Molecular Epidemiology of Community-Acquired Staphylococcus aureus in Families with and without Cystic Fibrosis Patients

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The major human pathogen Staphylococcus aureus, which asymptptomatically colonizes the anterior nares of humans, can also cause a wide spectrum of diseases [1]. Population genetic studies of bacterial pathogens have been successfully used to determine the variability within individual species and especially to determine the clonal relationships among strains associated with disease in comparison with commensal strains [2].

For the mostly hospital-associated methicillin-resistant S. aureus (MRSA), an intercontinental spread of certain clones is well documented [2, 3]. However, little is known about the clonal structure and the epidemiologic characteristics of methicillin-sensitive S. aureus (MSSA) from the community. The mean carrier rate of S. aureus in the nose of most populations studied is 37%, with a wide variation in carriage rates (reviewed by Kluytmans et al. [4]). The prevalence of S. aureus in the nares has been correlated with individual genetic background (e.g., type of HLA antigens [5]), certain underlying diseases (e.g., diabetes mellitus [6]), lifestyle (e.g., intravenous drug use [7]), or exposure to specific environments (e.g., hospitals [8]).

It is commonly accepted that the nasal carriage of S. aureus is a major risk factor for subsequent infections, as shown for different patient groups who were hospitalized or had undergone invasive measures, such as patients receiving peritoneal dialysis [4, 9].

In young children with cystic fibrosis (CF), S. aureus is the most frequent bacterial isolate, often preceding Pseudomonas aeruginosa infection [10, 11]. Pulmonary infections are the main cause for morbidity and mortality among these patients despite long-term antibiotic treatment. The reasons for the susceptibility of CF patients to S. aureus lung infection are a matter of dispute [12–14]. One might assume that CF patients are more susceptible to S. aureus in their noses and that the nose is the primary reservoir for pulmonary infection. However, little is known about the S. aureus colonization of the nares in CF patients. S. aureus–infected CF patients usually do not spend extended periods in hospitals. Thus, the S. aureus colonization in the nares and lungs is probably acquired outside the hospital, possibly from family members.

To resolve CF-specific epidemiologic questions, we investigated whether an underlying disease results in a higher frequency of S. aureus colonization of the nares, whether the nose is a reservoir for colonization of the lower airways in CF patients, and whether CF patients harbor S. aureus strains different from those in healthy persons. Because the population dynamics of S. aureus nasal commensal strains is generally unclear, we also determined prevalence, persistence, transmission rate, and clonal distribution of S. aureus strains in the community. All isolates were typed by pulsed-field gel electrophoresis (PFGE). This method is appropriate for addressing epidemiologic questions [15, 16] and for dissecting the clonal structure of strain populations [17].

Materials and Methods

Study design. We studied 128 members of 34 families with CF children and 79 members of 23 non-CF families from the state of Baden-Württemberg in southern Germany (group 1). Nose swabs were obtained from all persons on 4 separate occasions over 19
months. In addition, sputum was collected from the 38 CF patients. At each collection appointment, all CF patients received a questionnaire covering age, sex, antibiotic treatment in the 4 weeks prior to collection, and hospital admissions. To enlarge the group of CF patients, an additional 72 CF patients 1–25 years old and 72 age-matched healthy control subjects were analyzed once for S. aureus nose colonization (group 2). All specimens were cultured on sheep blood agar and mannitol salt agar. S. aureus colonies were identified with tube coagulase (BioMerieux, Nürtingen, Germany) and Staphaurex Plus (Murex, Burgwedel, Germany).

Methicillin resistance. Susceptibility to methicillin was determined by the standard disk method, on Mueller-Hinton agar plates containing 2% NaCl. Inhibition of growth was interpreted according to standard recommendations. The disks contained 5 μg of oxacillin.

Genome typing. PFGE was performed after restriction endonuclease digestion of whole chromosomal DNA with Smal, as described elsewhere [16]. The restriction fragments were separated by a contour-clamped homogeneous electric field (CHEF-DRII system; BioRad, Munich), in 0.5× Tris borate–EDTA buffer at 12°C and 200 V for 24 h, with switch times of 1–45 s.

