

# Multicenter Observational Study on Factors and Outcomes Associated with Various Methicillin-Resistant *Staphylococcus aureus* Types in Children with Cystic Fibrosis

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## Abstract

**Rationale:** Methicillin-resistant *Staphylococcus aureus* (MRSA) prevalence continues to increase in patients with cystic fibrosis (CF) in the United States, reaching 26.5% in 2012. Approximately 30% of strains are SCCmec (staphylococcal cassette chromosome mec) IV type, frequently USA300, which in the general population have different genotypic and phenotypic features than SCCmec II type.

**Objectives:** We hypothesized that risk factors for acquisition and outcomes in patients with CF differed for "health care-associated" (SCCmec II) versus "community-associated" (SCCmec IV) MRSA strains.

**Methods:** To determine the role of SCCmec type and Panton-Valentine leukocidin (PVL), MRSA isolates from patients not more than 18 years old at seven CF centers were typed and the association of potential risk factors and subsequent clinical course was assessed, using data provided by the CF Patient Registry.

**Measurements and Main Results:** Participants with chronic MRSA (295) had typeable isolates and clinical data; 205 (69.5%) had SCCmec II PVL(-), 39 (13.2%) had SCCmec IV PVL(-), and 51 (17.3%) had SCCmec IV PVL(+) strains. SCCmec IV, compared with SCCmec II,

increased during the study period, 1996–2010 ( $P = 0.03$ ). SCCmec II was associated with *Pseudomonas aeruginosa*-positive cultures and three or more clinic visits in the 6 months preceding the first positive MRSA culture (adjusted odds ratio, 2.05; 95% confidence interval, 1.13–3.74;  $P = 0.019$ ). Lung function and anthropometrics remained unchanged in the 6 months after initial MRSA detection compared with the 6 months prior. Although CF care increased for participants in both groups in the 6 months after MRSA detection, inhaled antibiotics were prescribed more frequently in those with SCCmec II strains and increased hospitalizations occurred in those with SCCmec IV PVL(-) strains compared with those with PVL(+) strains (adjusted difference, 34.10%; 95% confidence interval, 7.58–60.61;  $P = 0.012$ ). Participants in both groups had an increase in CF care in the 2 years after MRSA detection compared with the 2 years prior.

**Conclusions:** Increased exposure to CF clinics and *P. aeruginosa* may constitute risk factors for acquisition of SCCmec II MRSA strains. Clinical interventions increased 6 months and 2 years after initial MRSA detection regardless of SCCmec type.

**Keywords:** SCCmec type; Panton-Valentine leukocidin; risk factor; methicillin-resistant *Staphylococcus aureus*; clinic visits

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Lung infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) have markedly increased in patients with cystic fibrosis (CF) (1), reaching 26.5% prevalence in the United States in 2012 (2). Chronic MRSA is associated with a more rapid decline in lung function and increased mortality (3, 4). These epidemiologic studies did not account for the genetic lineage of MRSA, which is warranted in patients with CF based on differences in risk factors, clinical presentations, and outcomes in the general population (5).

Methicillin resistance is conferred by the *mecA* gene located within the staphylococcal cassette chromosome (SCC) (6). Several SCCmec types have been described (7), of which SCCmec II is more commonly found in so-called hospital-associated (HA)-MRSA infections, whereas SCCmec IV is more commonly found in the community-associated (CA)-MRSA infections in the United States (8). Differences in the clinical presentations include the invasive/systemic disease typically seen in patients with comorbidities acquiring HA-MRSA (9–12) whereas CA-MRSA typically causes skin and soft tissue infections in previously healthy individuals (13). SCCmec IV strains may also carry the putative virulence factor Pantón–Valentine leukocidin (PVL), which is commonly detected in USA300—the epidemic SCCmec IV strain in the United States (14). In some reports PVL status has been associated with more severe disease (15, 16), including in children with CF (17); however, the role of PVL remains debated (18).

Another key distinction between the SCCmec types is differential antibiotic resistance: SCCmec II encodes resistance to more antibiotic classes whereas the smaller SCCmec IV element encodes fewer antibiotic resistance genes. Thus, SCCmec IV strains are more likely to be susceptible to clindamycin, fluoroquinolone agents, and trimethoprim–sulfamethoxazole

(TMP–SMX). We previously reported similar differences in the antibiotic susceptibilities of MRSA isolates from children with CF; SCCmec II isolates had higher frequencies of resistance to clindamycin and ciprofloxacin compared with SCCmec IV isolates (19).

