Staphylococcus aureus Small-Colony Variants Are Independently Associated With Worse Lung Disease in Children With Cystic Fibrosis

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Background. Cystic fibrosis (CF) lung disease is associated with diverse bacteria chronically infecting the airways. Slow-growing, antibiotic-resistant mutants of Staphylococcus aureus known as small-colony variants (SCVs) have been isolated from respiratory secretions from European adults and children with CF lung disease using specific but infrequently used culture techniques. Staphylococcus aureus SCVs can be selected either by exposure to specific antibiotics or by growth with another CF pathogen, Pseudomonas aeruginosa. We sought to determine the prevalence, clinical significance, and likely mechanisms of selection of S. aureus SCVs among a US cohort of children with CF.

Methods. We performed a 2-year study of 100 children with CF using culture techniques sensitive for S. aureus SCVs, and evaluated associations with clinical characteristics using multivariable regression models.

Results. Staphylococcus aureus SCV infection was detected among 24% of participants and was significantly associated with a greater drop in lung function during the study (P = .007, adjusted for age and lung function at enrollment). This association persisted after adjusting for infection with other known CF pathogens, including P. aeruginosa and methicillin-resistant S. aureus. Evidence indicated that S. aureus SCVs were likely selected in vivo by treatment with the antibiotic trimethoprim-sulfamethoxazole and possibly by coinfection with P. aeruginosa.

Conclusions. Infection with SCV S. aureus was independently associated with worse CF respiratory outcomes in this pediatric cohort. As many clinical microbiology laboratories do not specifically detect S. aureus SCVs, validation and extension of these findings would require widespread changes in the usual laboratory and clinical approaches to these bacteria.

Keywords. cystic fibrosis; Staphylococcus aureus; small-colony variant; lung function; children.

Lung disease associated with chronic airway infection is the main determinant of longevity and morbidity in people with cystic fibrosis (CF) [1]. Staphylococcus aureus is the bacterium cultured most commonly from the respiratory tracts of children with CF [2], and the earliest descriptions of CF lung infections focused on this species [3]. Subsequently, Pseudomonas aeruginosa was increasingly isolated from children with CF, and studies established an association between P. aeruginosa and CF lung disease [4, 5], largely shifting the focus of CF microbiological research and therapy. Recently, increases in S. aureus prevalence, both methicillin susceptible and methicillin resistant (MRSA), have been described in CF [6]. Recent studies of children with CF noted similar inflammation and lung function decline...
during infection with *S. aureus* compared with *P. aeruginosa* [7–9]. These observations have led to a reexamination of *S. aureus* and its role in CF lung disease [10].

Phenotypic variants of *S. aureus* called small-colony variants (SCVs) emerge during many chronic infections [11], including in CF [12–17]. *Staphylococcus aureus* SCVs grow slowly on most laboratory media due to metabolic defects, which also confer resistance to many antibiotics [11]. *Staphylococcus aureus* SCVs can be selected in vitro either by long-term exposure to specific antibiotics [11, 18] or growth with *P. aeruginosa* [19, 20], both of which occur commonly in CF airways. However, these various conditions select for phenotypically different SCVs, with distinct metabolic defects and antibiotic resistance profiles.

As a result of their slow growth, *S. aureus* SCVs are difficult to detect unless specifically looked for. Currently, most clinical laboratories do not use culture methods required to detect SCVs or to estimate their prevalence and clinical impact. Recent studies of adults and children with CF in Europe [12–16] reported *S. aureus* SCV prevalences of between 8% [16] and 33% [13] and unadjusted associations with lower lung function [14, 15], but for several reasons those results cannot necessarily be extrapolated to other CF populations. For example, US and European CF centers differ substantially both in antibiotic treatment and prophylaxis practices [1, 2, 21, 22] and in rates of *P. aeruginosa* culture positivity [2, 6, 14, 22], 2 factors predicted to influence *S. aureus* SCV prevalence and antibiotic resistances. Children with CF also differ from adults in those 2 factors. We hypothesized that *S. aureus* SCVs would be common in US pediatric CF patients, but with different predicted antibiotic resistances from those found in previous (European) studies, warranting routine surveillance. To test these hypotheses, we performed a 2-year study of our hospital’s CF population using specialized culture methods and detailed logs of antibiotic use. We focused on children due to their relatively mild disease, high rates of *S. aureus*, relatively little antibiotic exposure, and less frequent *P. aeruginosa* infection compared with adults, strengthening our ability to determine associations with specific selection pressures and disease severity.

