Nasal Staphylococcus aureus Carriage Is Not a Risk Factor for Lower-Airway Infection in Young Cystic Fibrosis Patients

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Study design. Four specialized CF clinics (two in Muenster and one each in Duesseldorf and Essen) participated in this study. The demographic characteristics of the patients who participated are shown in Table 1.

There are basically three different strategies of S. aureus treatment, i.e., prophylactic therapy, treatment of positive cultures, and treatment only in the presence of symptoms. None of the four centers use continuous prophylactic
antistaphylococcal antibiotic therapy but do use treatment against _S. aureus_ if there are positive cultures and symptoms occur.

This study consisted of two parts. (i) The cross-sectional part assessed the prevalence of nasal and oropharyngeal carriage in young children with CF determined by analysis of the first specimens obtained after recruitment (n = 80). These data were compared to those on specimens from a control group from the Department of Ophthalmology comprising patients who underwent a short surgical procedure because of nasolacrimal duct obstruction (n = 109). The longitudinal part of this study analyzed the sequence of infection in children with CF from whom repeated specimens were available over a time period of at least 18 months (n = 62). Nasal and oropharyngeal swabs or sputum samples from patients were taken during their regular (quarterly) visits to the outpatient clinics. To assess nasal carriage, the anterior 1.5 cm of the nasal vestibule of both the left and right nares was rubbed with a sterile cotton swab.

Ethical approval for this study was obtained from the institutional review boards of all of the participating centers, and written informed parental consent was obtained in all cases.

**Questionnaire.** Parents were asked to complete a standardized questionnaire to determine risk factors for the acquisition of _S. aureus_ carriage at recruitment. Information was obtained regarding known risk factors for _S. aureus_ acquisition such as regular attendance at a children’s day care center, earlier hospitalization, use of antibiotics (orally, intravenously, or by inhalation), siblings (number, age, siblings with CF), relatives living in the same household (close contact with the child, chronic diseases of relatives or earlier hospitalization), and pets living in the household (15, 23, 29). Additionally, the parents were asked if the children used stuffed animals, had special favorite toys, or had contact with other CF patients.

**Laboratory methods.** All specimens were processed at the Institute of Medical Microbiology, Muenster. Primary cultures were performed with Columbia (Becton Dickinson, Heidelberg, Germany) sheep blood (Oxoid, Wesel, Germany) agar for gram-positive cocci or with Endo agar (Merck, Darmstadt, Germany) for gram-negative rods for 48 h at 35°C and with chocolate agar (Mast, Reinfeld, Germany) and interpreted according to published guidelines. Additionally, specimens were cultured in dextrose broth to enrich bacterial growth and streaked on blood and Endo agar after 48 h. Special culture conditions for the isolation of small-colony variants (SCVs) were used as previously described (11). Brieﬂy, primary cultures were streaked on brain heart infusion agar with 5% NaCl and on Schaedler agar, which is a rich medium agar used to culture anaerobic bacteria, and incubated for 48 h at 35°C. Identification of _S. aureus_ was conﬁrmed by Pasteurex slide test (Bio-Rad) and by tube coagulase testing. In the case of a discrepancy, PCR of the _S. aureus_ speciﬁc thermonuclease was performed (2). Susceptibility testing was carried out by disk diffusion on Mueller-Hinton agar (Mast) according to the guidelines of the Clinical and Laboratory Standards Institute (4). The antibiotics tested were penicillin, oxacillin, ampicillin, imipenem, cefaclor, cefazolin, cefotaxime, erythromycin, clindamycin, gentamicin, levofloxacin, trimethoprim-sulfamethoxazole, and rifampin. In the case of methicillin-resistant _S. aureus_ (MRSA), mupirocin was tested additionally.

**Persistent carriage.** The longitudinal part of this study allowed the determination of the patterns of _S. aureus_ carriage in both the nose and the oropharynx. Three different colonization patterns for nasal carriage have been described in the literature, i.e., persistent carriage, intermittent carriage, and noncarriage (29). Persistent carriage, colonization, or infection was defined as having _S. aureus_-positive cultures for at least 6 months based on a minimum of two positive cultures from the respective specimens taken at regular quarterly visits.