Among U.S. patients with CF infected with MRSA, about two-thirds harbor SCCmec II strains (20–22). Several studies show that patients remain infected with the same strain for many years; thus, typing at one time point is indicative of past and future MRSA type (22, 23). In a single-center study, we reported that children and adults with CF infected with SCCmec II strains were older, had longer duration of MRSA infection, and were more frequently coinfecting with *Pseudomonas aeruginosa* when compared with patients infected with SCCmec IV strains expressing PVL (24). In addition, in a multicenter study, we showed that pediatric patients with CF chronically infected with SCCmec II strains had a higher rate of treatment with oral antibiotics for protocol-defined pulmonary exacerbations than those with SCCmec IV strains (25). Thus, SCCmec type appears to be associated with different clinical outcomes in patients with CF.

In the current multicenter study, we evaluate risk factors for initial infection with the two MRSA SCCmec types (II vs. IV) among children with CF who remain persistently culture positive. We also evaluate risk factors for and outcomes associated with PVL among those children infected with SCCmec IV PVL(+) versus PVL(–) strains. We hypothesize that children with CF persistently infected with SCCmec II MRSA strains had more contact with the health care environment, before acquisition and/or more severe CF disease than those infected with SCCmec IV MRSA strains. In turn, we examine clinical outcomes after initial infection to evaluate whether SCCmec type and PVL status are related to subsequent changes in disease course.

## Methods

### Study Design, Sites, and Participants

We performed an observational, multicenter study to assess clinical characteristics and potential risk factors associated with acquisition of various MRSA strains based on SCCmec typing and the presence of the PVL gene. Seven geographically diverse, Cystic Fibrosis Foundation (CFF)–accredited pediatric centers, each treating more than 200 children and adolescents and having at least 20% MRSA prevalence, participated in this study. Eligible participants with CF were not more than 18 years of age and had two or more cultures positive for MRSA within at least one of the 2 years preceding enrollment. Participants in whom no MRSA-positive culture could be obtained at enrollment/consent into this study and/or at two subsequent CF clinic visits were excluded from analyses (Figure 1). Patients who had undergone lung or liver transplantation were not eligible to participate. Each center's institutional review board approved the study. Written consent was obtained from parents, and assent was obtained from children old enough to read.

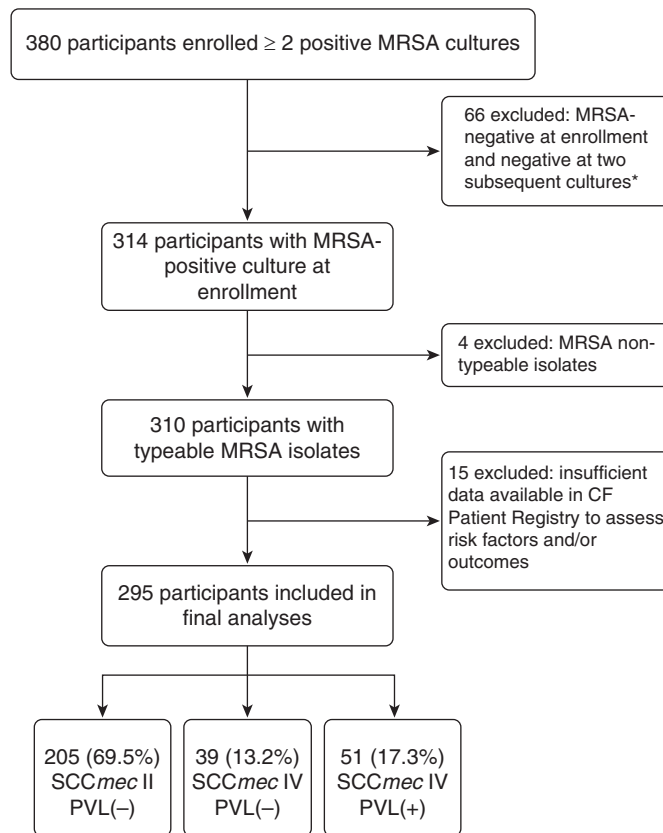
### Typing of MRSA Isolates

Participant respiratory cultures (deep pharyngeal swab, sputum, or bronchoalveolar lavage) were processed at each site's clinical microbiology laboratory according to CFF guidelines (26). Three colonies of MRSA from the same culture were sent to the core laboratory at the University of North Carolina (Chapel Hill, NC), where the SCCmec type and presence of the PVL gene were determined by PCR methods as previously described (22, 27).