Analysis of DNA relatedness. The gels were evaluated by use of WinCam3 software (Cybertech, Berlin). A similarity index was determined for strains by use of the dice coefficient. Clustering correlation coefficients were calculated by the unweighted pair group method. Strains showing only minor differences in the banding pattern (>87% similarity as assessed by the dice coefficient) were assigned to the same genome type (GT). GTs were evaluated blindly with the aid of the computer program, and all types were assigned to the same genome type (GT). GTs were evaluated blindly with the aid of the computer program, and all types were assigned to the same genome type (GT). GTs were evaluated blindly with the aid of the computer program, and all types were assigned to the same genome type (GT).

Phage typing. Phage typing was done in a reference laboratory (Robert Koch Institut, Wernigerode, Germany) by standard methods [18].

Statistical analysis. For statistical analysis, we used the paired Student’s t test and χ² test.

Results

Prevalence of S. aureus in the anterior nares. In order to determine whether CF patients are more susceptible to S. aureus colonization of the nares, we assessed the prevalence of S. aureus among CF patients and healthy control subjects. None of the CF patients had attended hospitals, but most (85%) had been treated with antistaphylococcal antibiotics in the 4 weeks preceding sampling (42% β-lactam antibiotics, 26% aminoglycosides, 7% macrolides, 36% trimethoprim-sulfamethoxazole). In total, 828 specimens were collected, and 271 isolates were identified as S. aureus. The mean prevalence of S. aureus nose colonization over 4 samplings among the families with CF children (32% ± 9%) did not differ significantly from that among families without CF patients (35% ± 5%; P ≈ .6). The prevalence of S. aureus nose colonization among CF patients who had received antibiotics <4 weeks before sampling was 29%, compared with 57% among those without such treatment (table 1). Also, in a second group of 72 CF subjects, the subjects without treatment showed higher carrier rates (83%) than did those who had received antibiotics (30%). In all, CF patients without antibiotic treatment had a significantly higher S. aureus carrier rate (66%) than did treated patients (29%; P < .001, χ² test) or healthy controls (32%; P < .001, χ² test).

Comparison of nose and sputum isolates from CF patients. Because the nose is assumed to be the primary reservoir for colonization of the lower respiratory tract, we compared S. aureus isolates from the nares and sputa of CF patients. Of 79 sputum samples available for analysis from 28 CF patients, 30 samples were S. aureus positive. In 22 (73%) cases, S. aureus was simultaneously isolated in the nares of patients. Genome typing by PFGE revealed that, in 86% of these cases (19/22), the nose isolates were identical to the corresponding sputum strain.

Transmission of S. aureus within families. To analyze the frequency of S. aureus strain transmission in the community, GTs obtained within a family were compared. In 55% of the CF families and in 62% of the non-CF families, a GT from 1 person was simultaneously isolated from ≥1 other family members, indicating that transmission of S. aureus strains occurs frequently within families. However, no difference in the transmission rates was detected between families with or without CF patients. We also analyzed whether CF patients colonized with S. aureus in the lungs transmitted the bacteria more often than persons colonized only in the nose; the sputum strain was concurrently isolated from another family member in only 27% (8/30) of the cases.

Persistence of S. aureus GTs in the anterior nares. To determine whether a colonizing strain persists over extended periods of time, it is lost spontaneously, or is replaced by another S. aureus strain, we typed and compared the nose isolates obtained at consecutive samplings from all subjects. After 3 months, 57% (8/14) of the colonized CF patients and 57% (32/56) of the colonized healthy controls still had the same strain in their noses (figure 1). After 19 months, the identical S. aureus type was detectable in only 21% (3/14) and 23% (8/56), respectively. Therefore, in both CF patients and healthy controls, persistence of S. aureus strains is generally short. Very little strain replacement (or none at all) occurred within the first 3 months. However, after 19 months, a notable number of sub-

Table 1. Prevalence of Staphylococcus aureus in the noses of cystic fibrosis (CF) patients with and without antibiotic (AB) treatment in comparison with healthy controls.

