Persistent Infection with Small Colony Variant Strains of *Staphylococcus aureus* in Patients with Cystic Fibrosis


In a 34-month prospective study to determine the prevalence of *Staphylococcus aureus* small colony variants (SCVs) in cystic fibrosis (CF) patients, *S. aureus* SCVs or SCVs plus normal *S. aureus* were recovered from 26 of 78 patients; 27 patients harbored only normal *S. aureus*. By pulsed-field gel electrophoresis, clonal identity was demonstrated of SCV and normal strains isolated at the same time and of multiple *S. aureus* SCV and normal strains in consecutive specimens from individual patients. All *S. aureus* SCVs were resistant to antifolate antibiotics, while the corresponding parent strains were susceptible, and in 11 of 12 SCV/normal pairs, gentamicin was less active against *S. aureus* with the SCV phenotype than against the normal isolate. Analysis of the underlying auxotrophism of SCVs revealed hemin, thymidine, and/or menadione dependencies. Thus, *S. aureus* SCVs are highly prevalent in respiratory secretions of CF patients, persist over extended periods, and may contribute to *S. aureus* persistence in CF patients.

Cystic fibrosis (CF) is an autosomal recessive genetic disorder, caused by a mutation in the cystic fibrosis gene affecting the CAMP-regulated transmembrane Cl⁻ channel, resulting in abnormal respiratory secretions [1]. CF is characterized by severe bronchopulmonary infections and inflammation of the airways, with a characteristic microflora (i.e., *Staphylococcus aureus* and *Pseudomonas aeruginosa*) contributing to progressive and ultimately fatal lung disease [2]. While in the preantibiotic era, lung infection due to *S. aureus* was the leading cause of death, treatment with efficient antistaphylococcal compounds and prophylactic therapy now effectively controls acute infections. Once colonized, however, often *S. aureus* cannot be cleared from the bronchial system despite the use of antimicrobials with high staphylocidal activity [3].

The pathogenesis of staphylococcal infection in CF patients is complex and incompletely understood. The decreased viscoelastic properties of mucus with reduced clearance of thickened secretions [1], increased bacterial adherence to airway epithelial cells [4], or a suggested receptor for pathogenic bacteria on the apical surface of epithelial cells of CF patients [5] have all been implicated in bronchial colonization and may also contribute to the persistence of the microorganisms in the lung tissue. Persistence of *S. aureus* in invasive infections other than CF, however, has recently been associated with the isolation of *S. aureus* subpopulations with a small colony variant (SCV) phenotype [6, 7]. In contrast to *S. aureus* exhibiting typical colony size, pigmentation, and hemolysis on Columbia agar (herein referred to as “normal” *S. aureus*), *S. aureus* SCV organisms grow as nonhemolytic, nonpigmented, and very small colonies and produce greatly reduced amounts of α-toxin and, consequently, have been shown to intracellularly persist in vitro systems [8]. Decreased susceptibility of SCVs may contribute to their isolation (typically after long-term antimicrobial therapy) because of selection within the patient.

We hypothesized that the recovery of *S. aureus* over long periods in CF patients, in spite of antibiotic therapy, may be the result of a metabolic alteration resulting in expression of the SCV phenotype. To address this hypothesis, special culture and identification procedures for determination of SCV prevalence had to be used, since *S. aureus* SCVs may be easily missed during microbiobiological examination of bronchial secretions from CF patients because of their fastidious growth characteristics and the frequent presence of *P. aeruginosa*. Furthermore, clonal identity of isolates was determined with molecular typing methods, and analysis of metabolic alteration resulting in SCV phenotype was assessed by defining specific growth requirements.

**Materials and Methods**

**Patients and specimens.** Specimens were obtained from 78 in- and outpatients with CF from April 1994 to January 1997 in our institution. The age of the patients ranged from 0.5 to 43 years. In general, specimens consisted of cultures of bronchial secretions;
however, if patients were too young or no bronchial secretions were produced, throat swabs were taken.

Culture techniques. Specimens were cultured on Columbia agar (Becton Dickinson, Heidelberg, Germany) for gram-positive cocci, on endo agar (Merck, Darmstadt, Germany) (35°C for 48 h) for gram-negative rods, on chocolate agar (Mast, Reinfeld, Germany) (24–48 h at 35°C with CO₂) for Haemophilus influenzae, and on Schaedler (Becton Dickinson) and brain-heart infusion (BHI; Merck) agar with 5% NaCl (anaerobically at 35°C for 48 h) to suppress P. aeruginosa and to support growth of S. aureus and SCVs of S. aureus.

