Carriage of *Staphylococcus aureus* among 104 healthy persons during a 19-month period

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SUMMARY

The present study was undertaken to investigate the frequency of the nasal carrier rate of *Staphylococcus aureus*. The investigation was performed on 104 healthy persons. The total number of swabs performed was 1498 and this resulted in isolation of 522 *S. aureus* strains. All strains have been identified, tested for antibiotic susceptibility, and phage-typed. The carrier-index (number of positive swabs/number of total swabs for each individual person) was compared with different sampling and culturing methods, phage type, age, and resistance to antibiotics. There was statistical difference in carrier rate according to sex (P < 0.05). Among the 104 persons 15 (14.4%) were persistent carriers, 17 (16.3%) intermittent carriers, 55 (52.9%) occasional carriers and 17 (16.3%) non-carriers. Among intermittent and occasional carriers the phage-type distribution was different from the *S. aureus* strains isolated from Danish hospitalized patients in 1992, while the persistent carriers had similar phage-type distribution.

INTRODUCTION

Since 1937 a large number of studies have consistently documented that the carriage of *Staphylococcus aureus* in the anterior nares is an important human reservoir for *S. aureus* [1–6]. Nasal carriage rates of *S. aureus* have been extensively studied in patients, hospital staff, and normal persons [7–14]. The nasal carriage rate in most adult populations at a given time has been reported to be between 30 and 50%, but cumulative rates after several swabbings may reach 90% or more [1, 7, 11, 14–16]. Between 20 and 35% of these subjects carry the organism consistently, 30–70% intermittently, and up to 20% of a given population are non-carriers [14]. The factors that lead to the establishment of a *S. aureus* carrier-state are not well elucidated; however, the association between the carriage of *S. aureus* and development of clinical infection has in certain situations been established [2–5, 9, 15, 17].

In Denmark no systematic *S. aureus* carrier studies have been performed in the last 35 years [3, 18]. Investigations 30–50 years ago demonstrated that many
patients shortly after hospitalization became colonized with *S. aureus* and carrier rates of 40% to around 90% were reported [3, 18, 19]. The colonizing strains were often ‘hospital staphylococci’, different in phage-type pattern and antibiotic resistance from the strains found outside hospital [20]. A present investigation in Denmark has shown that during recent years the strains that cause infections in hospitalized patients are similar to the strains isolated from patients with infections outside hospital with respect to phage type and antibiotic resistance [20]. This might indicate that today in the majority of the hospital-acquired infections, the patients are infected with their own strain. No investigation has, however, been performed recently to illustrate whether the colonization rate and/or the colonizing strains differ in patient/staff inside and outside hospital.

In order to study the *S. aureus* carrier rates a well-documented method for detection of carriage is essential. Such a method has been developed, and it has been documented that the procedure greatly influences the number of nasal carriers found in a given population, and the major key-points seem to be the swab material, the transportation medium, the medium for cultivation, and the incubation period [21]. A combination of charcoal swab, 6·5% NaCl broth enrichment medium and mannitol salt agar with incubation for 7 days has been described to give detection of a maximum number of *S. aureus* carriers [21].

The aim of the present study was to investigate if the carriers detected under optimal conditions differed in carrier-state and the type of *S. aureus* from carriers detected by minimal conditions.

**MATERIALS AND METHODS**

The healthy consenting normal persons who took part in this study were 86 female and 18 male staff members of three job categories; laboratory technician, academical staff and secretary. They all worked at Statens Seruminstitut but in seven distinct buildings. The type of work was (1) processing of bacteriological samples, (2) typing of staphylococci or (3) production of media without any contact with bacteriological samples. The median age was 46 years; range 23–69 years. Nasal samples were collected and plated by the same person over a 19-month interval from January 1992 to August 1993.

**Swabs and swabbing technique**

Swabs were obtained from the right or left anterior nares by using sterile, dry, cotton wool swab (Dansu A/S, Ganløse, Denmark) or sterile, dry, cotton wool impregnated with charcoal (prepared from dry cotton wool swab by boiling for 10 min in NaCl buffer pH 7·38 containing 5·2 mg active coal (Sigma, St Louis, USA) per swab). The whole anterior nares was swabbed by rubbing the swab twice around the inside of the nostril, while applying an even pressure and rotating the swab without interruption.

