3. Serum concentration of female sex hormone.* After an initial centrifugation, we stored serum samples at −80°C before batch quantification of E2 and P4 concentrations in the Clinical Central Lab of the hospital by electrochemiluminescence immunoassay (cobas®). Based on competition principle, the highly specific polyclonal (to E2) and monoclonal (to P4) antibodies can detect serum concentrations of E2 within the range of 5 to 4300 pg/mL[32] and P4 within the range of 0.030 to 60.0 ng/mL.[33]

### 2.4. Sample size and power consideration

Assuming a 40% detection rate in the menstrual phase, an estimated RR of 1.6 comparing periovulatory and menstrual phases,[12] and a correlation of 0.4 among repeated measures from the same individual, we aimed to recruit 15 intermittent carriers who would contribute, on average, 48 nasal swabs over the study period. With a conservative assumption for a 40% intermittent carriage rate among healthcare workers,[34,35] we sought to screen 38 to 40 females and to achieve a follow-up rate of 80% at the end of the observation. The associated type I error rate was designated at 5% for the sample size estimation.[36]

### 2.5. Statistical analysis

We first described and compared individual demographic and socioeconomic factors between persistent and intermittent carriers; the former was determined by positive results of all available swabs throughout the study period. Data from the only persistent carrier were excluded from the analysis on intermittent carriage. In exploratory analysis, we employed time series analysis techniques to assess whether temporal changes in carriage status showed any cyclic pattern as previously described elsewhere.[37] We further applied mixed-effects Poisson regression methods to evaluate the association between menstrual cycle phases and nasal *S aureus* carriage while addressing the intraperson correlation in multiple swab results from the same participant.[38]

Additionally, we performed recurrent event survival analysis with robust variance estimation methods to estimate incidence rates (IRs) for a transient loss and its subsequent gain of colonization, separately.[39] For incident loss of carriage, the participant began to enter the risk set when she was first found to harbor *S aureus* prior to the current loss of carriage, at which time the same woman entered into a separate risk set for a reappearance of nasal carriage. We used R for time series analysis and Stata for regression modeling.[40,41] We reported point estimates of RR, IR, and hazard ratio (HR) along with the associated 95% confidence intervals (CIs) for a 2-tailed significance level at 0.05.

## 3. Results

In total, we screened 56 nurses to evaluate for eligibility and carriage status. Based on a single swab, we excluded 41 noncarriers (including 2 ineligible volunteers) and enrolled 15 healthy carriers into the study (Fig. 1). The estimated nasal carriage rate in the screened population was 26.8% for *S aureus* and 14.3% for MRSA. The antibiogram, PFGE result, and molecular typing for the 15 isolates collected at screening are shown online (Supplementary file, Table S1 and Fig. S1, http://links.lww.com/MD/B73). In general, carriers and noncarriers were comparable regarding sociodemographic characteristics, work history, and personal hygiene practices (Supplementary file, Table S2, http://links.lww.com/MD/B73).

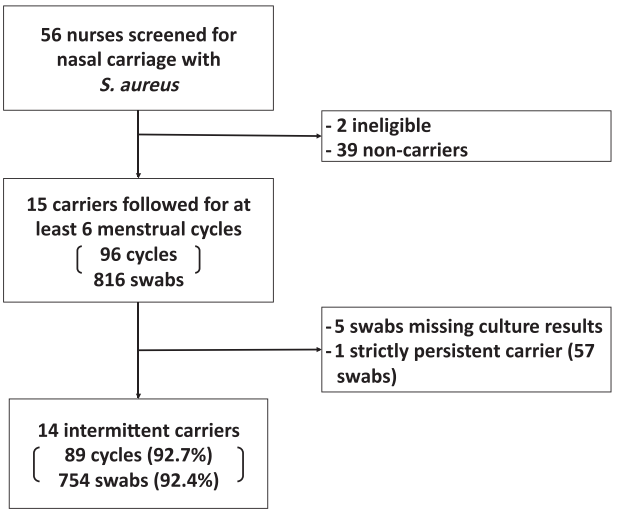


**Figure 1.** Participant flow at screening, at follow-up, and in analysis. After excluding 2 ineligible nurses and 39 noncarriers, we included 15 healthy carriers with nasal *Staphylococcus aureus* and followed them for at least 6 menstrual cycles. We further excluded data from 1 strictly persistent carrier in all analyses to evaluate factors associated with intermittent nasal carriage.

### 3.1. Characteristics of study population

Table 1 shows selected host characteristics and swab culture results for 15 carriers at enrollment and follow-up. Their median age was 35 (interquartile range [IQR]: 27–40) years; 80% of these participants held a college degree; the majority has worked in the same hospital unit for a median period of 5 years (IQR: 1.5–16). As most participants worked in the emergency or intensive care unit, over 80% of women reported regular use of antiseptic products for hand cleansing at baseline. Twelve participants reported habitual nostril cleansing (80%), either daily or weekly, whereas few applied antiseptic product for facial cleansing (2/15).

