Human Factor in *Staphylococcus aureus* Nasal Carriage

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Persistent nasal carriers and noncarriers of *Staphylococcus aureus* were inoculated with a mixture of different *S. aureus* strains. The majority of noncarriers and nearly all persistent carriers returned to their original carrier state after artificial inoculation. Furthermore, the majority of persistent carriers tested positive again for their original resident strain. Using a human nasal inoculation model, we here demonstrate that the human factor is an important determinant of *S. aureus* nasal carriage.

*Staphylococcus aureus* nasal carriage is a major risk factor for *S. aureus* infections (10, 22). Recently, cell wall lipoteichoic acid was described as the core factor for *S. aureus* nasal colonization (23). However, in earlier studies, no single staphylococcal factor essential for nasal colonization could be identified (1, 6, 15, 19). Furthermore, host factors (7, 8, 10) as well as environmental factors are recognized determinants of the *S. aureus* nasal carrier state (3, 16).

Three human nasal *S. aureus* carriage patterns can be distinguished: persistent carriage, intermittent carriage, and non-carriage (17). *S. aureus* density in the anterior nares is higher in persistent carriers (13), which may partly explain their increased risk for *S. aureus* infections (5). Variation among colonizing strains is higher for intermittent carriers (21), suggesting that the basic determinants of persistent and intermittent carriage are different. The biology of *S. aureus* nasal carriage remains incompletely understood, although the importance of various host factors has been demonstrated (2, 8, 9, 12). In seeking further clarification, we performed a study in which persistent carriers and noncarriers were inoculated intranasally with a mixture of *S. aureus* strains.

(Parts of these results have been presented at the International Society for Staphylococci & Staphylococcal Infections meeting, June 2000, Kolding, Denmark.)

In 1988, a cohort of healthy volunteers (staff members of the Departments of Medical Microbiology & Infectious Diseases and Virology of the Erasmus MC) was formed to investigate bacterial and human factors associated with *S. aureus* nasal carriage (21). The composition of this volunteer cohort was not fixed, in that outgoing personnel were considered lost to follow-up. Participants were excluded if they suffered from diabetes mellitus, skin diseases, chronic obstructive pulmonary disease, or cardiac valve abnormalities or if they were taking immunosuppressive agents. Eleven persistent carriers and eight noncarriers agreed to participate in the present study. All participants gave written informed consent, and the study was approved by the Medical Ethics Review Committee of the Erasmus Medical Center, Rotterdam, The Netherlands (METC Erasmus MC decision no. 156.137/1996/186).

For the noncarriers, a mixture of four different *S. aureus* strains was prepared; the mixture consisted of *S. aureus* 502A (a strain used in intervention studies in the 1960s and 1970) (11), *S. aureus* DU 5819 (a protein A-deficient Dublin strain, courtesy of T. Foster), *S. aureus* 274 (a strain from a persistent carrier), and *S. aureus* 1036 (a strain from an intermittent carrier). Strains were selected from different carriage classes to analyze whether they had different colonization capacities (21). The strains did not produce superantigens and did not show different in vitro growth characteristics (data not shown). For the persistent carriers, the same mixture of four *S. aureus* strains was used, but with each carrier’s own resident strain added.

Nasal swabs were obtained with cotton-wool swabs (Transwab, Corsham, United Kingdom) (21). The left and right anterior nares were swabbed four times around. The swabs were immediately placed in Stuart’s transport medium (Transwab) and kept at 4°C until quantitative culture on phenol red-mannitol-salt agar (PHMA) and in phenol red-mannitol-salt broth (PHMB). The PHMB was incubated at 37°C (7 days); the PHMA culture plates were incubated at 37°C (48 h) and at room temperature (5 days). Identification of *S. aureus* was based upon colony morphology and a catalase and latex agglutination test (Staphaurex Plus; Murex, Dartford, United Kingdom). The geometric mean CFU in the 26 postinoculation cultures was calculated by the formula \[
\frac{10 \log(CFU_1 + \ldots + 10 \log(CFU_{26} + 1))}{26}.\]

For each culture, 16 *S. aureus* colonies (maximum amount allowing for efficient molecular characterization), including all *S. aureus* morphotypes, were stored at −70°C. To obtain bacterial DNA, *S. aureus* isolates were grown overnight at 37°C on brucella
blood agar and processed as described by Boom et al. (4). DNA was stored at −20°C. Restriction fragment length polymorphisms of the coagulase and protein A genes were determined for strain identification purposes in the four S. aureus strains and all resident S. aureus strains from persistent carriers before inoculation (20). Furthermore, all S. aureus strains isolated 2 and 13 weeks after inoculation and/or from the last positive culture were genotyped by this method. Pulsed-field gel electrophoresis was performed to confirm the results (14).