### Data Collection

Data from the U.S. CFF Patient Registry were obtained from January 1996 through December 2012 and included demographic characteristics, cystic fibrosis

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**Figure 1.** Flow diagram of participants included in final analyses. \*Participants were excluded from further analysis as this study focused on those with persistently positive methicillin-resistant *Staphylococcus aureus* (MRSA) cultures. Participants who did not provide a positive MRSA culture did not differ from eligible participants for *Pseudomonas aeruginosa* or lung function, but had fewer MRSA-positive cultures overall (42).

transmembrane conductance regulator (CFTR) mutations, microbiology results, number of clinic visits, chronic CF medications, treatment with intravenous (IV) antibiotics, spirometry results, and growth parameters. Spirometry was ascertained in children at least 6 years old and expressed as FEV<sub>1</sub> % predicted (28, 29). Weight for length in children less than

2 years of age, or body mass index (BMI) percentiles in children 2 years of age or older, were calculated on the basis of Centers for Disease Control and Prevention reference values (30, 31). Hospital admissions (for any reason) and treatment with IV antibiotics in the home have been available in the CFF Patient Registry since 2003.

### Statistical Analyses

Year of MRSA acquisition during our study was stratified into three periods based on changes in MRSA prevalence and the emergence of USA300 (8, 14). Demographic and clinical characteristics were described using summary statistics and compared among participants with different SCCmec types and PVL status, using two-sided *t* tests for continuous variables or Fisher's exact test for categorical variables. Values for FEV<sub>1</sub> % predicted and BMI (ages ≥2 yr) or weight for length (<2 yr of age) were averaged over 2 years. Microbiologic factors included detection of "any" methicillin-susceptible *S. aureus* (MSSA) or *P. aeruginosa*, and chronic infection with MSSA or *P. aeruginosa* defined as more than 50% of cultures positive in the year before MRSA detection (32).

To assess short-term (i.e., within 6 mo) risk factors for acquisition and clinical outcomes of various MRSA types, chronic CF medications, clinic visits, hospitalizations, and treatment with intravenous antibiotics were compared among participants with different SCCmec types and PVL status. Paired summaries of the change from 6 months before and the 6 months after MRSA acquisition, and 95% confidence intervals (CIs) were reported. To test whether changes differed by SCCmec type or PVL status, an analysis of covariance was performed, adjusted for sex, age, and year of MRSA acquisition, study site, and presence of any *P. aeruginosa*. To test our primary hypothesis, a multivariate logistic regression model was fit to estimate the odds of acquisition of SCCmec MRSA type by health care exposures, that is, number of CF clinic

**Table 1.** Year of initial positive isolate by methicillin-resistant *Staphylococcus aureus* SCCmec type and Pantón–Valentine leukocidin status

Year of Initial Detection	SCCmec II (n = 205)	SCCmec IV (n = 90)	P Value	IV PVL (-) (n = 39)	IV PVL(+) (n = 51)	P Value
1996–2002	45 (22.0%)	9 (10.0%)	<b>0.002*</b>	7 (18.0%)	2 (3.9%)	<b>0.033*</b>
2003–2006	97 (47.3%)	35 (38.9%)		17 (43.6%)	18 (35.3%)	
2007–2010	63 (30.7%)	46 (51.1%)		15 (38.5%)	31 (60.8%)	

Definition of abbreviation: PVL = Pantón–Valentine leukocidin; SCCmec = staphylococcal cassette chromosome *mec*.

Boldface indicates significance at the 0.05 level.

Proportion of cultures and their distribution over the years of study are shown.

\*P value for Fisher's test of distributional differences between SCCmec types and PVL status.

visits or hospitalizations, adjusted for the aforementioned confounding variables.

Similarly, to assess longer term outcomes (i.e., within 2 yr) associated with SCCmec type and PVL status, the aforementioned variables were summarized as described above for participants in the 2 years before and after MRSA acquisition. In addition, changes in FEV<sub>1</sub>% predicted and BMI/weight-for-length percentiles were assessed. All analyses were performed with SAS version 9.2 (SAS, Cary, NC) and two-sided *P* values are reported.

