Reclassification of *Staphylococcus aureus* Nasal Carriage Types

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**Background.** Persistent nasal carriers have an increased risk of *Staphylococcus aureus* infection, whereas intermittent carriers and noncarriers share the same low risk. This study was performed to provide additional insight into staphylococcal carriage types.

**Methods.** Fifty-one volunteers who had been decolonized with mupirocin treatment and whose carriage state was known were colonized artificially with a mixture of *S. aureus* strains, and intranasal survival of *S. aureus* was compared between carriage groups. Antistaphylococcal antibody levels were also compared among 83 carriage-classified volunteers.

**Results.** Persistent carriers preferentially reselected their autologous strain from the inoculum mixture (*P* = 0.02). They could be distinguished from intermittent carriers and noncarriers on the basis of the duration of postinoculation carriage (154 vs. 14 and 4 days, respectively; *P* = 0.017, by log-rank test). Cultures of swab samples from persistent carriers contained significantly more colony-forming units per sample than did cultures of swab samples from intermittent carriers and noncarriers (*P* = 0.004). Analysis of serum samples showed that levels of immunoglobulin G and immunoglobulin A to 17 *S. aureus* antigens were equal in intermittent carriers and noncarriers but not in persistent carriers.

**Conclusions.** Along with the previously described low risk of infection, intermittent carriers and noncarriers share similar *S. aureus* nasal elimination kinetics and antistaphylococcal antibody profiles. This implies a paradigm shift; apparently, there are only 2 types of nasal carriers: persistent carriers and others. This knowledge may increase our understanding of susceptibility to *S. aureus* infection.

Nasal carriage of *Staphylococcus aureus* plays a key role in the epidemiology and pathogenesis of staphylococcal infection [1, 2]. Eradication of *S. aureus* from the nose has proved to be effective in reducing the incidence of staphylococcal infection [3–5]. This indicates that the anterior nasal region is a primary ecological reservoir of *S. aureus* [6, 7], although the throat and the perineum are also important reservoirs [8]. However, nasal recolonization may occur within weeks to months in those who have successfully been decolonized [9, 10]. In light of the emergence of antibiotic resistance and the lack of long-term elimination strategies against *S. aureus* nasal carriage, new approaches are needed for the prevention of staphylococcal disease. To develop new strategies, it is important to acquire additional knowledge about the underlying mechanisms of *S. aureus* nasal carriage.

Historically, individuals have been assigned to 1 of 3 groups with regard to carriage of *S. aureus*: persistent carriers (≈20% of individuals), intermittent carriers (≈30%), and noncarriers (≈50%) [1, 11, 12]. The prevalence of *S. aureus* nasal carriage varies, however, and is higher in young children [13], men [14], white persons [14], hospitalized patients [15], and a number of patient groups, including patients with diabetes mellitus [16], those undergoing hemodialysis [17] or chronic ambulatory peritoneal dialysis [18], those with *S. aureus* skin infection [19], and HIV-infected patients [1, 20]. It has been documented that certain individuals may carry their resident strain for extensive periods, sometimes
even for years [21]. How nasal carriage is established and maintained is still largely unknown [1, 7].

In addition to host factors, several bacterial carriage determinants have been studied. Cell wall teichoic acid, lipoteichoic acid, and fibronectin-binding proteins have been shown to represent major ligands in the adherence of S. aureus to epithelial cells [2, 22]. Recently, such a role for the protein clumping factor (Clf) B was ascertained by use of human and animal artificial colonization [23, 24]. Meanwhile, current knowledge still does not explain why some individuals are persistent carriers and others are intermittent carriers or noncarriers.

In this study, we provide additional insight into nasal carriage types. Differences in S. aureus survival in the human nose and differences in antistaphylococcal antibodies in healthy persistent carriers, intermittent carriers, and noncarriers were analyzed, and the specificity of the interaction between bacterial strains and individuals was determined.

**METHODS**

**Study Design: Artificial Nasal Colonization**

**Study population.** Volunteers with any of the following features were excluded from the study: known history of recurrent skin infections, eczema, presence of skin lesions, allergy to β-lactam antibiotics, endocarditis, chronic pulmonary disease, chronic sinusitis, diabetes mellitus, dialysis, current use of antibiotics or immunosuppressive medication, pregnancy, expected contacts with patients at high risk of staphylococcal disease during the study period (e.g., patients in intensive care units or undergoing dialysis), and any other conditions that in the opinion of the investigators would incur a risk to the participant or their contacts. Fifty-one healthy volunteers aged 18–65 years were included. Written informed consent was obtained, and participants were notified that an infectious diseases’ physician was on call for the entire study period. The study was approved by the institutional review board of the Erasmus MC Rotterdam (156.137/1996/186).