SCV identification. All pathogens from BHI and Schaedler agar were isolated in parallel on Columbia agar (24 h at 35°C) and on Schaedler agar (24 h at 35°C with CO₂). Very small, nonpigmented, nonhemolytic colonies on Columbia agar were suspicious for SCVs if they grew nearly normally on Schaedler agar. All SCVs were identified to be S. aureus with catalase, tube coagulase (bioMérieux, Marcy l’Etoile, France), Api ID 32 Staph (bioMérieux), and Pastorex Staph plus (Sanofi Pasteur, Marnes-la-Coquette, France). Presence of the S. aureus–specific nuc gene [9] was tested by polymerase chain reaction [10] to confirm the identification of S. aureus.

Genotypic analysis. To examine the clonal identity of phenotypically normal and S. aureus SCV isolates, we analyzed these strains with pulsed-field gel electrophoresis (PFGE) as described [11], except for the use of BHI broth, because SCVs do not grow well in tryptic soy broth (which is a medium containing fewer nutrients).

Susceptibility testing. Susceptibility for normal and S. aureus SCV isolates was tested by disk diffusion on Mueller-Hinton (MH) agar (Mast) according to the National Committee for Clinical Laboratory Standards (NCCLS) guidelines [12]. Because S. aureus SCVs grow too slowly on MH agar, susceptibility testing for SCVs was done on MH agar supplemented with blood. MICs were tested according to the NCCLS guidelines [13], except BHI broth was used because of the fastidious growth of SCV strains. The antibiotics tested were penicillin, ampicillin, oxacillin, imipenem, cefaclor, cefazolin, cefotaxime, erythromycin, clindamycin, chloramphenicol, gentamicin, ofloxacin, ciprofloxacin, trimethoprim-sulfamethoxazole, vancomycin, and teicoplanin. Penicillin, piperacillin, cefazidime, ciprofloxacin, gentamicin, and trimethoprim-sulfamethoxazole MICs were tested with Etest (AB Biodisk, Solna, Sweden) as recommended by the manufacturers on MH or MH-blood agar for normal S. aureus and SCVs, respectively. Twelve pairs of SCV and normal strains with indistinguishable bands in PFGE, isolated at the same time from 12 different patients with persistent SCV colonization, were compared with respect to their MICs.

Auxotrophism studies. Auxotrophy for hemin and NAD⁺ factor (Unipath, Basingstoke, UK) was tested by using standard disks, and for thymidine (Fluka Chemie, Buchs, Switzerland) and menadione (Sigma Aldrich Chemie, Deisendorf, Germany) by impregnating disks with 15 μL of thymidine at 100 μg/mL or menadione at 10 μg/mL. To determine single auxotrophy, test isolates were inoculated on chemically defined medium (CDM) agar [14] (compounds from Merck and from Boehringer Ingelheim, Heidelberg, Germany), impregnated disks were laid on top of the agar surface, and auxotrophism was determined as positive if a zone of normal growth surrounding the impregnated disks was detected after 18 h of incubation. Likewise, for determination of double auxotrophy, disks were tested on CDM agar supplemented with 100 μg of thymidine/mL, 10 μg of menadione/mL, or 1 μg of hemin/mL; for determination of triple auxotrophy, on CDM agar supplemented with 100 μg of thymidine/mL plus 10 μg of menadione/mL, 100 μg of thymidine/mL plus 1 μg of hemin/mL, or 10 μg of menadione/mL plus 1 μg of hemin/mL; and for determination of combined auxotrophism, on CDM agar supplemented with 100 μg of thymidine/mL, 10 μg of menadione/mL, and 1 μg of hemin/mL.