**Molecular typing.** Isolates were analyzed by pulsed-field gel electrophoresis (PFGE) after Small restriction of whole chromosomal DNA (8). The gels were analyzed both visually and by the computer program Quantity One (Bio-Rad Laboratories, Hercules, CA) and interpreted according to published guidelines (25, 26). Consecutive isolates from the same patient, with fragment patterns that were identical or showed only minor differences were assigned to the same clone or clonal lineage if the Dice coefficient was higher than 85% (26). Isolates with a similarity index below 85% were considered to belong to different clones.

**Statistical analysis.** Analysis of _S. aureus_ prevalence was carried out by standard statistical tests (median, Student’s t test, chi-square test). The Fisher exact test was used to calculate the correlation of nasal and oropharyngeal carriage. To assess risk factors for _S. aureus_ colonization, odds ratios (ORs) were calculated comparing cases with suitable controls. The bivariate analysis was followed by stratiﬁcation and logistic regression analysis with SPSS version 11.5.

## RESULTS

**Prevalence of nasal and oropharyngeal _S. aureus_ carriage in CF patients and healthy children.** The cross-sectional part of this study assessed the prevalence of _S. aureus_ carriage in the anterior nares and oropharynxes of 80 CF patients and 109 controls without respiratory disease. Twenty (25%) of 80 children with CF were positive for _S. aureus_ by both oropharyngeal and nasal cultures, whereas _S. aureus_ was cultured from the nose only in 8 children (10%) and from the oropharynx only in 15 children (19%). Thus, the overall prevalences of _S. aureus_-positive nasal and oropharyngeal cultures in children with CF were 35% (28/80) and 44% (35/80), respectively (Fig. 1). Both oropharyngeal and nasal cultures were positive in 10/109 control children (9%), and nasal or oropharyngeal cultures were positive for _S. aureus_ in 10 children each, resulting in a cumulative prevalence of nasal or oropharyngeal carriage of 27% (30/109) (Fig. 1). There was a statistically signiﬁcantly higher prevalence of _S. aureus_-positive nasal and oropharyngeal cultures in children with CF (OR, 2.4; conﬁdence interval [CI], 1.2 to 4.7; _P_ < 0.01) than in the control children (OR, 3.4; CI, 1.8 to 6.7; _P_ < 0.001).

The probability of oropharyngeal carriage was signiﬁcantly higher in nasal carriers than in noncarriers in both children with CF (OR, 6.2; CI, 2.2 to 17.0; _P_ < 0.001) and controls (OR, 7.9; CI, 2.6 to 23.6, _P_ < 0.001). In contrast to the children in the control group, who were colonized at both sites at similar frequencies, the oropharynx was signiﬁcantly more often colonized in children with CF.

![FIG. 1. Prevalence of _S. aureus_ nasal and oropharyngeal carriage in children with CF and control children. There was a statistically significantly higher prevalence of _S. aureus_ in children with CF than in healthy controls without respiratory disease in the nose (28/80 versus 20/109) and in the oropharynx (35/80 versus 20/109).](http://jcm.asm.org/DownloadedFrom/2017/06/25/TABLE_1. Demographic characteristics of the CF patients and controls in this study)

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>Age (yr)</th>
<th>No. (%) of girls</th>
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<td></td>
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<td>Median</td>
<td>Mean</td>
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<td>CF patients</td>
<td>80</td>
<td>3.6</td>
<td>3.8</td>
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<tr>
<td>Controls</td>
<td>109</td>
<td>1.6</td>
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### Table 1. Demographic characteristics of the CF patients and controls in this study
In our study populations, the median ages of acquisition of *S. aureus* oropharyngeal and nasal colonization were 4.18 and 4.45 years, respectively.