<table>
<thead>
<tr>
<th>Group</th>
<th>With AB</th>
<th>Without AB</th>
<th>Healthy controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>36/129 (28)</td>
<td>13/23 (57)</td>
<td>22/676 (33)</td>
</tr>
<tr>
<td>2</td>
<td>18/60 (30)</td>
<td>10/12 (83)</td>
<td>21/72 (29)</td>
</tr>
<tr>
<td>Total</td>
<td>54/189 (29)</td>
<td>23/35 (66)</td>
<td>243/748 (32)</td>
</tr>
</tbody>
</table>

NOTE. Group 1, 38 CF subjects and 169 healthy controls from CF and non-CF families; data are mean prevalence (%) over 4 samplings. Group 2, 72 CF patients and 72 healthy controls; data are prevalence (%) based on 1 sampling.

* CF patients without AB treatment showed significantly higher prevalence than did healthy controls (P < .001) or patients with AB treatment (P < .001; χ² test).
Discussion

We found that the prevalence of *S. aureus* among CF patients who had not received antibiotics was significantly higher than that among treated patients or healthy controls. This suggests that the altered physiology due to the primary mutation in the CFTR gene leads to a higher susceptibility for *S. aureus* colonization in the nares. It further suggests that the use of antibiotics influences the carrier status of CF patients, reducing the *S. aureus* prevalence in the nares of these patients. The higher susceptibility in the nose may be explained by mechanisms proposed for chronic lung infection in CF: The altered mucus composition and viscosity in the airways of CF patients leads to reduced mucociliary clearance [19]. The entrapment of *S. aureus* in the mucus overlying the ciliated cells [12] may facilitate the establishment of infection. Alternatively, there is experimental evidence that *S. aureus* may bind to the host epithelium via receptor-mediated adherence [13, 14, 20]. Altered receptor expression or mucus properties in the CF host may also account for higher *S. aureus* susceptibility of the nares. In summary, our results indicate that an underlying hereditary disease can lead to a higher *S. aureus* carrier rate in the nose.

In most CF patients, *S. aureus* strains from the nares and spuata were identical. One may assume that *S. aureus*–positive sputum samples reflect colonization of the lower airways rather than contamination of the specimens by nasal secretions. This assumption was supported by the inspection of primary culture plates, which revealed that more bacteria were usually recovered from the sputum samples than from nose swabs. Furthermore, Gilljam et al. [21] showed a good concordance for sputum cultures and endobronchial cultures obtained during bronchoscopy and concluded that sputum culture provides accurate information about the bacterial colonization of the lower respiratory tract of patients with CF. Therefore, the identity of *S. aureus* strains from nose and sputum indicate that the nose may be an endogenous source of colonization in the lower airways and of subsequent lung infection in CF patients, as shown for other groups of patients [4].

One source of strain acquisition and colonization of the nose was found to be the families of CF patients, since strain trans-

Figure 1. *Staphylococcus aureus* carriers identified at 1st sampling (14 cystic fibrosis [CF] patients and 56 healthy controls) were analyzed for persistence (black), loss (dotted), or replacement (gray) of genome types after 3 and 19 months.

Subjects had become colonized with a new GT (21% and 19%, respectively).

Distribution of *S. aureus* GTs among families without social contact. To assess the population dynamics of community-acquired strains, the clonal distribution of *S. aureus* among families with and without CF patients was analyzed. In total, 271 *S. aureus* nose isolates were assigned to 38 distinct GTs by PFGE. Strains showing minor differences in the banding pattern (differences in <3 bands or >87% similarity as assessed by the dice coefficient) were considered clonal [15, 16] and were divided into subtypes (figure 2). For example, GT2 was divided into subtypes 2.4, 2.6, and 2.17. Of the 38 GTs isolated, 30 were found within only 1 or 2 families. However, 8 GTs were isolated in ≥2 families without any interfamily social contact. GT2 was isolated from 14 families with CF patients and from 11 families without CF patients (figure 2). The 8 most common GTs accounted for 78% of all isolates (table 2); GT2 and GT36 were particularly widely distributed, suggesting a clonal spread of certain strains in the community. Common GTs were isolated from the noses of both CF patients and healthy controls. Therefore, no unique *S. aureus* GT was associated with CF, and the isolates from sputum samples also belonged to the same strain population. Methicillin-susceptibility testing revealed that none of the common GTs were MRSA. The only 3 MRSA isolates found in this study belonged to 2 rare GTs and were isolated in 2 CF families.