**Media**

All media were prepared at Statens Seruminstitut, Copenhagen, Denmark. Stuart’s transportation medium: sodium thioglycolate 0·9 g, sodium glycerophosphate 10 g, calcium chloride 0·1 g, methylene blue 2 mg, litex danagar
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hydrogel 15 g, deionized water 11. Adjustment to pH 7·4. The agar concentration might vary dependent on the break resistance. This medium was prepared according to Stuart and colleagues [22], with the exception that the agar concentration was higher than the 0·3 % Bacto agar used by Stuart and colleagues [22]. Broth enrichment culturing medium with 6·5 % NaCl: beef extract 400 g, glucose 0·3 g, NaCl 65 g, Na₂HPO₄ 2·0 g, peptone 100 g, distilled water 1·0 l and final pH 7·4. Mannitol salt agar: beef extract 1 g, peptone 10 g, NaCl 75 g, mannitol 10 g, agar 15 g, phenol red 0·025 g, distilled water 1 l, final pH 7·4, dissolved and autoclaved at 121 °C for 15 min according to Chapman [23]. 5 % horse blood agar: MgSO₄ 0·1 g, MnCl₂ 0·0067 g, Na₂HPO₄ 8·0 g, casein hydrolysate 5·0 g, yeast extract 3·0 g, peptone 5·0 g, KCl 6·67 g, detergent 0·01 g, agar 10 g, horse blood 50 ml, cysteine HCl 0·05 g, sodium pyruvate 2·0 g, distilled water 1 l, final pH 7·4, dissolved and autoclaved at 121 °C for 10 min. Agar plates: distilled water 1 l. beef extract 400 g, peptone 10 g, NaCl 3 g, Na₂HPO₄ 2 g, fatty acid 0·01 g, glucose 0·3 g, agar 10 g, final pH 7·4, dissolved and autoclaved at 121 °C for 15 min.

Culture method

Swabs were either inoculated directly on 5 % blood agar or mannitol salt agar, or placed in both enrichment medium with 6·5 % NaCl or Stuart’s transportation medium. Broth enrichment medium was incubated at 37 °C for 18 h, and Stuart’s transportation medium was stored at 4 °C for 7 days, before the inoculation on solid medium was performed. The plates were incubated at 37 °C overnight, and then at room temperature (to stimulate pigment formation) for up to 6 days. Thus, an incubation period of 7 days consisted of incubation overnight at 37 °C followed by 6 days at room temperature.

Identification of S. aureus

Colonies were isolated and checked by Gram-stained smears. All Gram-positive organisms were tested for catalase production after pure cultivation on agar plates, and colonies suspected by colony morphology to be staphylococci were tested for coagulase production by the citrate–plasma tube technique.

Phage typing

The method of Blair and Williams was used [24] with the 23 phages of the current international set and two local phages. The phages could be used at concentrations of routine test dilution (RTD), 100 × RTD and 1000 × RTD. Only isolates not typable at RTD were further typed at 100 × RTD, and if non-typable at 100 × RTD then typing at 1000 × RTD was performed. Subdivision into phage groups was accomplished according to Parker [25]. The present results were compared with results from the central register of all phage-typed S. aureus strains isolated from patients in Danish hospitals in 1992. In 1992 16725 S. aureus strains were phage-typed. Only one isolate was included per patient.

Pigment

The visual appearance of a yellow colour was regarded as pigmentation.
Definitions

A carrier index was defined as the number of positive swabs/number of total swabs for each person. We excluded persons with a total number of swabs below 6 over the period (7 persons).

Carriers

Persistent carriers comprise those with $0 \leq \text{carrier index} < 1$; intermittent carriers, $0 < \text{carrier index} \leq 0.8$; occasional carriers, $0.4 < \text{carrier index} \leq 0.1$; non-carriers, carrier index $= 0$.

S. aureus isolates and strains

An isolate is a *S. aureus* isolated by a given procedure. Isolates from the same patients with identical phage types were regarded and designated as a strain.