**METHODS**

**Study Design**

This study was performed at Seattle Children’s Hospital (SCH) between 2008 and 2010 and approved by the SCH Institutional Review Board. Eligible patients at enrollment were ≤16 years of age, with documented CF diagnosis, a minimum of 2 CF clinic visits and 2 respiratory cultures in the 12 previous months, and written informed consent/assent. Patients were excluded for previous lung transplant or comorbidities that would interfere with data interpretation (eg, prematurity, non-CF immunodeficiency, or congenital or structural lung disease). Of 137 eligible subjects, 103 were approached, and 100 were consented and observed prospectively for up to 2 years (average of 7.3 study visits per subject) during regularly scheduled quarterly CF clinic visits. Participants recorded their use of antibiotics and mucolytics in logs collected at study visits. Lung function measurements and culture results were obtained from the local CF clinical database.

**Bacterial Isolate Culture and Characterization**

Respiratory tract specimens (sputum, bronchoalveolar lavage, or oropharyngeal [OP] swabs) were cultured for traditional CF pathogens and SCV *S. aureus* at the Cystic Fibrosis Foundation–funded Therapeutics Development Network Center for CF Microbiology (TDN-CCFM) at SCH. Culture, species identification, and pulsed-field gel electrophoresis (PFGE) methods are described in the Supplementary Data.

**Sputum Analysis**

All expectorating participants were asked to provide 2 sputum samples per visit. When this occurred, the first sample was cultured by the TDN-CCFM. DNA extracts from the second were subjected to molecular microbiological analyses as described in the Supplementary Data.

**Statistical Analysis**

Data were summarized using descriptive statistics. Comparisons between patient subgroups were performed using *t* tests with unequal variances or *χ*² tests. Multivariable linear regression models with covariate adjustment compared differences in lung function between patients who were never versus ever SCV positive (the latter defined as having ≥1 positive culture) during the study. Multivariable logistic regression models with covariate adjustment were used to assess associations between SCV detection and preceding antibiotic use, isolation of *P. aeruginosa*, and pulmonary exacerbation (defined as new antibiotic administration for respiratory symptoms) during study follow-up. All analyses were exploratory and were not adjusted for multiple comparisons. Analyses were performed using Stata software, version 12.0 (StataCorp, College Station, Texas).

**RESULTS**

**Clinical Characteristics**

One hundred children with CF were studied for an average of 1.7 years (SD, 0.3 years; minimum, 0.5 years; maximum, 2.0 years). Subjects had a mean of 8.4 respiratory cultures during the study (SD, 2.9), of which 67.5% were OP swabs, 31% sputum, and 1% bronchoalveolar lavage. Study population demographic details are shown in Table 1.
Table 2 shows the proportion of subjects who were culture positive for specific bacteria during the study either intermittently or persistently (defined as having positive cultures during <50% and ≥50% of age quarters in which cultures were performed, respectively, similar to criteria used previously for *P. aeruginosa* [23]). Considering these categories together, 88% of subjects were culture positive during the study for any *S. aureus*, 26% for MRSA, and 24% for *S. aureus* SCVs. The SCVs from 9 subjects were also MRSA (37% of SCV-positive subjects). Forty-seven percent of the SCVs were isolated from OP swabs, and 53% from sputum. Figure 1 illustrates the patterns of culture positivity for *S. aureus* SCVs as a function of age among the 24 SCV culture-positive subjects during the study. In general, subjects tended to have alternating positive and negative culture positivity for *S. aureus* SCVs, strongly suggestive of repeated selection and enrichment for SCVs in each subject, incomplete detection by individual cultures, or both. Culture results at enrollment are shown in Supplementary Table 1.

Among cultures positive for *S. aureus* SCVs, 33% were negative for normal-colony *S. aureus*, indicating that no *S. aureus* would have been detected without SCV surveillance. Similarly, 28% of those cultures were negative for both of the most commonly cultured "standard CF pathogens" [2], normal-colony *S. aureus* and *P. aeruginosa*.