**Screening.** The volunteers were screened for nasal carriage of S. aureus over a mean duration of 6 months. Nasal swab samples were collected on 5–10 separate occasions during this 6-month period; samples were collected ≥1 week apart, but the usual interval was 3–4 weeks. Participants were labeled as persistent carriers if ≥80% of the cultures were positive for S. aureus; allowing 1 of ≥5 swab samples to test negative minimizes misclassification of carriage state because of culture or laboratory errors. Participants were labeled as noncarriers if all nasal cultures were negative for S. aureus; all other participants were labeled as intermittent carriers [25]. Immediately before the start of mupirocin treatment, a venous blood sample was obtained for measurement of antistaphylococcal antibodies and baseline safety assessments (C-reactive protein levels and leukocyte counts).

**Eradication.** After screening, participants self-administered mupirocin 2% nasal ointment (GlaxoSmithKline) twice daily for 5 days to eradicate resident S. aureus strains and other susceptible microorganisms present in the vestibulum nasi. Both oral and written instructions were given to the volunteers, and a visual presentation on the application of mupirocin ointment was given by the study staff. Nasal samples were obtained just before and 5 weeks after mupirocin treatment.

**Inoculation.** On the day of artificial colonization, all inclusion and exclusion criteria were rechecked. All participants were inoculated with a mixture of S. aureus strains with nearly indistinguishable in vitro growth rates: strain 502a, 2 strains randomly collected from persistent carriers (P1 and P2), a strain collected from an intermittent carrier (I), and in the case of persistent or intermittent carriers, the last cultured resident strain [26]. Inoculation took place under medical supervision. Both anterior nares were inoculated once with 1 × 10⁷ cells per bacterial genotype.

**Follow-up.** Nasal cultures were performed 1, 2, 4, 8, 16, and 22 weeks after inoculation. Restriction fragment-length polymorphism of the coagulase and protein A genes was used to type the cultured S. aureus strains [26]. Pulsed-field gel electrophoresis (PFGE) was performed according to methods described elsewhere [8] to confirm the results of restriction fragment-length polymorphism analysis. Participants received weekly medical examinations and were instructed to contact the investigator if they had putatively adverse reactions. At the end of the study, the safety assessments were repeated, and participants underwent their last medical examination.

**Microbiological Procedures**

Nasal samples were obtained by streaking both anterior nares with a sterile cotton swab (Transwab; Medical Wire and Equipment). All swab samples were processed within 24 h. Nasal swab samples were plated on a Columbia blood agar plate medium (Becton Dickinson) and were submerged in phenol red mannitol enrichment broth [27]. Plates were read after 1 and 2 days of incubation, and broth cultures were read after 7 days of incubation at 37°C. Broth cultures in which the color changed from red to orange-yellow were subcultured on blood agar plates. Identification of S. aureus was based on colony morphology and results of Gram stain, catalase test, and latex-agglutination test (Staphaurex Plus; Murex).

All strains included in the mixture were tested by polymerase chain reaction, as described elsewhere [28], for the absence of toxin production (i.e., toxic shock syndrome toxin 1 [TSST-1], enterotoxins A–R, and exfoliative toxins A and B). Antibiotic susceptibility testing was performed for all strains and involved testing for resistance to oxacillin, cefuroxim, gentamicin, vancomycin, rifampin, fusidic acid, ciprofloxacin, and mupirocin. All strains included in
the mixture had clearly distinct PFGE patterns, which facilitated unequivocal strain identification in samples after the in vivo nasal inoculation.

Because multiple S. aureus strains were inoculated, 16 colonies were randomly selected from each nasal culture positive for S. aureus during follow-up; colonies were stored separately in 15% glycerol storage medium at −20°C until genotyping. Final storage was at −80°C. The last strain found during the screening episode for persistent and intermittent carriers was typed by PFGE before it was added to the inoculation mix. S. aureus DNA was obtained as described by Boom et al. [29].