The clonality of the most frequently isolated GTs was confirmed by phage typing, which was done independently and blindly. All GT1 and GT7 isolates were assigned to phage type 94/96 and phage type 95, respectively. For the other GTs, phage typing was more variable, but in each case a given GT always belonged to 1 specific phage group [18] or was nontypeable (table 2).

We analyzed our data to determine whether common strains differ from rare strains in their capacity to colonize the nose, assuming that common strains are more persistent or more often transmitted. Persistence was defined as occurrence of the identical GT in 1 carrier at 2 successive samplings. Our results show that the 8 common GTs tend to be more persistent than the rare types (table 2). However, rates of persistence varied widely within the group of common strains. GT7 showed the highest persistence (60%), whereas GT2 was only persistent in 25% of the cases. Isolation of the identical GT in ≥1 other family member at a given time was defined as transmission. The transmission rate of common strains was higher than that of the rare strains, and again the group of common strains appeared very heterogeneous. The less persistent GT2 showed the highest transmission rate (table 2).
Figure 2. Pulsed-field gel electrophoresis after SmaI digestion of Staphylococcus aureus isolates from cystic fibrosis (CF) families 14, 41, and 34. λ, λ phage (molecular-weight marker in kilobases [kb]); cf, CF patient; s, sputum; f, father; c, healthy child; m, mother; 1–4, sample series. Similarity index was determined for each strain by use of the dice coefficient. Minor differences in banding pattern (differences in <3 bands or >87% similarity) were considered evidence of subtypes (STs) (e.g., STs of genome type [GT] 2 are GT2.17, GT2.6, and GT2.4).

Transmission between family members was frequent. CF patients colonized with S. aureus in the lower respiratory tract did not transmit the bacteria more often than CF patients and healthy persons colonized only in the nose. It is generally recommended that CF patients infected with P. aeruginosa in their lungs be isolated from uninfected patients, for instance during summer camps [22–24]. Similar measures are discussed for CF patients infected with S. aureus. Our results do not indicate that CF patients colonized with S. aureus in the lower respiratory tract transmit the bacteria more easily than CF patients or healthy controls colonized only in the nares. Therefore, our results do not support special measures involving the separation of S. aureus–colonized CF patients from uncolonized patients.

In general, no difference was found in the rates of transmission and persistence between the S. aureus nose isolates of CF patients and those of healthy persons. The persistence of

<table>
<thead>
<tr>
<th>GT</th>
<th>Phage type</th>
<th>No. of families&lt;sup&gt;a&lt;/sup&gt;</th>
<th>No. (%) of isolates</th>
<th>Persistence&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Transmission&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>94/96</td>
<td>6</td>
<td>17 (6)</td>
<td>6/13 (46)</td>
<td>3/6 (50)</td>
</tr>
<tr>
<td>7</td>
<td>95</td>
<td>8</td>
<td>34 (13)</td>
<td>14/23 (60)</td>
<td>4/8 (50)</td>
</tr>
<tr>
<td>2</td>
<td>Group I</td>
<td>25</td>
<td>58 (21)</td>
<td>12/48 (25)</td>
<td>15/25 (60)</td>
</tr>
<tr>
<td>36</td>
<td>Group II</td>
<td>19</td>
<td>57 (21)</td>
<td>23/43 (53)</td>
<td>8/19 (42)</td>
</tr>
<tr>
<td>19</td>
<td>Group III</td>
<td>10</td>
<td>19 (7)</td>
<td>5/16 (31)</td>
<td>3/10 (30)</td>
</tr>
<tr>
<td>16</td>
<td>NT</td>
<td>6</td>
<td>10 (4)</td>
<td>3/9 (33)</td>
<td>2/6 (33)</td>
</tr>
<tr>
<td>30</td>
<td>NT</td>
<td>5</td>
<td>11 (4)</td>
<td>5/10 (50)</td>
<td>2/5 (40)</td>
</tr>
<tr>
<td>54</td>
<td>Group III</td>
<td>4</td>
<td>6 (2)</td>
<td>2/5 (40)</td>
<td>0/4</td>
</tr>
<tr>
<td></td>
<td>All 8 common GTs</td>
<td></td>
<td>212 (78)</td>
<td>70/167 (42)</td>
<td>37/83 (45)</td>
</tr>
<tr>
<td></td>
<td>All 30 rare GTs</td>
<td></td>
<td>59 (22)</td>
<td>12/39 (30)</td>
<td>10/35 (28)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td></td>
<td>271 (100)</td>
<td>82/206 (40)</td>
<td>47/118 (40)</td>
</tr>
</tbody>
</table>