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Clinical Characteristics Associated With S. aureus SCVs

Among the 63 subjects ≥6 years who could perform spirometry and had S. aureus isolated during the study, SCV detection (ever vs never) was associated with significantly lower values of the lung function measure forced expiratory volume in 1 second percent predicted (FEV1 % predicted) FEV1% predicted at the beginning and end of the study (Table 3), with a trend toward greater decline in FEV1% predicted during the study. Change in FEV1% predicted was significantly associated with SCV detection in a linear regression model that adjusted for age and lung function at enrollment (Table 4, model 1). Controlling for P. aeruginosa and MRSA did not diminish the strength of that association (Table 4, models 2 and 3). Because of concern about day-to-day spirometric variability, we repeated this analysis using slope of FEV1% predicted (incorporating all study measurements, rather than only first and last), with similar results (Supplementary Table 2). In contrast, we found no significant association for either measure of lung function decline and MRSA (P = .536).

We investigated whether there were other differences between subjects who were culture positive versus culture negative for S. aureus SCVs. Although there was no association between age and SCV detection among subjects evaluated by spirometry (Table 3), among all subjects with S. aureus there was an association with increased enrollment age for those ever (n = 24) versus those never positive (n = 64) for SCVs during the study (11.1 vs 8.8 years, P = .016, not shown).

Frequency of CF respiratory exacerbation was not significantly different during the study period between subjects who were ever versus never S. aureus SCV culture positive (87% vs 77%, respectively, experienced an exacerbation; P = .447 adjusted for enrollment age, not shown). Results were similar for both P. aeruginosa (P = .583) and MRSA (P = .536).

Molecular Characteristics of S. aureus SCVs

The metabolic type and resistance profile of an SCV can usually be predicted from its selecting condition. For example, SCVs defective in production of the metabolic cofactors menadione or hemin can be selected by aminoglycosides and fusidic acid [11], resulting in high resistance to these and other antibiotics in vitro. Both types of SCV [19, 20] can also be selected by growth with P. aeruginosa. SCVs that are defective in the synthesis of thymidine can be selected by sulfonamide antibiotics such as trimethoprim-sulfamethoxazole (TMP-SMX), and they are resistant to TMP-SMX and other antibiotics in vitro [11].

All S. aureus SCVs from this study were therefore tested for improved agar growth by disks of menadione, thymidine, or hemin, or in increased CO2 (auxotrophy tests [11]; Supplementary Figure 1). We found that 95% were auxotrophic for thymidine, 7% for hemin, 1% for menadione, and 1% for CO2.
As shown in Table 5, SCV detection was significantly associated with TMP-SMX treatment during the preceding quarter; no significant association was found for aminoglycosides (Table 5) or coinfection with P. aeruginosa ($P = .066$, not shown). A significant association was also found for rifampin treatment, but this drug was usually administered with TMP-SMX, and further analysis indicated that TMP-SMX was the strongest predictor of SCV detection (Table 5). These results suggest that TMP-SMX strongly selects for S. aureus SCVs within CF airways, with predicted resistance to TMP-SMX and other antibiotics [11].

**Selection Pressures for S. aureus SCVs**

Closer analysis of the 6 subjects with hemin- or menadione-auxotrophic SCVs (25% of SCV-positive subjects, Figure 1) demonstrated that only half had recently been treated with aminoglycosides or TMP-SMX. However, 5 were culture positive for P. aeruginosa within 6 months before SCV isolation, and

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**Table 4. Small-Colony Variant Status as an Independent Predictor of Change in Lung Function Over the Study Period**