Antistaphylococcal Antibodies
Immunoglobulin (Ig) G and IgA antibodies directed against the following were quantified: S. aureus proteins ClfA and ClfB; surface protein G (SasG); iron-responsive surface determinants A and H; fibronectin-binding proteins A and B; serine-aspartate dipeptide repeat protein D and E; staphylococcal enterotoxins A, B, I, and M; TSST-1; chemotaxis inhibitory protein of S. aureus; staphylococcal complement inhibitor; and extracellular fibrinogen-binding protein. Antibodies were quantified simultaneously using a bead-based flow cytometry technique (xMAP; Luminex). Serum samples from the 51 volunteers were analyzed, along with additional serum samples from 13 persistent carriers, 1 intermittent carrier, and 18 noncarriers, as described elsewhere [30], to provide larger and more homogeneous groups. Carrier states for the latter 32 volunteers were determined from ≥3 nasal swab samples obtained at intervals of 2 weeks.

Methods have been described elsewhere [30–32]. In short, the purified proteins were coupled to different-colored microspheres and mixed to a working concentration of 3000 beads per color per well. Fifty microliters of serum, diluted 1:100 in phosphate-buffered saline, 1% bovine serum albumin, and 0.05% sodium azide (PBS-BN; pH 7.4), was incubated with the microspheres in a 96-well 1.2-μm polyvinylidene fluoride filter microtiter plate (Millipore) for 35 min at room temperature and washed, and the microspheres were then resuspended in 100 μL of assay buffer. Measurements were performed on the Luminex 100 instrument (BMD) with use of Luminex IS software (version 2.2). Tests were performed in duplicate, and median fluorescence intensity (MFI) values, reflecting quantitative antibody levels, were determined.

Statistical Analysis
The primary end point was survival of S. aureus in the nose after artificial colonization. Survival ended when results of ≥2 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 the nose at the end of the study were censored in the analysis. When data were missing, the last observation was carried forward. The secondary end point was the median number of colony-forming units (cfu) over the course of 22 weeks. The χ² test (or Fisher’s exact test for 2 × 2 tables) was used to compare proportions. For comparison of continuous data, the Mann-Whitney U test or Kruskal-Wallis test was used when appropriate. Differences were considered to be statistically significant when 2-sided P values were <.05.

RESULTS

Artificial Nasal Colonization
Screening. Fifty-one volunteers were included in this artificial nasal colonization study. Fifteen volunteers (29%) were classified as noncarriers (median age, 22 years; range, 19–53 years; 7 male and 8 female). For noncarriers, all nasal culture results were negative. Twenty-four volunteers (47%) were classified as intermittent carriers (median age, 25 years; range, 20–50 years; 11 male and 13 female). The median percentage of positive culture results in this group was 25% (range, 10%–78%). Twelve volunteers (24%) were classified as persistent carriers (median age, 22 years; range, 20–37 years; 5 male and 7 female). For 10 of 12 persistent carriers, all nasal cultures were positive for S. aureus. The percentages of positive culture results for the 2 other persistent carriers were 83% and 86%. In both of these volunteers, the result of a single nasal swab culture was negative.

Eradication. All volunteers, including noncarriers, received mupirocin nasal ointment for S. aureus decolonization. None of the volunteers reported any problems or adverse effects, and all reported having applied the ointment according to instructions. Five weeks after mupirocin treatment and just before artificial inoculation, 8 volunteers, 4 classified as intermittent and 4 as persistent carriers were 154 days among persistent carriers contained significantly more cfu per swab sample than did cultures of samples from intermittent carriers (P = .017). The cultures of samples from the persistent carriers contained significantly more cfu per swab sample than did cultures of samples from intermittent carriers or noncarriers that were positive for S. aureus. The median bacterial count during the 22 weeks after artificial inoculation was 0 cfu per sample (range, 0–26,000 cfu per sample) in noncarriers, 0 cfu per sample (range, 0–17,000 cfu per sample) in intermittent carriers, and 69 cfu per sample (range, 0–1,500,000 cfu per sample) in persistent carriers (P = .004). None of the vol-
teers experienced adverse effects, and all adhered to the study protocol for the duration of the study period.

Selection of strains. Seven (58%) of the 12 persistent carriers selected their own resident strain of the inoculation mixture, compared with 4 (17%) of 24 intermittent carriers \((P = .02)\). None of the intermittent carriers but 3 of the persistent carriers who still carried \(S. aureus\) after mupirocin treatment selected their own strain of the inoculum mixture. For these 3 persistent carriers, the bacterial counts after mupirocin treatment were low \((2, 4, \text{and} 4500 \text{ cfu per sample})\), compared with the counts 1 week after inoculation \((600, 1100, \text{and} 37,000 \text{ cfu per sample})\). Therefore, it was concluded that this finding indicated reacquisition of strains from the inoculum mixture, rather than re-colonization of strains in the nasal cavities.