Over the study period, we have observed 96 person-cycles, with a median of 6 menstrual cycles per woman (IQR: 6–7). On average, each woman contributed 54 swab samples (IQR: 50–60) over the course of the study (Table 1). The overall period prevalence of nasal carriage was 63.9% for *S aureus* and 36.0% for MRSA. Fifty-seven swabs from 1 participant were consistently positive for *S aureus* (methicillin-sensitive) across the cycles (Table 1) and were excluded from the following analysis.

### 3.2. Menstrual cycle phase and female sex hormones

We further excluded data from menstrual cycles without an identified ovulation time (39 swabs) and included 715 swabs from 84 person-cycles to explore the (within-person) menstrual cycle phase effect on intermittent carriage. We first aligned series of culture results within a menstrual cycle with each sampling time with respect to the ovulation day of a cycle. Since there were

**Table 1**

**Selected characteristics of 15 healthy *S aureus* nasal carriers by carrier type at baseline and follow-up.**

| | Carrier (N = 15) | | Persistent (N = 1) | | Intermittent (N = 14) | |
|---|---|---|---|---|---|---|
| **Baseline** | **n** | **%** | **n** | **%** | **n** | **%** |
| Age, y; median (IQR) | 35 | 27–40 | 39 | — | 32.5 | 27–40 |
| BMI, kg/m$^2$; median (IQR) | 20.8 | 20.0–23.3 | 23.4 | — | 20.8 | 20.0–22.8 |
| <18.5 | 1 | 7 | 0 | | 1 | |
| 18.5–25 | 12 | 80 | 1 | | 11 | |
| 25–30 | 1 | 7 | 0 | | 1 | |
| ≥30 | 1 | 7 | 0 | | 1 | |
| Education ≥college | 12 | 80 | 1 | 100 | 11 | 79 |
| Setting where patient contact occurred | | | | | | |
| ICU | 7 | 47 | 0 | 0 | 7 | 50 |
| ED | 7 | 47 | 1 | 100 | 6 | 43 |
| OPD | 1 | 7 | 0 | 0 | 1 | 7 |
| Time on current ward, years; median (IQR) | 5 | 1.5–16 | 1 | — | 7.5 | 2–16 |
| Symptoms of rhinitis in recent 6 months | 6 | 40 | 0 | 0 | 6 | 43 |
| Ever used antibiotics in prior 12 months | 8 | 53 | 0 | 0 | 8 | 57 |
| Type of antiseptic hand cleansers used | N = 13 | | | | N = 12 | |
| Povidone iodine | 6 | 46 | 1 | 100 | 5 | 36 |
| Povidone iodine and alcohol | 6 | 46 | 0 | 0 | 6 | 43 |
| Alcohol only | 1 | 8 | 0 | 0 | 1 | 7 |
| Regular nostril cleaning | 12 | 80 | 0 | 0 | 12 | 86 |
| Ever used antiseptic facial cleansers | 2 | 13 | 0 | 0 | 2 | 14 |
| Alcohol drinking | 2 | 13 | 0 | 0 | 2 | 14 |
| Exposure to second-hand smoke | 2 | 13 | 0 | 0 | 2 | 14 |
| Follow-up over the study period | | | | | | |
| Total no. of cycles followed | **96** | | 7 | | 89 | |
| Median no. of cycles followed, per subject (IQR) | 6 | 6–7 | 7 | — | 6 | 6–7 |
| Median duration of a cycle followed, days, per subject (IQR) | 30 | 27–33 | 27 | 27–33 | 30 | 28–33 |
| Total no. of swabs collected | **811** | | 57 | | 754 | |
| Median no. of swabs collected, per subject (IQR) | 54 | 50–60 | 57 | — | 54 | 50–60 |
| Median no. of swabs collected/cycle, per subject (IQR) | 8 | 8–9 | 8 | 8–9 | 8 | 8–9 |
| Swabs positive for *S aureus* | 518 | 63.9 | 57 | 100 | 461 | 61.1 |
| MRSA (+) swabs | 292 | 36.0 | 1 | 1.8 | 291 | 38.6 |
| Ever used systematic antibiotics | **1** | **7** | **0** | **0** | **1** | **7** |
| Ever cleansed nose | | | | | | |
| Using dry tissue paper, towel, or fingers, yes versus no | **11** | **73** | **1** | **100** | **10** | **71** |
| Rarely, 1–3/cycle, times | 5 | 33 | 1 | 100 | 4 | 29 |
| Frequently, ≥4/cycle, times | 6 | 40 | 0 | | 6 | 43 |
| Using water only, yes versus no | **7** | **47** | **0** | **0** | **7** | **50** |
| Rarely, 1–3/cycle, times | 4 | 27 | 0 | | 4 | 29 |
| Frequently, ≥4/cycle, times | 3 | 20 | 0 | | 3 | 21 |
| Using antiseptics, yes versus no | **2** | **13** | **0** | **0** | **2** | **14** |
| Rarely, 1–3/cycle, times | 1 | 7 | 0 | | 1 | 7 |
| Frequently, ≥4/cycle, times | 1 | 7 | 0 | | 1 | 7 |
| Ever had rhinitis symptoms in the previous 6 months | **3** | **20** | **0** | **0** | **3** | **21** |

BMI = body mass index, ED = emergency department, ICU = intensive care unit, IQR = interquartile range, MRSA = methicillin-resistant *Staphylococcus aureus*, OPD = outpatient department.
Bold values distinguish results of summary indicators from those of more detailed ones.

no identifiable periodic patterns by time series analysis (Supplementary file, Fig. S2, http://links.lww.com/MD/B73), we proceeded to Poisson regression modeling with specified random effects to account for within-person correlation of repeated measures.