Day of isolation

An incubation period of 2, 4 or 7 days consisted of incubation overnight at $37 \, ^\circ\text{C}$ followed by 1, 3 or 6 days at room temperature.

Design

A series of experiments was performed to compare the carrier-index (number of positive swabs/number of total swabs for each individual person) with different sampling and culturing methods, phage type, age, sex, profession and physical placement of job. When comparing different swabs or swabbing from different nostrils, the order of sampling was changed in half the cases; e.g., swabbing from right nostril using charcoal followed by cotton and the reverse order from left nostril. For each experiment swabs from all persons available on that day were performed without any other selection criteria.

Antibiotic susceptibility testing

The susceptibility of *S. aureus* to antibiotics was tested locally by an agar diffusion technique using Neosensitabs (Rosco diagnostica, Taastrup, Denmark) [26]. The tests included penicillin, streptomycin, tetracycline, gentamicin, erythromycin, methicillin, ciprofloxacin and fusidic acid.

Statistical analysis

When the data were mutual independent we used the Chi-square test. When the data were not mutual independent we used the one-tailed McNemar test. The Mann–Whitney $U$ test was used when the variable under consideration was measured on at least an ordinal (rank order) scale-test for independent samples.

RESULTS

Distribution of swabs and carrier indexes

After 12 swabbing rounds including 104 persons a total of 1498 swabs were obtained with a median of 14 swabs from each person, range 6–21. The carrier-index (number of positive swabs/number of total swabs for each person) of the 104
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Table 1. Distribution of carrier-index among males and females (%)

<table>
<thead>
<tr>
<th>Sex</th>
<th>Persistent carrier</th>
<th>Intermittent carrier</th>
<th>Occasional carrier</th>
<th>Non-carrier</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>6 (33)</td>
<td>4 (22)</td>
<td>6 (33)</td>
<td>2 (11)</td>
<td>18 (100)</td>
</tr>
<tr>
<td>Female</td>
<td>9 (11)</td>
<td>13 (15)</td>
<td>49 (57)</td>
<td>15 (17)</td>
<td>86 (100)</td>
</tr>
<tr>
<td>Total</td>
<td>15 (14)</td>
<td>17 (16)</td>
<td>55 (53)</td>
<td>17 (16)</td>
<td>104 (100)</td>
</tr>
</tbody>
</table>

Chi-square test

- $P = 0.01$
- $P = 0.45$
- $P = 0.07$
- $P = 0.5$

persons, indicating how often a person is carrying S. aureus, was distributed with a biphasic pattern. According to our definition for carriers (see Materials and Methods) 15 (14.4%) were persistent carriers, 17 (16.3%) intermittent carriers, 55 (52.9%) occasional carriers, and 17 (16.3%) non-carriers.

Age distribution

The median age was 46 years (range 23–69 years). No difference in the age distribution was observed when comparing female and male, or comparing the three different carrier groups and the non-carrier groups ($P > 0.05$; Mann–Whitney $U$ test) (data not shown); however, there were significantly more carriers among male as compared to female ($P < 0.01$; Chi-square test for trend), most pronounced among the persistent carriers (Table 1).

Carrier rate according to profession and type of work

By comparing three distinct professions (laboratory technician, academic staff, and secretaries), no difference could be observed in carrier rates or distribution of carrier indexes between professions or between persons with different type of work ($P > 0.05$; Chi-square test). Among the 104 persons 25 had their daily employment in the Staphylococcus Laboratory; compared to the rest of the group no difference could be observed among the 25 persons as regards carrier rates or distribution of carrier indexes ($P > 0.05$; Chi-square test) (data not shown).

Influence of culture technique on the carrier rate and the distribution of carrier indexes

In a previous study it was shown that cultivation on mannitol–salt agar for up to 7 days greatly increased the number of carriers detected compared to only 2 days incubation [21]. In Table 2 detection of growth of S. aureus on either day 2 or day 7 is summarized for persons with different carrier indexes. Among the persistent and the intermittent carriers detection occurred already at day 2, whereas among the occasional carriers significantly more S. aureus were detected at day 7 compared to day 2. When comparisons were performed for other changes in the sampling procedures (swabs, transportation medium, growth condition) significantly increased detection rates were again only found in the groups of occasional carriers (Table 3).