Intracellular persistence assay. The intracellular persistence assay was done as described [8] with some modifications. Aortic bovine endothelial cell monolayers were grown in Dulbecco’s modified Eagle medium (DMEM; Sigma Aldrich) with 10% bovine calf serum in 24-well tissue culture plates (Nunc, Roskilde, Denmark) to confluence (2 × 10⁵ cells/well). Overnight BHI broth cultures of bacteria were washed, adjusted to a concentration of 10⁷ bacteria/mL, and added to the monolayers. The infected monolayers were incubated for 3.5 h at 37°C with 5% CO₂ to allow adhesion and intracellular uptake of the bacteria. Then the monolayers were washed three times with DMEM and 10% calf serum to remove nonattached organisms. Next, 1 mL of DMEM with 10 μg of lysostaphin/mL was added to remove extracellular bacteria. Incubation in the presence of lysostaphin was continued for 20 min, 24 h, and 48 h. At these time points, the monolayers were washed three times with DMEM to remove lysostaphin. Endothelial cells were disrupted by addition of 1 mL of sterile water, and intracellular organisms were released. Aliquots (100 μL) of serial dilutions in sterile water were plated on BHI agar to determine the number of colony-forming units (cfu). The number of intracellular cfu at each time point was determined in duplicate: each point represents the mean of three experiments ± SE.

Statistical analysis. Fisher’s exact test was used to analyze SCV recovery as a function of trimethoprim-sulfamethoxazole therapy in patients of our study group [15]. Intracellular survival was analyzed by t test.

Results
Of the 78 patients, 53 (67.9%) harbored S. aureus in their respiratory specimens; of these 53, 27 (50.9%) had normal S. aureus and 26 (49.1%) had normal plus SCVs, SCVs alone, or pure cultures of normal alternating with pure cultures of SCVs. The median age of patients with SCV and patients with normal S. aureus was similar (13 years [range, 4–35] vs. 10 years [range, 3–40]). While colonization with S. aureus SCVs was demonstrated in 26 CF patients, in 24 patients SCVs were recovered from bronchial secretions and in 2 patients SCVs were recovered from throat swab cultures because of the impossibility of obtaining deep bronchial specimens. In consecutive specimens from 19 of the 26 patients with recovery of SCVs, these variants were isolated over a period of 2–31 months, indicating persistent SCV colonization. Altogether, 78 SCV strains and 79 normal strains of S. aureus could be analyzed with respect to their auxotrophism and clonality.

All SCVs showed a slightly delayed catalase reaction, were coagulase-positive in tube test by 24 h, were identified as S.
S. aureus by Api Staph ID, and agglutinated in Pastorex Staph plus test. The difference of normal and S. aureus SCV is exemplified in table 1 in a subset of SCV and normal S. aureus pairs with identical PFGE profiles recovered from 6 patients with persistent SCV colonization in the same bronchial secretion. The nuc gene was demonstrated by polymerase chain reaction in all SCVs. PFGE-revealed bands of S. aureus and SCV strains with the same profile were considered to be clonal [17]. In 2 patients, PFGE bands of S. aureus DNA were identical, suggesting cross-contamination, whereas all of the other strains differed between patients. Differences in numbers or size of restriction fragments were compatible with one genetic event. In 16 of 19 patients with persistent SCV colonization, restriction profiles of SCV and normal S. aureus from each patient revealed clonality of the strains. In 5 of these patients, in addition to the clonal SCV-normal pair, a second normal or SCV S. aureus strain could be isolated. Two patients lost a persistent SCV strain, which was isolated at the beginning of the study, during 4 and 12 months and started to harbor a second SCV/normal pair for 3 and 26 months, respectively. One patient harbored 3 different S. aureus SCV clones in his respiratory specimens. Three of 19 patients with persistent SCV colonization had only SCVs and no normal S. aureus in their specimens. Restriction profiles of several consecutively cultured normal and SCV S. aureus isolates of 1 of the 19 patients with persistent SCV colonization (patient 3 in table 1) are given in figure 1.