Antistaphylococcal Antibodies

Serum samples from 25 persistent carriers \((30\%)\), 25 intermittent carriers \((30\%)\), and 33 noncarriers \((40\%)\) were analyzed for antistaphylococcal antibodies directed against 17 \(S. aureus\) proteins. The MFI values reflecting the serum IgG levels for each person and each antibody isotype are shown in figure 2. For most of the antigens, there was no apparent quantitative difference in antibody level between persistent carriers, intermittent carriers, and noncarriers. However, the median serum level of IgG directed against TSST-1 and SasG was significantly higher in persistent carriers than in intermittent carriers or noncarriers \((P < .001 \text{ and} P = .019, \text{respectively})\). In addition, the median IgA serum level was significantly higher in persistent carriers than in intermittent carriers or noncarriers for TSST-1 \((P < .001)\), staphylococcal enterotoxin A \((P = .013)\), CIA \((P = .005)\), and chemotaxis inhibitory protein of \(S. aureus\) \((P = .006)\). MFI values did not differ significantly between noncarriers and intermittent carriers (table 1).

DISCUSSION

Although the amount of knowledge about host factors and bacterial determinants of \(S. aureus\) colonization is increasing, it remains unclear why certain individuals are persistent carriers, whereas others are intermittent carriers or noncarriers. This knowledge is important, because persistent carriers are at a higher risk for development of \(S. aureus\) infections \([5, 33]\). Although intermittent carriers carry \(S. aureus\) in the nose at times, their risk of infection is similar to that among noncarriers \([34]\). Important questions are: what drives intermittent carriage and why do intermittent carriers have a lower risk of autoinfections than do persistent carriers? An optimal model to study the interaction between bacteria and host is a human colonization model \([24, 26]\). In the present study, we identified carriage features specific for the different carriage types, including a large group of intermittent carriers, by performing an artificial inoculation study. We compared the overall survival of an artificially inoculated mixture of \(S. aureus\) strains in persistent carriers, intermittent carriers, and noncarriers. The median \(S. aureus\) survival was 4 days among noncarriers, 14 days among intermittent carriers, and >154 days among persistent carriers. This suggests that persistent carriers form a separate group, distinct from intermittent carriers and noncarriers. Furthermore, the cultures of swab samples from the persistent carriers contained significantly more cfu per sample than did cultures of samples from intermittent carriers and noncarriers that were positive for \(S. aureus\). This also suggests that persistent carriers stand out as a separate group. Furthermore, MFI values reflecting antibody levels against 17 \(S. aureus\) proteins never differed significantly between intermittent carriers and noncarriers, whereas differences were detected between persistent carriers and intermit-
Our data show that intermittent carriers and noncarriers have similar responses to inoculation with *S. aureus* and similar antistaphylococcal antibody responses. This suggests that either noncarriage is incidental and most humans are actually intermittent *S. aureus* carriers or intermittent carriers might actually be noncarriers who carry *S. aureus* only under environmental pressure.

Seven of the 12 persistent carriers selected their own strain of the inoculation mixture, compared with 4 of 24 intermittent carriers (P = .02). If the chances of selecting each strain of the inoculation mixture were equal, we would expect to find 2 of the 12 persistent carriers with their resident strain. Therefore, selection for the resident strain is not random in persistent carriers. When these data are combined with those presented by Nouwen...
et al. [26] in a similar human volunteer study, 11 of 19 persistent carriers selected for their own resident strain, whereas only 4 would be expected to do so if this effect was random ($P = .04$). These cumulative data help us to postulate that persistent carriage depends, at least in part, on an adequate biological match between human host and colonizing *S. aureus* strain.

We classified participants as persistent carriers, intermittent carriers, or noncarriers on the basis of nasal swab samples. We did not determine the *S. aureus* nasal carriage status of family members, pets, and other close contacts. This would have provided valuable information, because *S. aureus* strains that were not part of the inoculation mixture were detected after inoculation in all 3 groups. This suggests that colonization may be incidentally acquired during contacts with other *S. aureus* carriers, contaminated environments, or (pet) animals. These cross-colonization events happen frequently and present as random rather than specific events. Furthermore, 8 volunteers still carried *S. aureus* 5 weeks after treatment with mupirocin. This finding might be attributable to exposure to *S. aureus* strains from close contacts, but it may also be attributable to participants’ compliance with mupirocin treatment or the efficacy of that treatment, which differed among reported groups of individuals [4, 35].