Day of detection for 522 S. aureus isolates

The detection time was significantly later among occasional carriers compared with persistent and intermittent carriers independent of the methods used.
Table 2. Detection of *S. aureus* in nasal swabs according to carrier index, when comparing growth on mannitol-salt agar at day 2 and day 7 in 5 different experiments

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>No. of persons</th>
<th>No. of non-carriers</th>
<th>Day</th>
<th>Persistent carrier</th>
<th>Intermittent carrier</th>
<th>Occasional carrier</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>76</td>
<td>7</td>
<td>2</td>
<td>12 (100-0)</td>
<td>6 (54-5)</td>
<td>20 (43-5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>7</td>
<td>8 (100-0)</td>
<td>9 (63-6)</td>
<td>12 (58-7)</td>
</tr>
<tr>
<td>2</td>
<td>66</td>
<td>9</td>
<td>2</td>
<td>8 (100-0)</td>
<td>12 (81-8)</td>
<td>4 (10-5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>7</td>
<td>8 (100-0)</td>
<td>9 (91-8)</td>
<td>9 (23-7)</td>
</tr>
<tr>
<td>3</td>
<td>81</td>
<td>14</td>
<td>2</td>
<td>10 (76-9)</td>
<td>11 (68-8)</td>
<td>10 (26-3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>7</td>
<td>10 (76-9)</td>
<td>11 (68-8)</td>
<td>10 (26-3)</td>
</tr>
<tr>
<td>4</td>
<td>93</td>
<td>14</td>
<td>2</td>
<td>9 (75-0)</td>
<td>7 (43-8)</td>
<td>7 (13-7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>7</td>
<td>12 (100-0)</td>
<td>11 (68-8)</td>
<td>11 (21-6)</td>
</tr>
<tr>
<td>5</td>
<td>79</td>
<td>12</td>
<td>2</td>
<td>8 (88-9)</td>
<td>10 (71-4)</td>
<td>10 (22-7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>7</td>
<td>8 (88-9)</td>
<td>10 (71-4)</td>
<td>16 (36-4)</td>
</tr>
</tbody>
</table>

* Increased detection-rate (*P* < 0.005; McNemar test) as compared to day 2.

Table 3. Summary of comparison of the efficacy of different isolation procedures among occasional carriers

<table>
<thead>
<tr>
<th>Procedure 1</th>
<th>Procedure 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>One nostril</td>
<td>Both nostrils</td>
</tr>
<tr>
<td>Cotton swabs</td>
<td>Charcoal swabs</td>
</tr>
<tr>
<td>Direct plating</td>
<td>Stuart’s medium (7 days)</td>
</tr>
<tr>
<td>Direct plating</td>
<td>Broth enrichment medium</td>
</tr>
<tr>
<td>Blood agar (2 days)</td>
<td>Mannitol agar (2 days)</td>
</tr>
<tr>
<td>Mannitol agar (2 days)</td>
<td>Mannitol agar (4 days)</td>
</tr>
<tr>
<td>Mannitol agar (2 days)</td>
<td>Mannitol agar (7 days)</td>
</tr>
<tr>
<td>Mannitol agar (4 days)</td>
<td>Mannitol agar (7 days)</td>
</tr>
</tbody>
</table>

* Increased detection-rates for occasional carriers/total number of experiments

* *P* < 0.05; McNemar test.

Phage type of carrier strains compared to strains isolated from Danish hospitalized patients

Among the 104 persons 87 had swabs with *S. aureus* representing a total of 522 *S. aureus* isolates. Within each person phage types of all isolates were compared and isolates with identical phage type were put together as one strain, giving a
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Table 4. Carriers with more than one different S. aureus strain isolated during 19 months. Only including persons with number of positive S. aureus strains ≥ 2

