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Clinical Characteristics Associated with Isolation of Small-Colony Variants of *Staphylococcus aureus* and *Pseudomonas aeruginosa* from Respiratory Secretions of Patients with Cystic Fibrosis▽

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During a 3-month period, small-colony variant phenotypes of both *Staphylococcus aureus* and *Pseudomonas aeruginosa* were isolated from respiratory secretions of 8.2% and 9.2%, respectively, of 98 patients with cystic fibrosis, particularly those with advanced pulmonary disease and prolonged antibiotic exposure.

Infection of the respiratory tract with *Staphylococcus aureus* and *Pseudomonas aeruginosa* plays an important role in the pathogenesis of cystic fibrosis (CF) (7). Recently, subtypes of *S. aureus* and *P. aeruginosa* termed small-colony variants (SCV) have been isolated from the respiratory secretions of CF patients (3, 5, 12). *S. aureus* SCV have been associated with persistent infections, and they are more resistant to many antibiotics than normal *S. aureus* strains (9, 11, 13). The small, nonpigmented, nonhemolytic colonies, which may not be recovered in the routine clinical microbiology laboratory (10), were found in the respiratory secretions of 26 (33%) of 78 CF patients followed for 3 years (5). Characteristics of *P. aeruginosa* SVC (14, 16) include hyperadherence, enhanced biofilm formation, and increased antibiotic resistance (1, 2, 4, 6). These colonies were recovered from respiratory samples of 33 (38%) of 86 CF patients followed for 2 years (3). We wanted to prospectively assess the presence of SCV of both *S. aureus* and *P. aeruginosa* in respiratory secretions collected from CF patients during a 3-month period and to correlate microbiology findings with important clinical and laboratory parameters.

From 26 July to 29 October, 2000, conventional bacteriologic examinations of all respiratory specimens from our CF patients were complemented by a prospective screen for SCV of both *S. aureus* and *P. aeruginosa*. We examined 148 respiratory samples from 106 patients. Seven patients were excluded from analysis because of incomplete medical records, and one patient whose sputum grew SCV of both species was excluded for statistical reasons. Of the remaining 98 patients, 20 contributed more than one sample (two to six samples). For statistical reasons, we studied one sample per patient: either the first sample obtained or, for patients harboring SCV, the first sample that contained SCV. Clinical data and laboratory results were extracted from patients' charts using a standardized questionnaire. For parameters relating to weight (i.e., weight of an underweight patient as a percentage of predicted weight; the body mass index) and pulmonary function (percentage of predicted forced expiratory volume in 1 s [FEV_1] and partial pressure of arterial oxygen [PaO_2]), the highest value in the year preceding or following sample collection was evaluated. Antibiotic treatment for the preceding 36 months was studied. Patients without SCV served as controls. The study conformed to the policies outlined by the institutional review board of the University Hospital, Inselspital of Bern.

Specimens were cultured on Schädler agar under anaerobic conditions and on mannitol salt agar under aerobic conditions for 48 h at 35°C (5). All visible colonies were subcultured on Columbia agar at 35°C and on Schädler agar at 35°C under 5% CO_2. After 24 h, plates were screened for *S. aureus.* Colonies that were very small, nonhemolytic, and nonpigmented on Columbia agar but grew almost normally on Schädler agar were identified as SCV. Confirmation of *S. aureus* species identity was obtained by tube coagulase and DNase proof tests, followed by a DNA probe test (AccuProbe *Staphylococcus aureus* culture identification test; BioMérieux S.A., Marcy l'Étoile, France). Cultures on Columbia and MacConkey agar at 35°C were screened for *P. aeruginosa* after 24 and 48 h (3). Colonies measuring 1 to 3 mm in diameter after 48 h were subcultured on Columbia agar at 35°C and were identified as SCV if they retained their phenotype in at least two subcultures and were confirmed to be *P. aeruginosa* by gas chromatography (8). Statistical analysis was done in StatView, version 5.0 (SAS Institute Inc., Cary, NC). Differences between means were evaluated by the Mann-Whitney test or the Kruskal-Wallis test, as appropriate. Proportions were compared by Fisher's exact test. A P cutoff of ≤ 0.05 (two-tailed) was used for all analyses.

Eighty-four (86%) of 98 patients were infected with *P. aeruginosa* or *S. aureus* (Table 1). Coinfection with both species was found for 18 patients. SCV were cultured from 17 (17.4%) of 98 patients. *S. aureus* SCV were found in 8 (8.2%) patients, and *P. aeruginosa* SCV in 9 (9.2%) patients. Compared to patients without SCV, patients with SCV of both species had more-advanced pulmonary disease, as evidenced by a lower percentage of predicted FEV_1 ($P = 0.003$) and lower PaO_2 ($P = 0.002$). Those infected with *P. aeruginosa* SCV also had significantly more underweight ($P = 0.02$ for those younger than 18 years; $P = 0.04$ for those older than 18 years). Carriers