Table 2 displays the overall period prevalence and cycle phase-specific carriage rates as well as results of Poisson regression analysis. Among host characteristics and personal hygiene behavior, a body mass index (BMI) ≥30 kg/m$^2$ was associated with a substantial 60% reduction in risk for prevalent nasal carriage (crude RR: 0.39, 95% CI: 0.28, 0.55). In contrast to our hypothesis, the luteal phase prevalence for *S aureus* was 64.9% while that of the follicular and periovulatory phases was 58.7% and 63.1%, respectively. Yet, there was no statistical within-person difference among the 3 cycle phase-specific carriage rates in univariate Poisson regression model (both $P > 0.05$); multivariable adjustment for age and obesity status did not alter the results (Table 2). Likewise, the cycle phase-specific prevalence of MRSA was 35.6%, 36.3%, and 42.6% in the follicular, periovulatory, and luteal phases, respectively. When taking into the consideration the intraperson correlation, the risk for nasal carriage with MRSA versus methicillin-sensitive *S aureus* was 17% lower in the periovulatory phase (crude RR: 0.83, $P < 0.01$) and 8% lower in the luteal phase (crude RR: 0.92, $P$: 0.02) than in the follicular phase. Further age adjustment did not change the results (Table 2).

In a subgroup of women with available serum samples for 1 cycle, 4 showed intermittent changes in *S aureus* carriage status. Among these intermittent carriers, a high E2 (ng/mL)-to-P4 (pg/mL) ratio (log-transformed) was positively associated with staphylococcal carriage (crude RR: 1.49, 95% CI: 1.24, 1.80), even after age adjustment (adjusted RR: 1.46, 95% CI: 1.32,

**Table 2**

**Results of mixed-effects Poisson regression models on risk for nasal carriage with *S aureus* and MRSA by menstrual cycle phase among 14 healthy intermittent carriers (715 swabs).**

| | | | | 95% CI | | | | 95% CI | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Positive/total | % | Crude RR | LL | UL | *P* | Adjusted RR | LL | UL | *P* |
| Nasal swab (+) for *S aureus* | | | | | | | | | | |
| Overall | 448/715 | 62.7 | | | | | | | | |
| Age, y* | | | 1.01 | 0.97 | 1.06 | 0.525 | 1.02 | 0.97 | 1.07 | 0.478 |
| Obese (BMI ≥30 kg/m$^2$) | | | 0.39 | 0.28 | 0.55 | <0.001 | 0.38 | 0.29 | 0.49 | <0.001 |
| Menstrual cycle phase | | | | | | | | | | |
| Follicular | 122/208 | 58.7 | 1.00 | — | — | | 1.00 | — | — | |
| Periovulatory | 99/157 | 63.1 | 0.92 | 0.80 | 1.07 | 0.296 | 0.92 | 0.79 | 1.07 | 0.296 |
| Luteal | 227/350 | 64.9 | 1.00 | 0.90 | 1.12 | 0.979 | 1.00 | 0.90 | 1.12 | 0.984 |
| Nasal swab (+) for MRSA | | | | | | | | | | |
| Overall | 280/715 | 39.2 | | | | | | | | |
| Age, years* | | | 1.03 | 0.86 | 1.24 | 0.734 | 1.03 | 0.86 | 1.24 | 0.730 |
| Obese (BMI ≥30 kg/m$^2$) | | | 1.69 | 0.39 | 7.39 | 0.483 | | | | |
| Menstrual cycle phase | | | | | | | | | | |
| Follicular | 74/208 | 35.6 | 1.00 | — | — | | 1.00 | | | |
| Periovulatory | 57/157 | 36.3 | 0.83 | 0.73 | 0.94 | 0.004 | 0.83 | 0.73 | 0.94 | 0.005 |
| Luteal | 149/350 | 42.6 | 0.92 | 0.86 | 0.99 | 0.024 | 0.92 | 0.86 | 0.99 | 0.024 |

BMI = body mass index, CI = confidence interval, LL = lower limit, MRSA = methicillin-resistant *Staphylococcus aureus*, RR = relative risk (as estimated by incidence rate ratio in Poisson regression models), UL = upper limit.

* Age as a continuous variable centered at the median of 35 years.

1.62). Specifically, an E2:P4 ratio of 32 or greater (indicating E2: P4 ≥32,000) was associated with a nearly 1.5-fold increase in nasal staphylococcal carriage for a given woman (adjusted RR: 2.42, 95% CI: 1.72, 3.43). These positive relationships remained unchanged when data from the other 3 cycle-persistent carriers were included (data not shown).