<table>
<thead>
<tr>
<th>Number of different S. aureus strains</th>
<th>Carriers (% of total in the carrier group)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Persistent</td>
</tr>
<tr>
<td>1</td>
<td>6 (43)</td>
</tr>
<tr>
<td>2</td>
<td>5 (36)</td>
</tr>
<tr>
<td>3</td>
<td>3 (21)</td>
</tr>
<tr>
<td>4</td>
<td>0.00</td>
</tr>
<tr>
<td>5</td>
<td>0.00</td>
</tr>
<tr>
<td>Total</td>
<td>14</td>
</tr>
</tbody>
</table>

Table 5. Phage-type distribution for carrier-strains and strains isolated from Danish hospitals in 1992

<table>
<thead>
<tr>
<th>Hospital strains</th>
<th>All carrier strains</th>
<th>Persistent carriers</th>
<th>Intermittent carriers</th>
<th>Occasional carriers</th>
<th>Non-pigmented carrier strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n = 16725)</td>
<td>(n = 166)</td>
<td>(n = 26)</td>
<td>(n = 43)</td>
<td>(n = 97)</td>
<td>(n = 34)</td>
</tr>
<tr>
<td>80 Complex</td>
<td>1·6</td>
<td>0·6</td>
<td>3·7</td>
<td>0</td>
<td>0·00</td>
</tr>
<tr>
<td>Rest of group I</td>
<td>14</td>
<td>15·7</td>
<td>7·4</td>
<td>7·1</td>
<td>21·6*</td>
</tr>
<tr>
<td>Group II</td>
<td>23·2</td>
<td>14·5*</td>
<td>14·8</td>
<td>19</td>
<td>7·2*</td>
</tr>
<tr>
<td>Group III</td>
<td>12·5</td>
<td>5·4*</td>
<td>3·7</td>
<td>9·5</td>
<td>4·1*</td>
</tr>
<tr>
<td>83 A Complex</td>
<td>5·3</td>
<td>3</td>
<td>11·1</td>
<td>2·4</td>
<td>1</td>
</tr>
<tr>
<td>94·96 Complex</td>
<td>8·8</td>
<td>6·6</td>
<td>7·4</td>
<td>0·0*</td>
<td>9·3</td>
</tr>
<tr>
<td>Type 95</td>
<td>19·3</td>
<td>18·7</td>
<td>18·5</td>
<td>21·4</td>
<td>17·5</td>
</tr>
<tr>
<td>XI (Mixed-group)</td>
<td>6·5</td>
<td>18·1*</td>
<td>22·2*</td>
<td>21·4*</td>
<td>15·5*</td>
</tr>
<tr>
<td>XT (Non-typable)</td>
<td>8·8</td>
<td>17·5*</td>
<td>11·1</td>
<td>19</td>
<td>18·6*</td>
</tr>
</tbody>
</table>

* Significantly different (P < 0·05) as compared to hospitals strains.

total of 166 carrier strains. The number of carriers with different strains, i.e. more than one strain during the observation period, is shown in Table 4. Different strains were detected on the occasional carriers more often as compared to persistent and intermittent carriers (P < 0·01; Chi-square test for trend).

The phage-type patterns of the strains carried by the persistent, intermittent and occasional carriers are compared to the distribution in phage-type pattern among all strains isolated from Danish hospitalized patients in 1992 (Table 5). Among the carrier strains there was a lower prevalence of group II and group III strains, but a higher prevalence of the mixed and the non-typable group (Table 5).

Only strains from occasional carriers showed major differences compared to the hospital strains (Table 5), while strains from persistent and intermittent carriers only showed minor differences (Table 5).

Presence of pigment in 522 S. aureus isolates

A total of 484 (92·7%) of the 522 S. aureus isolates had pigmentation, and for the remaining 38 (7·3%) isolates there was no visual appearance of pigment. The non-pigmented strains were most often found among occasional carriers (data not
The non-pigmented carrier strains had a lower prevalence of group II strains and an increased prevalence of NI strains.

**Resistance to antibiotics**

Of the 166 different *S. aureus* strains from the 87 carriers, 137 (82.5%) were resistant to penicillin, compared to 15059 (87.5%) isolated from Danish hospitals in 1992 ($P > 0.05$; Chi-square test). Only 3 (1.6%) of the strains were resistant to tetracycline, compared to 488 (4.2%) isolated from Danish hospitals in 1992 ($P > 0.05$; Chi-square test). All 166 *S. aureus* were sensitive to the remaining antibiotics tested.