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Coefficient Estimate (Mean Difference in Change in FEV₁% Predicted Over Study Period)</th>
<th>95% Confidence Interval</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ever SCV positive on study</td>
<td>$-11.00$</td>
<td>$-18.81, -3.18$</td>
<td>.007</td>
</tr>
<tr>
<td>Age at enrollment</td>
<td>$-1.31$</td>
<td>$-2.43, -0.18$</td>
<td>.023</td>
</tr>
<tr>
<td>FEV₁% predicted at enrollment</td>
<td>$-0.28$</td>
<td>$-0.48, -0.08$</td>
<td>.007</td>
</tr>
<tr>
<td>Model 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ever SCV positive on study</td>
<td>$-11.32$</td>
<td>$-19.10, -3.55$</td>
<td>.005</td>
</tr>
<tr>
<td>Age at enrollment</td>
<td>$-1.51$</td>
<td>$-2.75, -0.26$</td>
<td>.019</td>
</tr>
<tr>
<td>FEV₁% predicted at enrollment</td>
<td>$-0.28$</td>
<td>$-0.48, -0.08$</td>
<td>.007</td>
</tr>
<tr>
<td>Ever <em>Pseudomonas aeruginosa</em> positive on study</td>
<td>$2.53$</td>
<td>$-4.75, 9.80$</td>
<td>.490</td>
</tr>
<tr>
<td>Model 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ever SCV positive on study</td>
<td>$-11.29$</td>
<td>$-19.61, -2.97$</td>
<td>.009</td>
</tr>
<tr>
<td>Age at enrollment</td>
<td>$-1.29$</td>
<td>$-2.44, -0.14$</td>
<td>.028</td>
</tr>
<tr>
<td>FEV₁% predicted at enrollment</td>
<td>$-0.28$</td>
<td>$-0.48, -0.08$</td>
<td>.008</td>
</tr>
<tr>
<td>Ever <em>Stenotrophomonas maltophilia</em> positive on study</td>
<td>$0.59$</td>
<td>$-5.92, 7.10$</td>
<td>.857</td>
</tr>
<tr>
<td>Model 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ever SCV positive on study</td>
<td>$-12.98$</td>
<td>$-21.55, -4.41$</td>
<td>.004</td>
</tr>
<tr>
<td>Age at enrollment</td>
<td>$-1.34$</td>
<td>$-2.51, -0.17$</td>
<td>.026</td>
</tr>
<tr>
<td>FEV₁% predicted at enrollment</td>
<td>$-0.26$</td>
<td>$-0.47, -0.06$</td>
<td>.013</td>
</tr>
<tr>
<td>Ever <em>Stenotrophomonas maltophilia</em> positive on study</td>
<td>$4.41$</td>
<td>$-2.67, 11.49$</td>
<td>.218</td>
</tr>
<tr>
<td>Model 5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ever SCV positive on study</td>
<td>$-10.91$</td>
<td>$-18.79, -3.03$</td>
<td>.008</td>
</tr>
<tr>
<td>Age at enrollment</td>
<td>$-1.28$</td>
<td>$-2.41, -0.16$</td>
<td>.026</td>
</tr>
<tr>
<td>FEV₁% predicted at enrollment</td>
<td>$-0.29$</td>
<td>$-0.49, -0.08$</td>
<td>.007</td>
</tr>
<tr>
<td>Any exacerbations on study</td>
<td>$-1.04$</td>
<td>$-7.55, 5.46$</td>
<td>.749</td>
</tr>
<tr>
<td>Model 6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ever SCV positive on study</td>
<td>$-11.92$</td>
<td>$-20.18, -3.66$</td>
<td>.006</td>
</tr>
<tr>
<td>Age at enrollment</td>
<td>$-1.29$</td>
<td>$-2.45, -0.12$</td>
<td>.031</td>
</tr>
<tr>
<td>FEV₁% predicted at enrollment</td>
<td>$-0.28$</td>
<td>$-0.49, -0.08$</td>
<td>.007</td>
</tr>
<tr>
<td>Use of TMP-SMX during the study</td>
<td>$2.19$</td>
<td>$-4.84, 8.92$</td>
<td>.517</td>
</tr>
</tbody>
</table>

Abbreviations: FEV₁%, percent predicted forced expiratory volume in 1 second; MRSA, methicillin-resistant *Staphylococcus aureus*; SCV, small-colony variant; TMP-SMX, trimethoprim-sulfamethoxazole.

* Approach as in Table 3, except with 6 separate linear regression analyses, each with different predictor variable sets, as shown. Adjusting for sex did not alter the results (not shown). Each potential confounding variable was evaluated by adding it to our base model (model 1). Due to sample size constraints, we did not evaluate all potential confounding variables simultaneously.