PFGE analysis revealed 38 different S. aureus clones in 26 patients with SCV carriage. By agar diffusion, resistance against penicillin was found in 27 of 38 clones. In addition, all SCV isolates were trimethoprim-sulfamethoxazole–resistant, while the normal isolates (except 1 methicillin-resistant S. aureus clone) were trimethoprim-sulfamethoxazole–susceptible. Four of 38 clones were resistant against gentamicin. The methicillin-resistant isolate was also resistant against erythromycin, lincomycin, gentamicin, netilmicin, and trimethoprim-sulfamethoxazole. Intermediate susceptibility was found in 14 clones for gentamicin, 3 clones for ciprofloxacin, and 2 clones for cefaclor. All other strains tested were susceptible to imipenem, cefazolin, cefotaxime, erythromycin, clindamycin, chloramphenicol, ofloxacin, vancomycin, and teicoplanin. For MIC determination, Etest with blood-containing medium yielded results similar to those obtained by conventional microdilution broth MIC testing using BHI agar, but Etest generally allowed a more discriminatory MIC determination for SCVs and was therefore further used. All normal S. aureus isolates except the methicillin-resistant isolate were trimethoprim-sulfamethoxazole–susceptible (MIC <0.125), while all SCVs were trimethoprim-sulfamethoxazole–resistant (MIC >32). Of 12 SCV-normal strain pairs, 11 of 12 SCVs had higher gentamicin MICs than did their corresponding normal S. aureus strain (table 2).

All 26 patients with SCVs had received trimethoprim-sulfamethoxazole prophylaxis, whereas only 10 of 27 patients with normal S. aureus received this treatment (P < .001). Patients with SCVs were treated longer with trimethoprim-sulfamethoxazole (median, 23.5 months) than were patients without SCVs but with normal S. aureus (median 18 months), but this difference was not significant. After trimethoprim-sulfamethoxazole was changed to another antimicrobial, in 11 patients it was possible to further isolate SCVs (3–32 months).

Table 1. Characteristics of 6 cystic fibrosis patients with persistent S. aureus SCV colonization and their SCV and normal (N) S. aureus strain pairs.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (y)</th>
<th>Clinical score*</th>
<th>Interventional antimicrobial therapy</th>
<th>Phenotype</th>
<th>Persistence of SCVs (months)</th>
<th>Elimination of SCVs</th>
<th>Hemolysis</th>
<th>Pigment</th>
<th>Coagulase</th>
<th>Catalase</th>
<th>Gentamicin MIC</th>
<th>TMP-SMZ MIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>23</td>
<td>30</td>
<td>AMC, IPM-TOB</td>
<td>N</td>
<td>SCV 5</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>4 h</td>
<td>++</td>
<td>0.75</td>
<td>0.094</td>
</tr>
<tr>
<td>2</td>
<td>18</td>
<td>34</td>
<td>AMC, CAZ-TOB</td>
<td>N</td>
<td>SCV 6</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>4 h</td>
<td>++</td>
<td>0.38</td>
<td>0.064</td>
</tr>
<tr>
<td>3</td>
<td>15</td>
<td>85</td>
<td>N</td>
<td>SCV 31</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>24 h</td>
<td>++/–</td>
<td>0.75</td>
<td>&gt;32</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>55</td>
<td>AMC, CAZ-TOB</td>
<td>N</td>
<td>SCV 31</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>24 h</td>
<td>++/–</td>
<td>1</td>
<td>&gt;32</td>
</tr>
<tr>
<td>5</td>
<td>12</td>
<td>65</td>
<td>CEF</td>
<td>N</td>
<td>SCV 31</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>24 h</td>
<td>++/–</td>
<td>4</td>
<td>&gt;32</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>85</td>
<td>N</td>
<td>SCV 25</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>24 h</td>
<td>++/–</td>
<td>1.5</td>
<td>&gt;32</td>
</tr>
</tbody>
</table>

NOTE. Data are for 6 N and SCV S. aureus strain pairs from 6 different patients with persistent SCV colonization, N and SCV isolated at same time with same pulsed-field gel electrophoresis (PFGE) pattern in each strain pair but different PFGE bands in strains from different patients.

* According to Shwachmann score [16].

1 In addition to trimethoprim-sulfamethoxazole (TMP-SMZ) prophylaxis, patients received indicated compounds as combination or sequential interventional therapy; AMC, amoxicillin/clavulanic acid; IPM, imipenem; TOB, tobramycin; CAZ, cefotaxime; CEF, cefaclor.

1 Hemolysis on Columbia agar.

1 Pigment on Columbia agar.

1 Time until positive reaction of tube coagulase test.
Auxotrophism for hemin could be demonstrated in 10, for menadione in 2, and for thymidine in 41 SCV strains. Double auxotrophy for thymidine plus hemin was found in 25 strains. NAD⁺ factor did not influence the growth.