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TABLE 1. Comparison between carriers of SCV and of normal colony types of *P. aeruginosa* and *S. aureus*

Clinical parameter[a]	Patients infected with *P. aeruginosa*			Patients infected with *S. aureus*		
	SCV	Normal colony type	*P*	SCV	Normal colony type	*P*
Total no.	9	53		8	32	
Age (yr)	19 (12–23)	16 (1–37)	0.09	16.5 (3–36)	8 (1–40)	0.04
Age at colonization with *P. aeruginosa* (yr)	7.5 (0.6–11.5)	5.0 (0.2–28.1)	0.45			
Age at colonization with *S. aureus* (yr)				4.1 (0.5–16)	4.2 (0.3–32.2)	0.35
Duration of colonization with *P. aeruginosa* (yr)	9.9 (5.5–18.4)	7.9 (0.1–23.4)	0.13	9.04 (0.3–12.9)	3.88 (0.25–16.2)[b]	0.08
Duration of colonization with *S. aureus* (yr)				14.9 (2.5–20.0)	2.4 (0.1–20.1)	0.007
Underweight (% of predicted weight) (<18 yr old)	14.0 (12.5–16.0)	2.7 (0–21.1)	0.02	6.8 (0–12.2)	1 (0–20.1)	0.4
Body-mass index (>18 yr old)	17.9 (13.6–20.0)	19.2 (16.6–21.9)	0.04	19.3 (16.6–21.9)	19.4 (18.2–21.1)	0.9
% of predicted FEV_1	39.0 (21–90)	65.5 (24–125)	0.01	57.5 (21–109)	92.0 (32–122)	0.01
PaO_2 (mm Hg)	64.0 (47.7–72.0)	71.5 (54.6–89.0)	0.004	66.8 (48.5–85.0)	74.0 (62–85)	0.07
Pretreatment with:						
i.v. aminoglycosides						
No. (%)	7 (77.7)	34 (64.1)	0.42	5 (62.5)	6 (18.1)	0.01
Duration (wks)	5 (0–11)	2 (0–16)	0.16	3 (0–16)	0 (0–4)	0.02
Topical aminoglycosides						
No. (%)	7 (77.7)	26 (49.0)	0.11			
Duration (wks)	31 (0–135)	0 (0–138)	0.33			
Cotrimoxazole						
No. (%)				7 (87.5)	11 (33.3)	0.01
Duration (wks)				42.5 (0–138)	0 (0–49)	0.001
Ciprofloxacin						
No. (%)	8 (88.8)	41 (77.3)	0.43			
Duration (wks)	27 (0–49)	6 (0–94)	0.07			

[a] Due to the small number of patients with SCV, summary measures are given as median (range). i.v., intravenous.
[b] $n = 24$. Eight of 32 carriers of the normal phenotype of *S. aureus* were not coinfected with *P. aeruginosa*.

of *S. aureus* SCV were significantly older and had been infected with *S. aureus* longer than those with the normal phenotype. The age at colonization was not different. Carriers of *S. aureus* SCV were pretreated more often and longer with systemic aminoglycosides ($P = 0.02$) and cotrimoxazole ($P = 0.001$) than patients with normal *S. aureus*. In contrast, no significant difference in antibiotic exposure was found between patients with *P. aeruginosa* SCV and those with normal phenotypes.

S. aureus SCV have been isolated particularly from sites of chronic infections of bone, skin, and soft tissue (9, 11, 15), while *P. aeruginosa* SCV have been best studied in CF (3). We report the first attempt to simultaneously isolate SCV of both *S. aureus* and *P. aeruginosa* from CF patients. Compared to a study that included multiple respiratory samples of CF patients followed for 34 months (5), we found a lower proportion of patients positive for *S. aureus* (41.8% versus 67.9%) and a lower proportion of *S. aureus* SCV (19.5% versus 49.1%), a difference that may be explained by our 3-month study period focusing on one respiratory specimen per patient. Similarly, and possibly for comparable reasons, a higher rate of *P. aeruginosa* SCV was found in the respiratory samples of *P. aeruginosa*-positive CF patients (38.4% versus 14.5%) (3). In our cohort, coinfection with SCV of both bacteria was rare. The combined frequency of 17.4% for SCV of *S. aureus* and *P. aeruginosa* in this short-term study indicates that laboratories should be encouraged to actively look, on routine agar plates possibly complemented by Schädler agar, for SCV in CF patients.

In accordance with findings reported for patients infected with *P. aeruginosa* SCV (3), respiratory function tests were significantly worse, and values for arterial oxygenation were lower, for patients from whom SCV of both species were isolated. It remains unknown whether advanced pulmonary damage is a risk factor for the emergence of SCV or whether chronic infection with SCV contributes to the decline in lung function. Further studies will be needed to better define whether and how SCV of both species contribute to the progressive pulmonary damage characteristic of CF. Finding the anatomical and functional niche in the airways occupied by SCV of both species might be an important first step.

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