* For covariates coded as yes/no (such as culture positivity), this is the mean difference in change in FEV₁% predicted over the study period between subjects coded as yes versus those coded as no. For continuous covariates, this is the mean difference per 1 unit increase in covariate (eg, for age, the mean difference per 1 year increase in age).

* Includes data for 59 participants who had antibiotic data reported. Similar results were obtained when adjusting for total quarters of TMP-SMX use.
Any aminoglycosides  

nonsignificant results (not shown).

antistaphylococcal activity (as defined in Supplementary Table 3) also gave

monobactams). Restricting analysis to

compared with clonal, normal-colony

aeruginosa revealed all to be relatively resistant to growth inhibition by

resistance to aminoglycosides and other antibiotics [19]. Fur-

P. aeruginosa pyrosequencing. Thus, coinfection with

the sixth had 3 sputum samples that tested positive for P. aeru-

left; results reflect 409 quarters evaluated among 83 participants with relevant culture data and prior antibiotic usage during the study.

b Each of the 6 separate logistic models assessed whether SCV detection was associated with use of a specific antibiotic class reported during the preceding age quarter. All models used robust variance estimates, accounted for repeated observations per participant, and adjusted for age quarter when cultured.

c Includes true β-lactams and structural relatives (ie, carbapenems and monobactams). Restricting analysis to β-lactams with significant antistaphylococcal activity (as defined in Supplementary Table 3) also gave nonsignificant results (not shown).

d The effect of each antibiotic after adjusting for the other antibiotic. In 14 of 16 instances of rifampin use during the study, TMP-SMX was used concurrently.

the sixth had 3 sputum samples that tested positive for P. aeruginosa by quantitative polymerase chain reaction (qPCR) and pyrosequencing. Thus, coinfection with P. aeruginosa may select for specific S. aureus SCV metabolic types, with predicted resistance to aminoglycosides and other antibiotics [19]. Furthermore, testing of a subset of thymidine- auxotrophic SCVs revealed all to be relatively resistant to growth inhibition by P. aeruginosa compared with clonal, normal-colony S. aureus isolates from the same patients (Supplementary Figure 2). Therefore, coinfection may frequently provide additional selection pressure for all S. aureus SCV types.

In CF, TMP-SMX is commonly used to treat MRSA [24], Burkholderia cepacia species [22], or Stenotrophomonas maltophilia [1, 22]. Given the results shown in Table 4 (model 3) and the rarity of B. cepacia (Table 2), it remained possible that S. aureus SCVs were a marker for S. maltophilia. We therefore repeated our analyses of the relationship between FEV1 and SCVs controlling for S. maltophilia and found no change in the SCV effect (Table 4 and Supplementary Table 2, model 4). Thus, the relationship between S. aureus SCVs and respiratory outcomes was not simply attributable to other commonly cultured CF pathogens, including MRSA, P. aeruginosa, or S. maltophilia.

The interrelated associations between S. aureus SCVs, TMP- SMX, and lung function in this study raised the possibility that TMP-SMX treatment was itself a risk factor for worse lung function. TMP-SMX was used in 24% of CF exacerbation treatments including oral antibiotics in our study, and by 38% of study subjects, much less frequently than β-lactams and similar to many other antibiotics (Supplementary Table 3). As shown in Table 4 and Supplementary Table 2 (model 6), adjusting our analyses for any TMP-SMX use during the study did not change the strength of the association between SCV positivity and change in FEV1% predicted. Similarly, adjusting for the occurrence of antibiotic-treated exacerbations during the study did not change this association (model 5). Thus, the association between SCVs and worse respiratory outcomes also was not simply due to the use of TMP-SMX or of antibiotics in general.

SCV Genetic Typing

We used PFGE to determine whether SCVs emerged in vivo from preexisting normal-colony lineages. From each SCV culture-positive subject, we analyzed at least 1 SCV (60 SCVs from 24 subjects) and at least 2 normal-colony S. aureus isolates (75 isolates from 24 subjects). The results in Supplementary Figure 3 demonstrate that the S. aureus SCVs belonged to 14 distinct PFGE groups. (Although the focus of this analysis was on clonality within each study subject, a molecular epidemiological description of the S. aureus within the study population, and comparison with S. aureus in other CF populations, will be presented elsewhere.) Five lineages of genetically related S. aureus SCV isolates were found in ≥3 subjects, suggesting that specific SCV lineages were more common than others. However, in the vast majority of subjects (21/24), an SCV was isolated subsequent to or concurrent with a clonally related normal-colony S. aureus isolate, suggesting that SCVs were generally selected in vivo rather than transmitted as SCVs between subjects. In the remaining 3 cases, no clonally related normal-colony S. aureus isolate was identified from the same subject as an SCV. Two of these SCVs were genetically indistinguishable from SCVs isolated from other subjects, suggesting that, while uncommon, transmission of SCVs or acquisition from a common source could have occurred between subjects.