### 3.3. Host characteristics and behavior for incident loss or gain of carriage

To identify other host factors associated with the dynamic changes in carriage status, we included all swabs from 14 intermittent carriers in the recurrent event survival analysis. In sum, there were 164 transient status changes, including 82 losses (IR: 4.74 per 100 carriage-days) and 82 incident gains of nasal carriage (IR: 5.47 per 100 carriage-days), with a median of 4 losses (IQR: 2–5; maximum: 11) and 4 subsequent gains (IQR: 2–5; maximum: 10) per subject. The median time to an interim loss of carriage was 10 days (95% CI: 8, 11), whereas the median time to a recolonization was 6 days (95% CI: 5, 9).

***3.3.1. Risk factors for a loss of carriage.*** Estimated rates for short-term disappearance of nasal carriage were similar by several personal hygiene habits collected at baseline, such as frequency of nostril cleansing and whether using povidone with or without alcohol for hand cleansing (Table 3). However, women in the age group of 30 to 40 years appeared to be relatively stable carriers, with a very low rate of decolonization (IR: 1.5 per 100 carriage-days), as compared to those aged 20 to 30 years (HR: 0.26, 95% CI: 0.09, 0.80; Table 3).

Also, an increasing BMI was linearly associated with a correspondingly reduced rate for loss of carriage; overweight women (BMI ≥25 kg/m$^2$) showed a 50% lower hazard, whereas those underweight (BMI <18.5 kg/m$^2$) had a more than 50% higher hazard than those with normal BMI (*P* for trend <0.001). Furthermore, working in an adult intensive care unit (MICU) seemed to enhance women's rate of losing staphylococcal carriage (IR: 8.3 per 100 carriage-days) as compared to others working in the ER (IR: 4.8 per 100 carriage-days) or in the neonatal or pediatric ICUs (IR: 4.2 per 100 carriage-days); yet the association attenuated with additional age adjustment.

At follow-up, a few participants who reported using wet methods, such as wet paper tissue or towels, showed an increased rate for losing staphylococcal carriage (9.1 per 100 carriage-days) when compared to those who used dry methods only (4.0 per 100 carriage-days). This positive association persisted after adjustment for age (adjusted HR: 1.97, 95% CI: 1.21, 3.20), BMI (adjusted HR: 1.73, 95% CI: 1.05, 2.85), and working unit (adjusted HR: 2.14, 95% CI: 1.31, 3.50). However, due to the colinearity of the above-mentioned host characteristics, we did not proceed to additional multivariable adjustment.

***3.3.2. Risk factors for a gain of carriage.*** Table 4 displays estimated rates and comparison results for incident recolonization by selected host factors. In contrast to incident loss data, 30- to 40-year-old women (HR: 0.53, 95% CI: 0.21, 1.34) or overweight women (HR: 0.91, 95% CI: 0.54, 1.51) had a comparable hazard for regaining nasal carriage to their corresponding counterparts (Table 4). Healthcare workers from MICU (IR: 2.28 per 100 carriage-days) and women who denied nostril cleansing habit at baseline (IR: 2.38 per 100 carriage-days) appeared to have the lowest rate of recolonization among all. Meanwhile, wet cleaning of nostril was associated with a nearly 1.5-fold increase in the hazard for reappeared nasal carriage (HR: 2.47, 95% CI: 1.31, 4.63) as compared to that in women who never did so during the follow-up period. Additional adjustment for host characteristics such as age (adjusted HR: 2.24), BMI (adjusted HR: 2.19), or hospital ward (adjusted HR: 1.93) resulted in a much weaker yet still significant association (Table 4).

## 4. Discussion

Among 56 healthy female healthcare workers, we have identified 15 nasal staphylococcal carriers, among whom 14 were intermittent carriers. We quantified up to 40% of variation in

**Table 3**

**Estimated incidence rates and hazard ratios for a repeated loss of nasal carriage with *S aureus* by selected host characteristics and behavior at baseline and follow-up in 14 intermittent healthy carriers.**