**DISCUSSION**

By optimizing the culture technique for detection of *S. aureus* carriage a carrier rate as high as 83.7% was obtained when both intermittent and occasional carriers are included. This is higher than comparable previous Danish [3, 18] and foreign studies [27–29]. We detected significantly more occasional carriers, and these results were in good accordance with the previously described importance of the culture technique [21]. As a result of the Danish work from 1960 [3] the use of dry cotton swabs was regarded as optimal. The Norwegian work from 1966 [4] compared the use of dry and moistened swabs, and no difference could be observed in the quantity of staphylococci in the nose.

Based on these results we did a methodological study to compare the dry cotton swabs with charcoal swabs and found a significantly better result by the use of charcoal swabs (McNemar’s two-tailed test; $P < 0.05$) [21].

The clinical importance of the finding of more occasional carriers by optimizing the culture technique is yet unknown. In certain diseases or following certain operations the nasal carriage state may predispose to infection [17]. However, it is not known how many of these persons are occasional carriers. Occasional carriers were detected after longer incubation as compared to persistent carriers. The explanation could be that the occasional carriers harboured fewer staphylococci as compared to other carrier groups or also that the strains from these persons were less commonly pigmented. It is important to comment on pigmentation, because maybe some of the lower carrier rates among the occasional and intermittent carriers could result from the presence of non-pigmented strains, which could be easily missed. The phage-type distribution shows that only strains from occasional carriers differ from hospital-acquired *S. aureus* strains, while strains from persistent and intermittent carriers only showed minor differences (Table 5). Occasional carriers more often had different strains isolated on different occasions, indicating only transitional carriage. The observed increased carrier rate among males was most pronounced for the persistent carriers (Table 1). Although Lamikanra and colleagues [29] described a higher carrier rate among females, others have found no difference between males and females, and the difference found in this study has never been described before.

As found in other studies with comparable age groups we found no difference in carrier rate according to age [14, 29]. Among the healthy persons we observed no difference in carrier rate because of profession or job with or without *S. aureus*. This indicates that staff who handle staphylococci, both for typing and in the
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routine microbiology laboratory, had the same carrier rates as staff without contact with staphylococci (i.e. secretaries and staff involved in media production).

As formerly reported, *S. aureus* strains of group II in Denmark have increased during the years 1961–91 [31], and in 1992 they comprised 23·2% of all *S. aureus* strains isolated (Table 5). However, the lower prevalence of group II strains among carriers could be caused by a low ability of group II strains to colonize but a higher ability for establishing infection compared to other phage groups. However, we do not know the prevalence of group II strains among carriers in the same period. Overall, the difference in phage-type distribution could be caused by a difference in colonization-resistance among carriers and it is possible that *S. aureus* mainly lead to infection among persistent carriers. Another explanation could be that *S. aureus* strains which cause infection are able to establish a persistent carrier-state. The ability to colonize the nasal cavity should then be considered as a virulence factor. The non-pigmented carrier strains had a lower prevalence of group II strains and an increased prevalence of NI strains (Table 5).

Why some people are carriers and others are non-carriers is unknown. Contributing to the carrier-state could be the structure and ecology in the nose, nasal secretion, and possible environmental factors excluding the profession. The carrier state of *S. aureus* could also be related to genetic predisposition [32] or systemic IgG antibody production or local IgA production. If *S. aureus* are unable to split local IgA [33], that could contribute to the explanation of why *S. aureus* are able to establish a carrier state among some people and only cause invasive infection in a small number of cases.

Whether nasal carriage of *S. aureus* can be used to identify patients more susceptible to these infections seems to depend on the quantity in the nose and the ability to disperse the organism. Further studies are needed to illustrate a possible relation between the different carrier groups and the quantity of *S. aureus* in the nose, especially combined with colonization and adhesion studies which perhaps can show if occasional carriers are real carriers or not. If not, this is in good accordance with the biphasic appearance of index distribution among carriers and non-carriers.

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