Abundance of S. aureus SCVs

Because of their poor growth, it is possible that S. aureus SCVs are less abundant in vivo than their normal-colony counterparts, with implications for in vivo growth and pathogenicity and for detection. We therefore performed S. aureus species-specific qPCR on 54 sputum samples from 27 S. aureus–positive study subjects (range, 1–6 samples per subject), including 13

<table>
<thead>
<tr>
<th>Model</th>
<th>Predictor</th>
<th>OR, SCV Detection</th>
<th>95% CI</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Any aminoglycosides during preceding quarter</td>
<td>1.69</td>
<td>0.81–3.49</td>
<td>.160</td>
</tr>
<tr>
<td>2</td>
<td>Any quinolones during preceding quarter</td>
<td>0.85</td>
<td>0.33–2.16</td>
<td>.733</td>
</tr>
<tr>
<td>3</td>
<td>Any β-lactams during preceding quarter</td>
<td>0.71</td>
<td>0.33–1.52</td>
<td>.376</td>
</tr>
<tr>
<td>4</td>
<td>Any TMP-SMX during preceding quarter</td>
<td>9.51</td>
<td>4.70–19.24</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>5</td>
<td>Any rifampin during preceding quarter</td>
<td>8.93</td>
<td>3.03–26.34</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>6</td>
<td>Any TMP-SMX during preceding quarter</td>
<td>8.06</td>
<td>3.74–17.40</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; OR, odds ratio; SCV, small-colony variant; TMP-SMX, trimethoprim-sulfamethoxazole.

A small-colony variant was identified in 22% of culture-positive subjects, with implications for in vivo growth and pathogenicity and for detection. We therefore performed S. aureus species-specific qPCR on 54 sputum samples from 27 S. aureus–positive study subjects (range, 1–6 samples per subject), including 13
Subjects with *S. aureus* SCVs during the study. *Staphylococcus aureus* abundance was similar by qPCR whether SCVs were present or not (mean, 3.27 [SD, 4.24] × 10^6 colony-forming units [CFU]/mL equivalents vs 4.52 [SD, 8.22] × 10^6 CFU/mL equivalents, respectively), even among the 6 samples collected when SCVs but no normal-colony *S. aureus* were cultured (mean, 5.25 [SD, 5.07] × 10^6 CFU/mL equivalents), suggesting that SCV and normal-colony *S. aureus* cells are similarly abundant in CF airways.

**DISCUSSION**

In our study, *S. aureus* SCVs were common, detected in about one-quarter of participants, and were independently associated with substantially worse lung function outcomes. This effect persisted when controlling for enrollment disease severity, age, and other relatively well-studied CF pathogens, including *P. aeruginosa*, *S. maltophilia*, and MRSA of all colony sizes. Analyses indicated that the *S. aureus* SCVs were selected in vivo most often by the antibiotic TMP-SMX, and less frequently by coinfection with *P. aeruginosa*. Because few clinical laboratories currently identify or report SCVs, and no optimal therapeutic approach to SCVs has been identified, these results underscore the need for both widespread surveillance and additional clinical and laboratory studies of these variants.

Our study was limited by its single-center nature. There currently is no gold standard method for detecting *S. aureus* SCVs; the techniques we used were similar to those used in other studies, but other methods with higher sensitivity and/or specificity may yet be identified. The agreement between our qPCR and culture findings was reassuring regarding this issue. Because OP culture for *S. aureus* is not a perfect predictor of lower airway culture [25], future studies will be required to determine the relationship between culture positivity for SCVs in the upper and lower airways and whether detection in either location alone correlates with outcomes.