| Characteristics | No. of events | Carriage-days (×100) | Incidence rate (per 100 carriage-days) | 95% CI* | | Hazard ratio | 95% CI† | |
|---|---|---|---|---|---|---|---|---|
| | | | | LL | UL | | LL | UL |
| Baseline | | | | | | | | |
| Age, y | | | | | | | | |
| 20–30 | 40 | 7.14 | 5.60 | 3.59 | 8.56 | 1.00 | | |
| 30–40 | 9 | 5.86 | 1.54 | 0.61 | 4.85 | **0.26** | **0.09** | **0.80** |
| ≥40 | 33 | 4.3 | 7.67 | 5.23 | 11.36 | 1.23 | 0.76 | 1.98 |
| BMI | | | | | | | | |
| <18.5 | 10 | 1.02 | 9.80 | | | **1.57** | **1.16** | **2.12** |
| 18.5–24.99 | 64 | 12.49 | 5.12 | 3.51 | 7.64 | 1.00 | | |
| ≥25 | 8 | 3.79 | 2.11 | 1.09 | 5.31 | **0.50** | **0.32** | **0.78** |
| Working unit | | | | | | | | |
| ER | 38 | 7.96 | 4.77 | 2.67 | 9.17 | 1.00 | | |
| NICU/PICU | 35 | 8.26 | 4.24 | 2.46 | 7.44 | 0.93 | 0.50 | 1.72 |
| MICU | 9 | 1.08 | 8.33 | 5.17 | 13.36 | **1.68** | **1.09** | **2.59** |
| Frequency of nostril cleaning | | | | | | | | |
| Daily | 58 | 10 | 5.80 | 4.12 | 8.14 | 1.00 | | |
| Occasionally | 17 | 5.21 | 3.26 | 1.03 | 14.02 | 0.60 | 0.24 | 1.51 |
| Never | 7 | 2.09 | 3.35 | 0.38 | 25.55 | 0.75 | 0.23 | 2.43 |
| Povidone for hand cleaning‡ | | | | | | | | |
| No | 35 | 10.41 | 3.36 | 2.02 | 5.97 | 1.00 | | |
| Yes | 32 | 5.4 | 5.93 | 3.25 | 11.05 | 1.67 | 0.92 | 3.04 |
| Past symptoms of rhinitis§ | | | | | | | | |
| No | 52 | 7.78 | 6.68 | 5.26 | 8.55 | 1.00 | | |
| Yes | 30 | 9.52 | 3.15 | 1.59 | 6.66 | 0.58 | 0.29 | 1.15 |
| Exposure to smoke | | | | | | | | |
| No | 67 | 13.89 | 4.82 | 3.18 | 7.48 | 1.00 | | |
| Yes | 15 | 3.41 | 4.40 | 1.68 | 11.12 | 0.85 | 0.49 | 1.50 |
| MRSA status¶ | | | | | | | | |
| No | 39 | 6.49 | 6.01 | 4.25 | 8.53 | 1.00 | | |
| Yes | 43 | 10.81 | 3.98 | 2.21 | 7.55 | 0.88 | 0.50 | 1.54 |
| At follow-up | | | | | | | | |
| Menstrual phase‖ | | | | | | | | |
| Follicular | 28 | 4.71 | 5.94 | 3.99 | 9.02 | 1.00 | | |
| Periovulatory | 14 | 3.56 | 3.93 | 2.02 | 8.31 | 0.70 | 0.40 | 1.25 |
| Luteal | 37 | 8.31 | 4.45 | 2.91 | 6.92 | 0.78 | 0.49 | 1.25 |
| Nostril cleansing | | | | | | | | |
| Never | 16 | 5.04 | 3.17 | 1.13 | 10.51 | 0.90 | 0.35 | 2.30 |
| Dry methods only | 31 | 7.70 | 4.03 | 2.36 | 6.99 | 1.00 | | |
| Wet methods only | 29 | 3.19 | 9.09 | 7.55 | 10.91 | **1.96** | **1.21** | **3.16** |
| Antiseptics only | 6 | 1.34 | 4.48 | | | 1.09 | 0.71 | 1.68 |
| MRSA status (before loss)¶ | | | | | | | | |
| No | 39 | 5.61 | 6.95 | 4.81 | 9.98 | 1.00 | | |
| Yes | 43 | 9.72 | 4.42 | 2.38 | 8.54 | 0.75 | 0.39 | 1.44 |

BMI = body mass index, CI = confidence interval, ER = emergency room, ICU = intensive care unit, LL = lower limit, MICU = adult ICU, MRSA = methicillin-resistant *Staphylococcus aureus*, NICU = neonatal ICU, PICU = pediatric ICU, UL = upper limit.

* Jackknife CIs were estimated to account for repeated events within the same individual; missing estimates were due to the small number of events in the specific subgroup.

† Robust variance estimation method was applied to account for repeated event clustering within the same individual.

‡ Missing data in 2 subjects.

§ Within previous 6 months.

¶ Included only culture-positive person-time at risk.

‖ Two events occurred in ovulation-undefined menstrual cycles and were not included in the estimation.

Bold values were meant to signal results that have a *P*-value <0.05.

nasal carriage rates over 6 menstrual cycles; only 1 woman was a consistent carrier as determined by 57 consecutively positive swabs. Over the 18-month study period, there were no clustered MRSA infections identified among participating ward units reported to the hospital infection control team.

While the study did not find evidence for associations between menstrual cycle phases and nasal carriage status, we noticed that a higher BMI was negatively associated with prevalent carriage with *S aureus*. This particular finding was in contrast to a recent report by Befus et al, who found a positive association between obesity and prevalence of staphylococcal carriage among middle-aged female inmates.[42] Particularly, our prevalence results were inconsistent with those of incidence analysis, in which women with an increased BMI were more likely to retain their staphylococcal carriage than those with a normal-range BMI. Discordances in results from prevalence and incidence data explained for the necessity of presenting both within the same study whenever possible so as to avoid reporting bias.

