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 & Staphyloccal 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 and were replaced by incoming personnel. All volunteers were screened initially with 12 quantitative nasal swab cultures performed at 1-week intervals. After this initial establishment of S. aureus nasal carriage status, the volunteers were rescreened regularly with four quantitative nasal swab cultures at 1-week intervals. For the present study, only volunteers in nasal carriage status, the volunteers were rescreened regularly with four quantitative nasal swab cultures done were included. Long-term persistent carriers were defined as those with all preceding cultures positive, and long-term noncarriers were defined as those with all preceding cultures negative. 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 (Tran-swab, 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 (Tran-swab) 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 \[10 \log(CFU_1 + 1) + 10 \log(CFU_2 + 1) + \ldots + 10 \log(CFU_{26} + 1))/26.\] 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 $-20^\circ$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 $10^9$ 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 $10^9$ 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). Also, the 26-week log geometric mean number of CFU was higher in persistent carriers (median, 2.9 [range, 0.6 to 3.7] versus 0.7 [range, 0 to 3.6]), nearly reaching statistical significance ($P = 0.069$).

Six out of eight noncarriers became noncarriers again: four within 2 weeks after inoculation (noncarriers 224, 317, 244, and 207), and two after 19 (noncarrier 302) and 23 (noncarrier 311; inoculation strain *S. aureus* 502A) weeks (Fig. 2). Noncarrier 302 remained persistently positive with inoculation *S. aureus* strain 274 until week 11, was intermittently positive between weeks 12 and 18, and reverted to the noncarrier state again at week 19 (Fig. 2). Two noncarriers still had positive nasal cultures at the end of the study. Noncarrier 233 had positive cultures up to week 8, negative cultures between weeks 9 and 20, and then cultured positive again until the end of the study. This person first tested positive for *S. aureus* DU 5819, while ultimately a foreign *S. aureus* strain not included in the inoculation mixture was identified. Noncarrier 249 remained persistently positive after inoculation until the end of the study with inoculation *S. aureus* strain 274 and thus had become a persistent *S. aureus* nasal carrier (Fig. 2). Two of the noncarrier volunteers developed minor self-limiting skin lesions, noncarrier 311 colonized with *S. aureus* 502A and noncarrier 249 colonized with *S. aureus* strain 274. No antibiotic treatment was necessary, and all laboratory parameters remained com-

![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).](http://iai.asm.org/)

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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).

**FIG. 2.** Postartificial nasal inoculation culture results for 11 long-term persistent and 8 non-$S. aureus$ nasal carriers during 26 weeks of follow-up. ID, identification number of individual participants. At time points 2 and 13 days postinoculation of the last positive culture, all $S. aureus$ strains cultured were genotyped. The various genotypically distinct $S. aureus$ strains cultured are identified by colors and codes. The original resident strains of the persistent carriers are colored orange and coded O (“own”). The four $S. aureus$ strains used in the inoculation mixture are colored (mint) green and coded i1 (inoculation $S. aureus$ strain 502A), i2 (inoculation $S. aureus$ strain 274 [persistent-carrier strain]), i3 (inoculation $S. aureus$ strain 1036 [intermittent-carrier strain]), and i4 (inoculation $S. aureus$ strain DU 5819 [protein A-deficient strain]). Five unique foreign $S. aureus$ strains, which were neither resident strains from persistent carriers nor inoculation strains from the inoculation mixture, were cultured in five participants. These foreign $S. aureus$ strains are colored blue and coded f1, f2, f3, f4, and f5. Multiple genotypically distinct $S. aureus$ strains can thus be cultured at each point in time during follow-up. The shading of colors indicates the total number of CFU of $S. aureus$ per culture: dark coloring, more than 100 CFU; light coloring, 1 to 99 CFU; no coloring, cultures negative at that point in time.
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 intrinsically unique foreign strains not included in the mixture. Three mupirocin-susceptible resident population of bacteria. This finding suggests that noncarriers are not protected by a 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