## Results

### Study Population and MRSA Type

From October 2008 to April 2010, 380 potentially eligible participants with a history of MRSA infection were enrolled. After exclusion of 85 participants as detailed in Figure 1, 295 participants were included in this study. Distribution of SCCmec types and PVL status is shown in Table 1. The proportion of participants with SCCmec IV strains was lowest among those with initial MRSA detection from 1996 to 2002 and increased over time; 51% of all SCCmec IV strains were detected from 2007 to 2010 (Table 1). Similarly, the presence of the PVL gene among SCCmec IV strains increased over time; from 1996 to 2002, 3.9% of strains were PVL(+) whereas from 2007 to 2010, 60.8% of strains were PVL(+).

### Characteristics Associated with Acquisition of Various MRSA Types

The mean age for all participants at the time of their first MRSA-positive culture was 7.0 (SD 4.2) years and was similar among participants with different SCCmec types. The distributions of age, sex, and CFTR genotypes were also similar among those who acquired different SCCmec types; 56.1% of those with SCCmec II versus 47.8% of those with SCCmec IV MRSA strains were F508 del homozygote.

In the 2 years before the first MRSA-positive culture, anthropometric percentiles were similar for those who acquired SCCmec II versus SCCmec IV (Table 2). Lung function, available for 110 participants with SCCmec II and for 44 with SCCmec IV isolates, was also similar in the two groups.

In the 6 months before first MRSA-positive culture, more participants who

acquired SCCmec II strains had at least one *P. aeruginosa*-positive culture (Table 2). However, the proportion of participants with chronic *P. aeruginosa* did not differ by SCCmec type. Differences in *P. aeruginosa*-positive cultures were not secondary to more frequent assessment as the number of cultures was similar for participants with SCCmec II versus SCCmec IV isolates (mean [SD], 3.1[1.7] vs. 3.0 [1.9]).

Among participants with SCCmec IV, none of the above-described characteristics

were associated with the PVL gene (data not shown).

In the 6 months before first MRSA-positive cultures, the use of chronic CF medications and days of intravenous antibiotic treatment of pulmonary exacerbations were similar in the two groups (Table 2). However, a greater proportion of participants who acquired SCCmec II strains had three or more CF clinic visits compared with those who acquired SCCmec IV. This observation persisted when adjusted for the covariates

**Table 2.** Demographic and clinical characteristics associated with various methicillin-resistant *Staphylococcus aureus* types in children with cystic fibrosis

Characteristic	SCCmec II (n = 205)	SCCmec IV (n = 90)	<i>P</i> Value
Age group, yr: n (%)			
<3	42 (20.5%)	16 (17.8%)	0.86*
3 to <6	42 (20.5%)	21 (23.3%)	
6 to <10	87 (42.4%)	36 (40.0%)	
≥10 to 18	34 (16.6%)	17 (18.9%)	
Sex, n (%)			
Female	106 (51.7%)	39 (43.3%)	0.21
Health status 24 mo before MRSA: n, mean (SD)			
FEV <sub>1</sub> % predicted <sup>†</sup>	110, 89.1 (16.8)	44, 84.6 (20.4)	0.16
BMI or weight/height percentile <sup>‡</sup>	196, 44.4 (27.0)	80, 44.9 (27.2)	0.90
Microbiology before MRSA acquisition, n (%)			
MSSA any (6 mo prior)	58 (28.3%)	22 (24.4%)	0.57
MSSA chronic <sup>§</sup> (1 yr prior)	35 (17.1%)	16 (17.8%)	0.87
<i>P. aeruginosa</i> any (6 mo prior)	96 (46.8%)	27 (30.0%)	<b>0.007</b>
<i>P. aeruginosa</i> chronic <sup>§</sup> (1 yr prior)	61 (29.8%)	18 (20.0%)	0.088
CF medications 6 mo before MRSA acquisition			
Chronic use of inhaled antibiotics, n (%)	72 (35.1%)	25 (27.8%)	0.23
Chronic use of DNase, n (%)	67 (32.7%)	30 (33.3%)	0.99
Chronic use of macrolides, n (%)	34 (16.6%)	12 (13.3%)	0.60
Days of IV antibiotics per IV course <sup>  </sup> ††: n, mean (SD)	47, 13.4 (8.1)	21, 10.2 (6.7)	0.12
Health care exposure 6 mo before MRSA acquisition			
CF clinic visits, n (%)			
0	20 (9.8%)	18 (20.0%)	<b>0.007</b>
1 or 2	108 (52.7%)	52 (57.8%)	
≥3	77 (37.5%)	20 (22.2%)	
≥1 hospitalization, <sup>  </sup> n (%)	44 (27.5%)	20 (24.7%)	0.76
Hospital days per admission <sup>  </sup> ††: mean (SD)	9.0 (6.8)	8.0 (5.5)	0.57

*Definition of abbreviations:* BMI = body mass index; CF = cystic fibrosis; IV = intravenous; MRSA = methicillin-resistant *Staphylococcus aureus*; MSSA = methicillin-susceptible *Staphylococcus aureus*; *P. aeruginosa* = *Pseudomonas aeruginosa*; SCCmec = staphylococcal cassette chromosome *mec*. Boldface indicates significance at the 0.05 level.