**Table 4**

**Estimated incidence rates and hazard ratios for a repeated recolonization with nasal *S aureus* by selected host characteristics and behavior at baseline and follow-up in 14 intermittent healthy carriers.**

| Characteristics | No. of events | Carriage-days (×100) | Incidence rate (per 100 carriage-days) | 95% CI* | | Hazard ratio | 95% CI† | |
|---|---|---|---|---|---|---|---|---|
| | | | | LL | UL | | LL | UL |
| Baseline | | | | | | | | |
| Age, y | | | | | | | | |
| 20–30 | 38 | 7.88 | 4.82 | 2.71 | 8.61 | 1.00 | | |
| 30–40 | 10 | 4.27 | 2.34 | 0.83 | 7.45 | 0.53 | 0.21 | 1.34 |
| ≥40 | 34 | 2.83 | 12.01 | 7.23 | 19.01 | 1.61 | 0.88 | 2.95 |
| BMI | | | | | | | | |
| <18.5 | 10 | 0.63 | 15.87 | | | **1.95** | **1.34** | **1.51** |
| 18.5–24.99 | 64 | 11.98 | 5.34 | 3.26 | 8.81 | 1.00 | | |
| ≥25 | 8 | 2.37 | 3.38 | 1.56 | 7.61 | 0.91 | 0.54 | 1.51 |
| Working unit | | | | | | | | |
| ER | 41 | 4.83 | 8.49 | 4.39 | 16.91 | 1.00 | | |
| NICU/PICU | 32 | 6.20 | 5.16 | 3.17 | 8.57 | 0.65 | 0.42 | 1.00 |
| MICU | 9 | 3.95 | 2.28 | 1.89 | 2.78 | **0.36** | **0.23** | **0.55** |
| Frequency of nostril cleaning | | | | | | | | |
| Daily | 56 | 8.34 | 6.71 | 4.29 | 10.48 | 1.00 | | |
| Occasionally | 18 | 3.28 | 5.49 | 1.30 | 27.4 | 1.04 | 0.43 | 2.48 |
| Never | 8 | 3.36 | 2.38 | 2.14 | 2.73 | **0.50** | **0.35** | **0.72** |
| Povidone for hand cleaning‡ | | | | | | | | |
| No | 35 | 6.14 | 5.70 | 2.94 | 11.54 | 1.00 | | |
| Yes | 32 | 6.21 | 5.15 | 2.60 | 10.34 | 0.81 | 0.50 | 1.31 |
| Past symptoms of rhinitis§ | | | | | | | | |
| No | 52 | 7.07 | 7.36 | 4.29 | 12.36 | 1.00 | | |
| Yes | 30 | 7.91 | 3.79 | 1.83 | 8.26 | 0.68 | 0.34 | 1.32 |
| Exposure to smoke | | | | | | | | |
| No | 67 | 13.24 | 5.06 | 3.13 | 8.24 | 1.00 | | |
| Yes | 15 | 1.74 | 8.62 | 3.33 | 24.5 | 1.48 | 0.99 | 2.21 |
| MRSA status¶ | | | | | | | | |
| No | 39 | 5.50 | 7.09 | 3.50 | 13.49 | 1.00 | | |
| Yes | 43 | 9.48 | 4.54 | 2.42 | 8.95 | 0.72 | 0.40 | 1.31 |
| At follow-up | | | | | | | | |
| Menstrual phase‖ | | | | | | | | |
| Follicular | 16 | 3.53 | 4.53 | 2.48 | 8.61 | 1.00 | | |
| Periovulatory | 23 | 3.69 | 6.23 | 3.94 | 10.33 | 1.26 | 0.63 | 2.54 |
| Luteal | 41 | 6.78 | 6.05 | 3.86 | 9.67 | 1.39 | 0.77 | 2.49 |
| Nostril cleansing | | | | | | | | |
| Never | 15 | 5.90 | 2.54 | 1.09 | 6.82 | 1.00 | | |
| Dry methods only | 32 | 5.40 | 5.93 | 2.70 | 12.59 | 1.83 | 0.89 | 3.78 |
| Wet methods only | 29 | 2.70 | 10.74 | 6.70 | 17.10 | **2.47** | **1.31** | **4.63** |
| Antiseptics only | 6 | 0.98 | 6.12 | | | **2.38** | **1.30** | **4.37** |
| MRSA status¶ | | | | | | | | |
| No | 38 | 2.75 | 13.8 | 7.69 | 23.1 | 1.00 | | |
| Yes | 44 | 4.70 | 9.36 | 4.06 | 21.0 | 0.82 | 0.50 | 1.34 |

BMI = body mass index, CI = confidence interval, ER = emergency room, ICU = intensive care unit, LL = lower limit, MICU = adult ICU, MRSA = methicillin-resistant *Staphylococcus aureus*, NICU = neonatal ICU, PICU = pediatric ICU, UL = upper limit.

* Jackknife CIs were estimated to account for repeated events within the same individual; missing estimates were due to the small number of events in the specific subgroup.

† Robust variance estimation method was applied to account for repeated event clustering within the same individual.

‡ Missing data in 2 subjects.

§ Within previous 6 months.

¶ Included only culture-positive person-time at risk.

‖ Two events occurred in ovulation-undefined menstrual cycles and were not included in the estimation.