**Dynamics of *S. aureus* infection in young children with CF.** Culture results of nasal and oropharyngeal specimens from 62 children with CF (40% girls) with a median age of 3.5 years who were monitored for at least 18 months were analyzed. The median observation period was 33 months (range, 18 to 65 months). The mean numbers of nasal and oropharyngeal cultures per patient were 9.5 and 11 specimens, respectively. *S. aureus* isolates were cultured from 196/587 (33%) nasal and 305/659 (46%) oropharyngeal swabs.

In 17 (27%) children, *S. aureus* was already cultured from the nose and airways at recruitment. In 29 (47%) children, *S. aureus* colonization could be observed for the first time during the observation period. In 9 (14%) children, *S. aureus* was cultured at the same time from the nose and the oropharynx, in 14 (23%) patients only from the oropharynx at first and later also from the nose, and in 6 children from the nose followed later by positive cultures from the oropharynx. The only site of *S. aureus* recovery during the study period was the oropharynx or the nose in 14 patients (23%) or 1 patient (2%), respectively. Only one patient never carried *S. aureus* throughout the study period. For 24 patients with nasal and oropharyngeal carriage and for 10 patients with oropharyngeal carriage only, data on their forced expiratory volume in 1 s were available. The lung functions of the two groups were not different (mean, 93.83 versus 95.3; *P* = 0.813).

In 11 patients, in addition to oropharyngeal swabs, 52 sputum samples were positive for *S. aureus*. Most of the clones isolated from the sputum samples were also recovered from the oropharynx (47/52, 90%), indicating colonization or infection of the lower airways with the same *S. aureus* clone as recovered from the oropharynx.

In summary, during the longitudinal part of this study, *S. aureus* was cultured from the upper airways and the nose in 46 children, from the oropharynx only in 14 children, and from the nose only in 1 patient (Fig. 2). Thus, the most prevalent site for *S. aureus* in CF patients was the oropharynx (60/62 [97%]), with a significantly lower prevalence in the nose (47/62 [76%]; *P* < 0.001).

**Persistent carriage of *S. aureus* in the nose and oropharynx.** Persistent positive nasal and oropharyngeal cultures were observed in 31/62 (50%) and 46/62 (74%) children, respectively, with a median persistence of 23 months (mean, 27 months; range, 6 to 46 months) and 29 months (mean, 27 months; range, 6 to 50 months). While 30/31 (97%) patients with persistent nasal carriage were also persistently colonized in the oropharynx, only 30/46 (64%) with persistent oropharyngeal carriage were also persistent nasal carriers. Interestingly, the number of patients with persistent *S. aureus* carriage in the oropharynx was statistically significantly higher than that of patients with persistent *S. aureus* carriage in the nose (*P* = 0.001).

**Dynamics of *S. aureus* carriage assessed by molecular techniques.** Molecular analysis of all of the cultured *S. aureus* isolates by PFGE allowed us to determine the population dynamics of *S. aureus* clones. One hundred seven different clones from the nose and 155 different clones from the oropharynx were isolated from 62 patients, with mean numbers of 2.3 (range, 1 to 8) and 2.6 (range, 1 to 11) clones per patient for each site (Table 2). Eighty-five of these clones were isolated from both sites (79% of the nasal clones, 55% of the oropharyngeal clones). Fourteen clones appeared first in the nares, as exemplified for one patient in Fig. 3A; 31 appeared first in the oropharynx, as shown for a second patient in Fig. 3B; and 40 clones were isolated from both sites at the same time, as demonstrated for a third patient in Fig. 3C. Overall, the number of different and persisting clones was statistically significantly higher in the oropharynx than in the nose (Table 2).
While nearly all of the clones isolated from the nose also appeared either at the same time or later in the oropharynx (82%), clones isolated from the oropharynx were less likely to be isolated from the anterior nares (57%).