\**P* value for Fisher's test of distributional differences between SCCmec types.

<sup>†</sup>For participants 6 years of age or older.

<sup>‡</sup>Weight/height percentiles in children less than 2 years of age and BMI percentiles in those 2 years of age and older.

<sup>§</sup>Chronic defined as greater than 50% of cultures in 12 months positive for the pathogen.

<sup>||</sup>Evaluable in n = 160 SCCmec II and n = 81 SCCmec IV with data after 2003.

<sup>††</sup>Combined inpatient and outpatient intravenous therapy.

\*\*Mean and SD reported among those with event/hospitalization (excluding 0s).

(adjusted odds ratio, 2.05; 95% CI, 1.13–3.74;  $P = 0.019$ ). The frequency of hospitalization and days of hospitalization were not associated with SCCmec type when adjusted for covariates (adjusted odds ratio, 0.99; 95% CI, 0.52–1.89;  $P = 0.97$ ).

### Changes in Clinical Parameters from 6 Months before to after Detection of Various MRSA Types

The association of SCCmec II versus SCCmec IV MRSA with changes in clinical care and outcomes from 6 months before to 6 months after initial MRSA detection is shown in Table 3. Differences between the two groups, adjusted for covariates, are also shown. The use of chronic CF medications increased within both groups, with the exception that the use of inhaled antibiotics remained unchanged in those with SCCmec IV strains but increased significantly in those with SCCmec II strains. The greatest percent increase occurred for use of DNase for both groups. The number of CF clinic visits increased regardless of SCCmec type.

*P. aeruginosa*-positive cultures, hospitalizations, and duration of hospitalization were similar before and after MRSA detection and were similar by SCCmec type. There were no significant changes in lung function or anthropometric parameters.

Among participants with SCCmec IV, those with PVL(-) strains had a significant increase in the use of DNase (15.38%; 95% CI, 1.4, 29.37) and the chronic macrolide agents azithromycin and clarithromycin (12.82%; 95% CI, 1.84, 23.8). In contrast, those with PVL(+) strains had a significant increase in the use of inhaled antibiotics (7.84%; 95% CI, 0.21, 15.48). However, none of these differences were significant between the groups when adjusted for covariates. Participants having at least one hospitalization significantly increased among those with PVL(-) (17.95%; 95% CI, -0.07, 35.97) compared with those with PVL(+) strains (-9.80%; 95% CI, -24.96, 5.35) and this difference remained significant after adjusting for covariates (adjusted difference, 34.10%; 95% CI, 7.58, 60.61;  $P = 0.012$ ).

### Changes in Clinical Parameters from 2 Years before to after Detection of Various MRSA Types

Lung function, but not anthropometric measures, significantly declined in the 2 years after MRSA detection compared with the 2 years prior, regardless of SCCmec status (Table 4). Similarly, CF medication use and CF clinic visits also significantly increased in all participants compared with 2 years prior. In contrast, hospitalizations and IV antibiotic days per IV course significantly increased only for those with SCCmec II strains, and *P. aeruginosa*-positive cultures increased significantly in those with SCCmec IV strains. Whereas the number of cultures increased in each group, similar numbers of cultures were obtained before and after detection of MRSA; the mean (SD) cultures/participant/24 months was 5.4 (3.3) versus 5.3 (3.9) for those with SCCmec II versus SCCmec IV strains, respectively ( $P = 0.98$ ) before MRSA detection and 8.6 (3.9) versus 9.1 (3.8) for those with SCCmec II versus SCCmec IV strains, respectively ( $P = 0.30$ ) after MRSA acquisition. None of the changes that occurred within SCCmec type were

**Table 3.** Changes in clinical parameters 6 months before compared with after detection of various methicillin-resistant *Staphylococcus aureus* types