In the current study, we revealed that host-*S. aureus* interactions are highly specific because of the apparent reacquisition of the autologous strain in persistent carriers. Furthermore, intermittent carriers and noncarriers have similar *S. aureus* elimination kinetics and similar antistaphylococcal antibody patterns. Moreover, the median numbers of cfu yielded on cultures after artificial inoculation were clearly lower in these groups than in the persistent carriers. It was also shown elsewhere that persistent nasal carriers have an increased risk of *S. aureus* infection, whereas intermittent nasal carriers have the same risk of infection as noncarriers [34]. This implies that a paradigm shift is required; apparently, there are only 2 human types of nasal *S. aureus* carriers: persistent carriers and others. It is important to develop novel ways of identifying persistent carriers, such as by human genomic polymorphisms [36, 37].

### Table 1. Median fluorescence intensity (MFI) values reflecting antigen-specific immunoglobulin (Ig) G and IgA levels in persistent carriers (PC), intermittent carriers (IC), and noncarriers (NC).

<table>
<thead>
<tr>
<th><em>S. aureus</em> protein (antibody isotype), carrier state</th>
<th>MFI value, median (range)</th>
<th>PC vs. NC</th>
<th>PC vs. IC</th>
<th>IC vs. NC</th>
<th>PC vs. IC and NC</th>
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<tbody>
<tr>
<td><strong>TSST-1 (IgG)</strong></td>
<td></td>
<td></td>
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<tr>
<td>PC</td>
<td>12,123 (100–18,322)</td>
<td>&lt;.001</td>
<td>.003</td>
<td>NS</td>
<td>&lt;.001</td>
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<tr>
<td>IC</td>
<td>5973 (57–18,136)</td>
<td></td>
<td></td>
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<tr>
<td>NC</td>
<td>4714 (42–17,053)</td>
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<tr>
<td><strong>TSST-1 (IgA)</strong></td>
<td></td>
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<tr>
<td>PC</td>
<td>754 (87–3130)</td>
<td>.011</td>
<td>&lt;.001</td>
<td>NS</td>
<td>&lt;.001</td>
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<tr>
<td>IC</td>
<td>134 (10–3139)</td>
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<tr>
<td>NC</td>
<td>110 (20–3506)</td>
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<td><strong>SasG (IgG)</strong></td>
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<tr>
<td>PC</td>
<td>56 (29–485)</td>
<td>.042</td>
<td>.037</td>
<td>NS</td>
<td>.019</td>
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<tr>
<td>IC</td>
<td>127 (32–556)</td>
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<tr>
<td>NC</td>
<td>110 (21–413)</td>
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<td><strong>SEA (IgA)</strong></td>
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<tr>
<td>PC</td>
<td>48 (19–1070)</td>
<td>.017</td>
<td>.056</td>
<td>NS</td>
<td>.013</td>
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<tr>
<td>IC</td>
<td>31 (16–277)</td>
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<tr>
<td>NC</td>
<td>29 (17–284)</td>
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<tr>
<td><strong>ClfA (IgA)</strong></td>
<td></td>
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<tr>
<td>PC</td>
<td>1197 (108–3997)</td>
<td>.008</td>
<td>.028</td>
<td>NS</td>
<td>.005</td>
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<tr>
<td>IC</td>
<td>524 (74–2430)</td>
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<tr>
<td>NC</td>
<td>441 (87–3156)</td>
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<td><strong>CHIPS (IgA)</strong></td>
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<tr>
<td>PC</td>
<td>5513 (1004–12,309)</td>
<td>.032</td>
<td>.006</td>
<td>NS</td>
<td>.006</td>
</tr>
<tr>
<td>IC</td>
<td>3445 (156–11,644)</td>
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<tr>
<td>NC</td>
<td>2525 (27–11,532)</td>
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</table>

**NOTE.** CHIPS, chemotaxis inhibitory protein of *Staphylococcus aureus*; ClfA, clumping factor A; NS, not statistically significant; SasG, surface protein G; SEA, staphylococcal enterotoxin A; TSST-1, toxic shock syndrome toxin 1.

* Differences in antigen-specific MFI values between groups were considered to be statistically significant at $P < .05$ (Mann-Whitney U test).
Acknowledgments

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References