NOTE: GT, genome type; NT, not typeable.
<sup>a</sup> GTs were isolated from >1 family member. In 23 families, only 1 common GT was isolated; in 27 families, multiple common GTs were isolated.
<sup>b</sup> Data are no. of individuals carrying identical GT at 2 successive samplings/no. of samples (%).
<sup>c</sup> Data are no. of identical GTs in >1 other family member at given time/no. of samples (%).
a given \textit{S. aureus} strain in the nares was assessed by genome typing of the isolates. After 3 months, 57\% of all carriers still harbored the same strain in the nose; however, after 19 months, only 21\% of the carriers remained colonized with the same strain, and strain replacement was observed in 23\%. These results agree closely with those of a Danish study, which found that, after 19 months, 20\% of carriers still harbored the same phage type in their noses [25]. Further studies are needed to clarify whether the spontaneous loss or replacement of a strain is associated with specific host factors or with certain characteristics of the bacteria. Production of distinct autoinducers from \textit{S. aureus} strains leads to a heterologous inhibition of virulence factors [26], and this mechanism has been mentioned as a possible bacterial strategy to inhibit invasion by strains of the same species. Such strain interference may be the factor that determines whether a strain is replaced or persists in a natural habitat.

The 271 \textit{S. aureus} nose isolates were found to belong to 38 distinct GTs. There was a close correlation between genome typing and phage typing: A given GT always belonged to the same lytic group. When Pantucek et al. [27] divided \textit{S. aureus} isolates into intraspecies restriction groups on the basis of similarities in the restriction pattern obtained by \textit{SmaI} digestion, they found a good correlation between lytic and restriction groups.

We also analyzed the clonal distribution of \textit{S. aureus} in the community. Most of our GTs were isolated within just 1 family. However, 8 GTs were widely distributed in families without any social contact with each other. We also isolated the most common clones from this study in epidemiologically unrelated patients from Saxony, France, and Italy, indicating that these strains are not limited to the Baden-Württemberg region [16, 28]. Our results indicate the predominant occurrence of a limited number of \textit{S. aureus} clones within the community. All our frequently isolated GTs were MSSA. Although the clonal spread of MRSA is well documented [2, 3], little information is available for MSSA. Only 2 well-defined phage types (94/96 and 95) [29, 30] have been shown to spread from epidemiologically unrelated sources. However, the clonal analysis of a wide range of strains is limited by the poor typeability and discriminatory power of phage typing. On the other hand, genome typing by PFGE is hindered by the lack of a universal system of strain definition. Until a uniform typing procedure [31] is established, the comparison of our results with other epidemiologic studies will be hampered by the inconsistent definition of types and clones.

The molecular and evolutionary basis for the predominance of certain clones remains to be determined. A Danish study of \textit{S. aureus} strains of phage type 95 indicated that this specific type may have a greater colonization capacity [32]. Our results confirm that this clone (GT7) has the highest persistence, which may explain its wide distribution. Other common clones may be more easily transmitted (e.g., GT2). The molecular basis for the differences among various clones’ capacities for persistence and transmission remains to be determined. Other evolutionary mechanisms (horizontal gene transfer, recombination, and chromosomal rearrangements) also contribute to the diversity within \textit{S. aureus} and can complicate the recognition of a clonal structure [33].

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References