Bold values were meant to signal results that have a *P*-value <0.05.

Moreover, we were able to characterize intermittent carriers according to their carriage duration. We noted that 1 group of carriers, who were underweight and tended to cleanse their nostrils using wet towels, frequently lost and regained staphylococcal carriage. The other group, either working in the MICU or reportedly never having cleansed their nostrils, had a moderate (or above the average) rate of losing carriage yet a relatively low (or below the average) rate of recolonization. Still another group, comprising of women aged 30 to 40 years and those with a BMI ≥25 kg/m$^2$, regained carriage at a similar rate to others but were the least likely to decolonize. Whether these host characteristics could facilitate risk stratification among the majority of staphylococcal carriers (being intermittent ones) and how differences in average carriage durations translate into risks for clinical diseases need to be further addressed in a large study.

Although the current study enrolled only women, who are generally at a lower risk than men for harboring *S aureus* in the anterior nares,[2] we hypothesized that the changing nature of female sex hormones within a menstrual cycle and the between-

visit variation in personal hygiene behavior might shed light on alternative mechanisms underlying the intermittent nature of nasal carriage with *S aureus*. The correct classification for intermittent versus persistent carriage and the precise measurement for fluctuating sex hormones of a cycle were key to meaningful interpretations of the study findings.

While the earliest interest in investigating estrogen effects on pathogen colonization began in the genital tract epithelium, study results on humans[43–45] have been as conflicting as those on cell lines.[46] Early animal models, however, showed some consistency in findings that estrogen at the high-range level could predispose the host animals to bacterial colonization[47] or infection.[48,49] With later studies revealing that sex steroid receptors also existed in the nasal cavity[50] and that menstrual cycle affected allergic reactions of the nose[51,52] and the skin,[53] Winkler et al were among the first to use the karyopyknotic index (to represent women's estrogen level) and compared nasal carriage rates in both premenopausal and postmenopausal women.[11]

Previously, investigators have mostly relied on surrogates to reflect physiological variations in sex hormones within a menstrual cycle or during pregnancy and few have taken advantages of repeated, intensive sampling to study the dynamic inter-relationship of nasal carriage and female sex hormones (Table 5). In the current analysis, when we similarly grouped culture results by convention and statistically addressed the intraperson correlation, we found no association between nasal *S aureus* carriage and cycle phases, either contemporaneously or in lagged analysis (data not shown). However, results of subgroup analysis suggested otherwise.

Lagged analysis on serum concentration of E2 also showed its predictive value for nasal carriage with *S aureus* by a 2-week leading time (data not shown), suggesting that cycle phase categorization could lose significant information while assessing hormonal effects over time. When we further replaced hormonal measurements with cycle phase indicators in subgroup analysis, we failed to reproduce the associations found with serum concentrations of sex hormones. Putting it altogether, our seemingly inconsistent findings suggested that direct measurements of sex hormones in the serum were required to fully understand their potential immunomoderating effects on nasal staphylococcal carriage.

In addition to the possible immunomodulating effects directed by estrogen[12] or P4,[52] indirect influences on the microenvironment of anterior nares by sex hormones are also likely to play a role in the dynamics of staphylococcal carriage. In contrast to what we have learned from animal models and in vitro studies that sex hormones can fine-tune both innate and adaptive immunity in the female genital tract,[56] how cyclic sex hormones may moderate the skin immunity of the anterior nares remains unexplored.

Recently, the importance of other commensal bacteria in the anatomical niche for *S aureus* has gained momentous recognition with the advancing sequencing technology. For instance, Bessesen et al have shown that in a matched case–control study, there was a negative association between MRSA colonization and co-colonization with *Streptococcus mitis*, which inhibited MRSA growth similarly to the effect by adding catalase.[57] In another case–control study, Yan et al demonstrated that 2 strains of *Corynebacterium* spp. were associated with a high and low abundance of *S aureus*, separately.[58] Both studies and others[59–61] suggest that the interspecies interaction could vary among carriers by carrier's distinct nasal microbial signature, molding of which by either physiological or behavioral perturbations may provide opportunities for controlling and preventing prolonged carriage with pathogens such as *S aureus*.

### 4.1. Limitations

Several limitations should be considered while interpreting the study results. First of all, findings from this highly selected study population may not be readily generalizable. As noticed, all participants worked in either the emergency department or ICUs where universal precautions (including hand hygiene practices) were already in place. The hypothesized hormonal effects might be too subtle to sustain beyond what individual behavior might have had on the changing rates of nasal carriage in this particular study population. Given the number of collected samples, and the observed carriage rate in the follicular phase, ad hoc power analysis revealed a statistical power of mere 55% to detect the theoretical risk difference between the middle and the early cycle phase based on the literature. Alternatively, assuming the observed OR of colonization comparing the periovulatory and the menstrual phases was true (OR = 0.92), we would need to follow nearly 5080 person-cycles to obtain a statistical power of 80% in order to detect such small difference in carriage risks. Such intensive follow-up scheme for a considerable period of time would be challenging had we chosen females in the general population.