**Susceptibility.** During this study, susceptibility testing was performed with 824 *S. aureus* isolates cultured from the anterior nares (250 isolates), the oropharynx (436 isolates), or sputum samples (64 sputum samples from 12 patients; 138 isolates). Thirty-five (anterior nares, 13; oropharynx, 22) MRSA isolates (4%) were detected in five children from one center. MRSA isolates were also resistant to clindamycin and erythromycin, including resistance to either gentamicin (*n* = 21) or levofloxacin (*n* = 14). β-Lactamase expression was detected in 674/789 (85%) methicillin-susceptible *S. aureus* (MSSA) isolates (anterior nares, 194/237 [82%]; oropharynx, 354/424, [83%]; sputum samples, 91/138 [66%]). Resistance to gentamicin was observed in 10/789 MSSA strains (anterior nares, 1; oropharynx, 6; sputum samples, 3), to levofloxacin in 1 MSSA isolate (sputum samples, 1), to clindamycin and erythromycin in 70/789 (9%) MSSA isolates (anterior nares, 23/237 [10%]; oropharynx, 38/424 [9%]; sputum samples, 9/138 [7%]), and to trimethoprim-sulfamethoxazole in 39/789 (5%) MSSA isolates (anterior nares, 5 [1 SCV]; oropharynx, 10 [2 SCVs]; sputum samples, 24 [21 SCVs]). Neither MRSA nor MSSA isolates were rifampin resistant. None of the MRSA isolates was mupirocin resistant. Sequential isolates from individual patients did not increase in resistance during the study period, except in the cases in which MRSA strains replaced MSSA strains (three patients) or in the cases in which SCVs emerged (eight patients).

**Risk factors for *S. aureus* carriage.** Forty-three questionnaires completed by the parents of 29 boys and 14 girls were included for further analysis, reflecting a response rate of 69%. The epidemiological and microbiological data from this subgroup were representative of the whole group: The median age of the children was 3.5 years, and the median observation period was 33 months. *S. aureus* was cultured from 142/434 (33%) nasal and 220/495 (44%) throat swabs. The use of a pacifier or oral or inhaled antibiotics, regular attendance at a children’s day care center, a hospital stay, or using a stuffed animal was not associated with persistent *S. aureus* carriage at either site. However, in the logistic regression analysis having a pet at home was associated with nasal carriage (OR, 4.5; CI, 1.0 to 21.1; *P* = 0.05).

### DISCUSSION

The cross-sectional part of this study demonstrated that significantly more children with CF harbored *S. aureus* in both the anterior nares and oropharynx than did those in the control group. The carrier rate of our control group is consistent with the study by Peacock et al. in which 21% of the children monitored from birth were colonized at 6 months of age (19). Our data indicate that CF by itself represents a risk factor for...
S. aureus acquisition and thus also infection. While the control group had similar carriage rates in the oropharynx and anterior nares, positive cultures of the oropharynx significantly exceeded carriage rates in the anterior nares of children with CF. This finding is in contrast to many other studies, as reviewed in reference 15, where the anterior nares were the main site of S. aureus colonization in humans.

Nasal carriage has been demonstrated to be a risk factor for consecutive S. aureus infection (28, 29). In these studies, nasal carriage of S. aureus preceded infection, which led to the implementation of preventive strategies to eradicate nasal carriage, thereby decreasing the number of S. aureus-induced infections. To observe if such a sequence of colonization and infection also occurs in CF patients required a longitudinal study design. However, many patients already carried S. aureus in both the oropharynx and the nose at the beginning of this study. In those patients who were culture positive at only one site, the rate of S. aureus detection was considerably higher in oropharyngeal than in nasal cultures. Only in a small number of patients have the anterior nares been identified as the first site of colonization. The observed sequence of S. aureus colonization was confirmed by molecular techniques. While almost all of the clones cultured from the nose later appeared in the oropharynx, only a small fraction of the clones cultured from the oropharynx occurred in the nose. Therefore, the sequence of S. aureus colonization differs in CF patients from that in other patients and healthy persons with a higher prevalence and persistence of S. aureus in the oropharynx than in the nose.