All persistent carriers were treated with mupirocin nasal ointment (Bactroban; GlaxoSmithKline, Zeist, The Netherlands) two times daily for 5 days. The noncarriers did not receive mupirocin treatment. Ten weeks later, with nasal swab cultures negative, all participants were inoculated. Inoculation was performed using cotton-wool swabs drenched in PHMB containing 10⁹ CFU of each strain/ml. For each nostril, one swab was firmly applied against the inner side of the anterior nares and turned around four times. In this way, the strains were inoculated in a total amount of 10⁹ CFU. At the time of inoculation, blood was drawn for the determination of the erythrocyte sedimentation rate, C-reactive protein, leukocyte count and differentiation, and antistaphylococcal antibodies. These tests were repeated when required. Nasal cultures were performed weekly during the study period. All participants with positive cultures at the end of the study were offered mupirocin nasal ointment (Bactroban).

The primary end point was survival of S. aureus in the nose after artificial colonization. Survival was considered ended when at least two consecutive nasal swab cultures were negative. Kaplan-Meier curves and the log rank test were used to compare S. aureus survival curves. Participants still carrying S. aureus in their noses at the end of the study were considered censored in the analysis. The secondary end point was the geometric mean count of CFU over 26 weeks. Percentages and continuous data were compared by Fisher’s exact test and Mann-Whitney’s test, respectively.

After artificial inoculation with a mixture of S. aureus strains, median nasal survival of S. aureus was 186 days in persistent carriers versus 35.5 days in noncarriers (P = 0.0427; log rank test).

FIG. 1. S. aureus survival after artificial nasal inoculation in long-term persistent S. aureus nasal carriers and nasal noncarriers. Kaplan-Meier curves of S. aureus nasal survival in persistent carriers (solid line) and in noncarriers (broken grey line) are shown. Survival ended when at least two consecutive nasal swab cultures were negative. After artificial inoculation with a mixture of S. aureus strains, the median nasal survival of S. aureus was 186 days in persistent carriers versus 35.5 days in noncarriers (P = 0.0427; log rank test).
pletely normal. No side effects were noted in the persistent-carrier group ($P = 0.1637$).

In the persistent-carrier group, seven persons became persistent carriers again after artificial nasal inoculation: four carrying their own resident strains (persistent carriers 322, 211, 216, and 248) and three carrying unique foreign strains not included in the inoculation mixture (persistent carriers 318, 303, and 228). These new strains were all genetically different and did not represent a laboratory contamination. Three persistent carriers became intermittent carriers with their own resident strains (persistent carriers 326, 316, and 321), and one person reverted to the noncarrier state (persistent carrier 240) (Fig. 2).

The present results identify the importance of host factors in determining the $S. aureus$ nasal carrier state in healthy adults. Half of the noncarriers became noncarriers again within 2 weeks after inoculation. Only one noncarrier became a persistent carrier, which coincided with the occurrence of minor self-limiting skin lesions. These data suggest that most noncarriers are inherently resistant to colonization, but when $S. aureus$ carriage is imposed, minor skin lesions can develop. Bacterial interference may be an explanation of the noncarrier state: when an ecological niche is already occupied by other bacteria, $S. aureus$ does not seem to have the means to establish a local population (18). Recent data indicate that when the noncarriers were treated with mupirocin prior to inoculation, elimination was as efficient: only 1 out of 16 volunteers was found to be still colonized after 16 weeks (data not shown).
This finding suggests that noncarriers are not protected by a mupirocin-susceptible resident population of bacteria.

Among the 11 persistent carriers, 7 became persistent carriers again: 4 with their own resident strains and 3 with genetically unique foreign strains not included in the mixture. Three persons became intermittent carriers, all with their own resident strains. Only one person reverted to the noncarrier state. Given the opportunity, persistent carriers will select for an optimally fitting S. aureus strain, either from the inoculation mixture or from their environment (21). Including the intermittent carriers, 7 out of 11 volunteers were colonized with their original resident strains again.

So far, no single common genetic or phenotypic characteristic segregating successful from less successful or nonsuccessful colonizing S. aureus strains has been identified (1, 7, 8, 15, 19). However, lipoteichoic acid has been recently implicated as an essential bacterial factor for S. aureus nasal colonization in a rat model (23). Here we conclude that, in addition, host characteristics significantly codetermine the S. aureus carrier state and that optimal fit between host and bacteria seems to be important. Further research should focus on identifying the specific host and bacterial factors involved. New strategies for the prevention of S. aureus nasal carriage and endogenous S. aureus infection could then be developed.

We gratefully acknowledge the volunteers that agreed to participate in this study. Without their willing participation, none of what is presented here could actually have been documented in such detail.

REFERENCES


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