Second, we chose to follow only healthcare workers whose single-time swab culture was positive at screening due to the limited funding and difficulty in accrual. Accordingly, we could have missed a substantial number of intermittent carriers. Although we expected some behavioral changes by these included participants knowing their carriage status at baseline, we found little evidence for different personal hygiene practices while comparing women's behavior at follow-up with that at baseline (before knowing the carriage status). Women who reported having never cleansed their nostrils still refrained from doing so over the study period.

Also, we have assumed that the study participants carried only single, dominant strain of *S aureus* throughout the study period and that the observed carriage pertained to the same single and only strain as identified by the first sample in the study. While such assumption was not fully supported by ad hoc PFGE studies, which showed pulsotype switches in 3 participants (or 8 out of 81 selected cycle-representative swabs, 9.9%), current results were not altered when we limited the analysis on the other 11 participants. The observed frequency of type switch at the cycle level was compatible with that in a cohort of community-dwelling carriers over a 24-month period.[62]

Our observations may in fact suggested that whether the single-strain assumption was valid or not did not matter; as long as the sampling procedure became frequent enough, staphylococcal colonization was not always but only intermittently detectable. The observed variation in nasal carriage with *S aureus* could have resulted from sampling variation per se, rather than from any host hormonal or behavioral influences causing low bacterial loads. However, the fact that host characteristics such as age, BMI, and personal hygiene practices unequally correlated with the transient gain and loss of carriage status suggested that random sampling errors were unlikely to completely explain for the observed dynamics. Lastly, the lack of serum concentrations of E2 and P4 from all participants and throughout the study period has precluded comprehensive assessments on possible dynamic interactions of intermittent carriage and female sex hormones within a menstrual cycle.

**Table 5**

**Summary of selected previous investigations on effects of female sex hormones on nasal or genital carriage of *S aureus* in healthy individuals.**

| Year | Author | Study population | Design | Statistical method | Results | Summary of findings |
|---|---|---|---|---|---|---|
| Nare | | | | | | |
| 1990 | Winkler et al[11] | 479 women | Cross-sectional, single sample per subject | Chi-square test | Nasal carriage rate was higher for women with high KIs (40.7%) than for those with intermediate (27.03%) and low (25.1%) KIs (*P*: 0.026). *S aureus* carriage rate was higher in the middle (31%) and the last-third of the cycle (35%) than in the first one-third of the cycle (14%, *P*: 0.008) | Levels of sex hormones as reflected by the KI were associated with *S aureus* nasal carriage rates |
| 2012 | Zanger et al[12] | 720 women, 460 men | Cohort, 2 samples per subject | Logistic regression | OCP users and men had higher odds for *S aureus* colonization than non-OCP users (adjusted OR: 1.88, *P*: 0.007) | Estrogen might increase women's risk for *S aureus* colonization |
| Genital tract | | | | | | |
| 1982 | Martin et al[43] | 145 women (aged 15–32 years, mean 23.7 years) | Cross-sectional, single nasal and vaginal sample per subject; multiple samples for a subgroup of women | Multiple 2-sample (independent) *t* test | 60% of vaginal carriers for *S aureus* (n: 9) also carried nasal *S aureus*, whereas 23% of vaginal noncarriers (n: 30) had nasal *S aureus*. Bacterial colony counts were higher in isolates obtained during than after menstruation ($P < 0.02$) | Menstruation may increase the number of vaginal *S aureus* in carriers |
| 1982 | Noble et al[44]; Smith et al[45] | 52 women (aged 17–37 years) | Cohort, 2 samples per subject (menstrual phase and midcycle) | McNemar test | Cervical colonization of *S aureus* was more frequent during menstruation (17%) than at midcycle (5.8%, $P < 0.05$) | Significant association of menses with *S aureus* colonization of the cervix |
| 1984 | Chow et al[54] | 495 women (mean age 22.8 years) | Cross-sectional, single sample per subject | Unclear | Prevalence of vaginal carriage with *S aureus* was 3.7% in the first to 7th days, 6.8% in the 8th to 14th days, 8.3% in the 15th to 21st days, and 6.7% in the 22nd or later days of the cycle | No association of menstrual phases with vaginal colonization of *S aureus* |
| 2013 | Anderson et al[55] | 47 pregnant and 16 nonpregnant women (aged 17–35 years) | Cohort, 4 serial samples per subject | Cochran–Mantel –Haenszel test | No *S aureus* was detected in nonpregnant women, whereas 8.5%, 2.2%, 0%, and 4.2% of swabs taken from pregnant women at the <14, 14–28, and >28 weeks' gestation and postpartum visit, respectively, showed positive for *S aureus* | There was no significant difference in vaginal or cervical *S aureus* carriage rate between pregnant and nonpregnant women |

KI = karyopyknotic index, OCP = oral contraceptive pill, OR = odds ratio.

In conclusion, we found that nasal staphylococcal carriage could vary substantially over time in healthy carriers; yet such dynamic intraperson changes were not statistically associated with menstrual cycle phases. Notably, we identified that host age and a high BMI were correlated with a tendency toward persistence in healthy intermittent carriers.

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