The high rate of S. aureus detection in this study underscores the fact that S. aureus is acquired very early in CF patients. It is conceivable that the recruited group, with a median age of 3.6 years, may have already been too old for determination of the acquisition sequence and the age at which S. aureus was acquired. Interestingly, the ages at which S. aureus colonization of the oropharynx and the nose was acquired by the study population investigated here were 4.14 and 4.48 years, respectively. However, studying infants diagnosed by neonatal screening may potentially yield different results with a lower S. aureus acquisition age and another sequence of S. aureus colonization. It is also possible that the sampling interval at the quarterly clinic visits, with a relatively low sampling frequency, may have led to a lower rate of nasal carriage detection preceding oropharyngeal carriage. However, a very short transient period of nasal carriage would not be clinically relevant, since this could not be addressed with a topical treatment strategy.

The prevalence of S. aureus in the nose was higher in CF patients than in the control group. However, the prevalence of S. aureus in the nose was exceeded by its prevalence in the oropharynx in CF patients. This may be explained by additional changes present in the airways such as impaired mucociliary clearance or a changed presentation of receptors for bacterial binding present on epithelial cells (10). It may also be due to expectoration of lower airway secretions containing S. aureus through the oropharynx. Interestingly, a recent study of non-CF patients and hospital personnel also reported a higher frequency of oropharyngeal colonization compared to the nares, albeit in lower bacterial numbers (18). These results are in contrast to other studies published so far but raise the question of whether differences in culture technique may affect the rate of S. aureus detection. Furthermore, the fact that in this study the tonsils, which have been shown to be a source of recurrent S. aureus carriage in some cases (27), and not the oropharynx, were swabbed may have resulted in this high S. aureus prevalence in the throat.

Susceptibility results did not differ for isolates cultured from the anterior nares or from the oropharynx. However, significantly fewer isolates recovered from sputum samples expressed β-lactamases compared to isolates recovered from the anterior nares or from the oropharynx, indicating that different selective pressures act upon S. aureus, depending on the source of the isolate. Importantly, the susceptibility of sequential S. aureus isolates to antibiotics did not change during the observation period, except in the cases where MRSA replaced MSSA isolates or in the cases where SCVs emerged.

Interestingly, the questionnaire which evaluated risk factors for S. aureus carriage revealed that having a pet at home appeared to enhance nasal carriage in children with CF. While transmission of S. aureus between humans because of domestic animals has been shown before (24), pets have not been recognized so far as a risk factor for S. aureus carriage in children with CF. This result may have important implications for the regular life of CF patients.

Since most young children with CF do not produce sputum, most of the specimens that we investigated were oropharyngeal swabs. It has been shown that oropharyngeal cultures represent good negative but not good positive predictive values for bacteria present in the lower airways (21). Therefore, it is difficult to draw conclusions about the number of patients with S. aureus infections of the lower airways. To do so, it would have been necessary to study bacterial cultures of bronchoalveolar lavage fluid, a method which would have been unsuitable for repetitive clinical use in children. However, it was not the intention of our study to determine the rate of S. aureus infection in CF patients but rather to study the sequence of S. aureus colonization.

The results of our study have implications for treatment strategies for CF patients. Our data identified the oropharynx as the most prevalent and often the first site of S. aureus carriage in young CF patients. Treatment strategies to prevent infections by eradication of nasal carriage will therefore not be an option for CF patients. Our data may also be transferred to the management of MRSA eradication in CF. In non-CF patients, MRSA eradication procedures usually combine nasal eradication with the topical use of antiseptics, leading in most cases to loss of the pathogen. However, considering the high prevalence of S. aureus in the upper airways, it is very unlikely that MRSA in CF patients will be eradicated by standard hygiene procedures.

In conclusion, our findings demonstrate that CF patients carried S. aureus mostly in the oropharynx and not in the nose, in contrast to other patient groups and healthy individuals. Furthermore, persistent carriage rates were higher in the oropharynx than in the nose. Therefore, it is highly unlikely that CF patients will benefit from strategies aimed at local eradication of nasal carriage to prevent S. aureus airway infections.

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REFERENCES