Ingestion of α-toxin–producing S. aureus by endothelial cells results in damage of the endothelial cell monolayer [18]; thus, S. aureus is released to the extracellular milieu and is lysed by extracellular lysostaphin. To determine the intracellular persistence of S. aureus SCV strains with their decreased production of α-toxin compared with normal S. aureus strains, the inraendothelial cell persistence assay was done with a clinical isogenic normal and S. aureus SCV isolate pair. S. aureus isolates were allowed to invade endothelial cells for 3.5 h. After an additional 20 min of incubation in the presence of lysostaphin, subsequent removal of lysostaphin by washing, and disruption of the endothelial cells with sterile water, 0.59%

| Table 2. Comparison of antibiotic susceptibility of 12 isogenic S. aureus strain pairs of SCV and normal (N) phenotype. |
|-----------------|-----------------|------------------|-----------------|-----------------|------------------|------------------|
|                 | SCV              | N                |                 |                 |                 |                 |
|                 | MIC₅₀ | MIC₉₀ | Range          | MIC₅₀ | MIC₉₀ | Range          |
| Piperacillin     | 1      | 6     | 0.064–32       | 1.5   | 8     | 0.19–32        |
| Penicillin       | 0.047  | 12    | 0.016–24       | 0.125 | 16    | 0.016–32       |
| Ceftazidime      | 2      | 3     | 0.5–3          | 4     | 6     | 3–8            |
| Ciprofloxacin    | 0.094  | 0.5   | 0.032–6        | 0.19  | 1     | 0.064–4        |
| Gentamicin       | 1.5    | 3     | 0.25–4         | 0.38  | 0.75  | 0.5–0.75       |
| TMP-SMZ          | >32    | >32   | >32            | 0.032 | 0.125 | 0.004–0.125    |

NOTE. Data are for 12 N and SCV S. aureus strain pairs from 12 different patients with persistent SCV colonization. N and SCV were isolated at same time with same pulsed-field gel electrophoresis pattern for each strain pair.
and 10.00% of normal and S. aureus SCV, respectively, were recovered from the intracellular site. The ratio of viable organisms of SCV versus normal phenotype recovered after 24 h and 48 h of coincubation with lysostaphin was >1000-fold and >100-fold, respectively (figure 2; P < .05, unpaired t test).

Discussion

In this study, for the first time, a high prevalence of S. aureus SCVs in specimens from a defined patient population could be demonstrated. Because of the atypical colony morphology of these variants, prerequisite for this finding was the application of extended conventional culture and identification techniques. Furthermore, it could be demonstrated that S. aureus SCVs from our CF patients exhibit profoundly decreased susceptibility against antimicrobials such as trimethoprim-sulfamethoxazole and aminoglycosides compared with that of isogenic S. aureus isolates with the normal phenotype. Finally, it could be shown that the recovery of S. aureus SCVs in consecutive patient specimens over extended time spans reflects persistence of clonally identical strains and that parallel or consecutive cultures of phenotypically different S. aureus populations occur in spite of clonal identity of the isolates.

SCVs of S. aureus have been well described for >40 years [19, 20], yet their significance has been limited by the fact that their occurrence in clinical specimens has been rarely reported [7]. Renewed interest in S. aureus SCV pathogenicity gave insight into selection for and persistence of S. aureus SCVs in eukaryotic cells [8, 21] and the underlying metabolic alteration [7, 22]. Recently, a site-directed S. aureus hemB mutant was demonstrated to exhibit a typical SCV phenotype, sharing other SCV characteristics, such as aminoglycoside resistance and enhanced intracellular persistence compared with the parent strain [22]. Furthermore, in small yet well-documented case series, the clinical features of persistent and relapsing infection could be directly related to the SCV phenotype [6, 23]. As a result of these in vitro and clinical observations, the following hallmarks of S. aureus SCVs have emerged [7]: S. aureus SCVs are associated with refractory and persistent infection, emergence of S. aureus SCVs is favored by prior exposure to antimicrobials, and the type of phenotypic variation likely reflects impaired respiratory metabolism resulting from alterations in the electron transport system, with the consequence of decreased ATP production and reduced membrane potential.