*Staphylococcus aureus* SCVs have been associated with diverse chronic infections [11]. Although several studies of European CF patients have been performed, the prevalence and clinical associations of SCVs in the current pediatric study population, in which antibiotics are rarely administered long-term for antistaphylococcal prophylaxis or treatment, extend and clarify those previous studies. Kahl et al [12, 13] found an *S. aureus* SCV prevalence of 33% over up to 6 years among German CF patients (adults and children) who had frequently received long-term TMP-SMX treatment and prophylaxis; most isolates were also thymidine-auxotrophic. However, that study did not correlate culture results with clinical outcomes. Besier et al [14] found *S. aureus* SCVs over 12 months among 17% of 267 German adults and children with CF who had frequently received long-term and prophylactic TMP-SMX. They found that subjects with *S. aureus* SCVs had lower lung function, advanced age, and more TMP-SMX exposure, but they did not report the effect of controlling for other variables, such as *P. aeruginosa*. Schneider et al [15] reported similar findings among 98 Swiss CF patients over 3 months. Interestingly, the study by Besier et al [14], in which thymidine-auxotrophic isolates were relatively less common (63%) than in the current study, found *S. aureus* SCVs to be more common with *P. aeruginosa* coinfection. Thus, *P. aeruginosa* coinfection may strongly select for *S. aureus* SCVs in specific contexts, as suggested here.

The rarity of clinical laboratory culture for SCVs indicates that physicians are frequently unaware of these highly antibiotic-resistant infections in selecting treatments for their CF patients, underestimating the presence both of all *S. aureus* and of a subtype that correlates with worse respiratory disease. Therefore, routine *S. aureus* SCV surveillance would provide more complete information to guide treatment. During the study, many subjects continued to receive antibiotics to which SCVs are predicted to be resistant, such as TMP-SMX and aminoglycosides, indicating that treating physicians may have made different treatment choices had these results been available.

The relationship between *S. aureus* SCVs and lower lung function that we and others [14, 15] observed suggest at least 2 possible explanations: (1) SCVs are significant pathogens in CF, or (2) SCVs emerge more frequently in children with more aggressive lung disease, who receive more antibiotics either targeting traditional pathogens or simply as a response to their more troubling presentation. We did not find evidence beyond lung function outcomes for the first possible explanation in our study; for example, no association was found between SCVs and exacerbations (which may indicate different determinants of CF exacerbations and chronic spirometric decline), and too few subjects provided sputum to identify differences in inflammatory measures. With respect to the second possible explanation, *S. aureus* SCVs may merely be one of many indicators of higher antibiotic burdens, perhaps as indicated by lower enrollment FEV₁ among SCV culture-positive subjects. Similarly confounded relationships have been suggested for other antibiotic-resistant bacteria such as MRSA [26] and multiply antibiotic resistant *P. aeruginosa* [27, 28]. Interventional studies would likely be much more powerful than observational studies in clarifying these causal relationships, as well as in answering whether having *S. aureus* SCV culture information and/or specifically treating *S. aureus* SCVs would improve outcomes. In the meantime, the wider adoption of culture for *S. aureus* SCVs, and their report in surveillance data [2] and databases [29], would greatly facilitate addressing these questions.

In the absence of further study, insufficient information exists to advise changes in current treatment approaches. For example, it may be tempting to avoid treatment of CF patients...
with TMP-SMX based on these findings, but our results give little indication of any clinical advantage of this strategy (particularly because TMP-SMX treatment was not associated with worse lung function). Although these results suggest that SCVs should be detected and characterized to aid management, whether the therapeutic goal should be prevention, eradication, suppression, or episodic exacerbation treatment is not yet clear. Regardless, additional work will be required to determine how best to measure susceptibilities of clinical S. aureus SCVs in order to more rationally direct therapy.

Supplementary Data

Supplementary materials are available at Clinical Infectious Diseases online (http://cid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Acknowledgments. We thank the study participants and their families for their invaluable contributions; M. Elliott, K. Worrell, A. Genatossio, and J. Foster for assistance with this study’s clinical portion; and D. Vandevanter for helpful discussions.

Financial support. This work was supported by grants from the Cystic Fibrosis Foundation (HOFFMA07P0) and the American Thoracic Society (CF-07-003).

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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