Normally, patients with S. aureus infections respond to antibiotic therapy, unless the focus of infection has not been drained. In CF, bronchial colonization generally begins during infancy, with S. aureus as one of the first pathogens [24], and often persists throughout the clinical course [25] despite the use of antistaphylococcal therapy on a prophylactic or intermittent basis. The frequent isolation and persistence of S. aureus in our CF patients, as demonstrated by clonal identity in PFGE, confirms these observations, while the high prevalence of SCV isolates adds evidence for a specific role of these variants in chronic staphylococcal disease. It has been argued that S. aureus SCVs may intracellularly persist because of their greatly reduced α-toxin production and thus may escape host defense and antibiotic therapy [8, 18]. Interestingly, all the clinical SCV isolates characterized in this study were phenotypically α-toxin–negative, and their potential for intracellular persistence could be exemplified with 1 SCV-normal S. aureus strain pair. On the other hand, it recently has been demonstrated that uptake of P. aeruginosa by mutant cystic fibrosis transmembrane regulator (CFTR) epithelial cells is decreased in vitro [26]. Accordingly, it has been hypothesized that phagocytosis of bronchial pathogens by these nonprofessional phagocytes may clear bacteria from normal lung, a host defense mechanism possibly impaired in CF. To delineate the role of S. aureus SCV intracellular persistence in CF disease, further studies are necessary to examine the uptake and survival in bronchial epithelial cells.

S. aureus SCVs can be selected by antimicrobials, as shown by exposure of normal S. aureus to subinhibitory concentrations of gentamicin [8]. In vivo, emergence of SCVs has been strongly associated with antibiotic use [6, 7, 23]; however, SCVs may also be isolated after prolonged antibiotic-free intervals [6, 7]. All of our patients with SCVs received trimethoprim-sulfamethoxazole prophylaxis, and 11 of 19 patients with persistent SCV colonization received interventional aminoglycoside therapy. However, and in contrast to previously reported thymidine-dependent S. aureus variants [27], in addition to thymidine auxotrophism, both hemin and menadione auxotrophism were demonstrated in our study. Notably, SCVs were isolated in our patients even after extended trimethoprim-sulfamethoxazole–free intervals (3–31 months) and remained in
vitro as stable SCV phenotype after primary culture and multiple passages in antibiotic-free medium. Hence, while antibiotic exposure of CF patients may contribute to SCV selection or induction, the variants then persist, even in the absence of selective antibiotic pressure. Moreover, after converting into the SCV phenotype, S. aureus from CF patients acquire phenotypic resistance against compounds such as aminoglycosides by decreased antibiotic uptake [28] or against antibiotics by acquiring the ability to use exogenous nucleotide sources (as available in quantity in the pus of CF patients) [27]. This decreased susceptibility may then contribute to clinical persistence despite continued use of compounds such as trimethoprim-sulfamethoxazole.

Considering the special phenotypic SCV characteristics, it is not surprising that almost all SCV isolates reported thus far have been recovered from defined infectious foci, such as soft tissue abscesses or osteomyelitis, or from sterile body fluids [23, 29], while in complex specimens, such as bronchial fluid from CF patients, they are difficult to detect despite their high prevalence. Yet, S. aureus is a significant pathogen in CF [30], contributing to a chronic inflammatory state in the lung tissue with high neutrophil concentrations and resulting enzymatically triggered tissue degradation [1]. Failure to appropriately identify and treat S. aureus is therefore of particular relevance for patients with chronic lung disorders such as CF. Our study strongly suggests that in CF patients with negative culture results for S. aureus obtained under routine culture conditions, these pathogens must be actively sought by use of appropriate selective media and growth conditions. Furthermore, the decreased susceptibility of S. aureus SCVs against antimicrobials typically used for S. aureus prophylaxis or treatment in CF patients requires the identification of these variants even in the presence of normal S. aureus in the specimens.

In conclusion, SCVs of S. aureus can be frequently isolated in CF patients, especially after long-term trimethoprim-sulfamethoxazole or interventional aminoglycoside treatment, suggesting selection or induction of hemin, thymidine, and menadione auxotrophs. These SCVs persist over long periods with or without concomitant normal S. aureus, suggesting that SCVs are important in maintaining persistent S. aureus colonization or infection. While further evaluation of the biochemical and genetic factors resulting in S. aureus SCV populations is warranted for enhanced understanding of their pathogenic role, diagnostic microbiologic procedures for specimens from patients possibly harboring S. aureus SCVs need to be adapted for accurate identification and susceptibility testing.

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References