	Change within SCCmec II (n = 205)			Change within SCCmec IV (n = 90)			Adjusted* Difference between SCCmec Type (SCCmec II–SCCmec IV)		
	n	Mean	95% CI	n	Mean	95% CI	Mean Difference	95% CI	P Value
<b>Health status</b>									
FEV <sub>1</sub> % predicted	102	-0.1	-2.23, 2.04	34	-1.45	-5.15, 2.25	2.49	-2.26, 7.25	0.30
BMI or weight/stature percentile	180	-0.56	-2.74, 1.62	68	2.87	-0.68, 6.41	-2.48	-6.78, 1.82	0.257
<b>CF medications</b>									
Chronic inhaled antibiotics	205	17.56%	<b>9.75, 25.37</b>	90	4.44%	-1.76, 10.65	12.87%	-0.44, 26.19	0.058
Chronic DNase		18.05%	<b>11.35, 24.75</b>		11.11%	<b>3.14, 19.08</b>	7.50%	-4.62, 19.62	0.22
Chronic azithromycin		8.29%	<b>4.25, 12.34</b>		7.78%	<b>2.14, 13.42</b>	0.00%	-7.43, 7.31	0.98
Days of IV antibiotics per IV course <sup>††</sup>	160	2.62	<b>0.18, 5.06</b>	81	2.06	-0.51, 4.63	1.48	-2.59, 5.54	0.48
<b>Microbiology</b>									
Any <i>P. aeruginosa</i>	205	-1.46%	-7, 4.07	90	-1.11%	-8.47, 6.25	-4.75%	-14.09, 4.59	0.32
<b>Health care exposure</b>									
CF clinic visits	205	0.84	<b>0.52, 1.16</b>	90	1.00	<b>0.44, 1.56</b>	-0.030	-0.65, 0.60	0.94
≥1 hospitalization	205	6.83%	-0.19, 13.85	90	2.22%	-9.52, 13.96	4.16%	-9.78, 18.11	0.56
Days inpatient per hospitalization	160	0.72	-0.35, 1.78	81	1.02	-0.87, 2.92	-0.39	-2.53, 1.75	0.72

**Definition of abbreviations:** BMI = body mass index; CF = cystic fibrosis; CI = confidence interval; IV = intravenous; MRSA = methicillin-resistant *Staphylococcus aureus*; *P. aeruginosa* = *Pseudomonas aeruginosa*; SCCmec = staphylococcal cassette chromosome *mec*.

Changes are shown for characteristics before compared with after MRSA by SCCmec types and adjusted changes. Boldface indicates significance at the two-sided 0.05 level.

\*Comparisons by SCCmec type are adjusted for site, sex, age, year of MRSA onset, and *P. aeruginosa* during the 6 months after MRSA acquisition.

<sup>†</sup>Evaluable in n = 160 SCCmec II and n = 81 SCCmec IV with data after 2003.

<sup>††</sup>Combined inpatient and outpatient intravenous therapy.

**Table 4.** Changes in clinical parameters 2 years before, compared with after, detection of various methicillin-resistant *Staphylococcus aureus* types

	Change within SCCmec II (n = 205)			Change within SCCmec IV (n = 90)			Adjusted* Difference between SCCmec Type (SCCmec II–SCCmec IV)		
	n	Mean	95% CI	n	Mean	95% CI	Mean Difference	95% CI	P Value
Health status									
FEV <sub>1</sub> % predicted	110	−2.40	<b>−3.99, −0.72</b>	43	−2.64	<b>−5.29, −0.01</b>	−0.17	−4.50, 4.17	0.94
BMI or weight/stature percentile	196	0.22	−2.00, 2.43	80	1.32	−2.19, 4.83	−0.022	−5.74, 5.69	0.99
CF medications	205			90					
Chronic inhaled antibiotics		32.20%	<b>24.56, 39.83</b>		14.44%	<b>2.88, 26.01</b>	12.31%	−7.10, 31.72	0.21
Chronic DNase		27.32%	<b>21.02, 33.62</b>		25.56%	<b>15.85, 35.26</b>	11.16%	−3.62, 25.94	0.14
Chronic macrolides		24.88%	<b>18.76, 31.00</b>		21.11%	<b>12.52, 29.71</b>	14.27%	−1.51, 30.07	0.076
Days of IV antibiotics per IV course <sup>†‡</sup>	160	1.66	<b>0.09, 3.23</b>	81	2.45	−0.57, 5.47	−0.60	−4.53, 3.33	0.76
Microbiology	205			90					
Any <i>P. aeruginosa</i>		1.00%	−7.09, 9.04		17.78%	<b>6.64, 28.91</b>	−12.25%	−27.76, 3.25	0.12
Health care exposure									
CF clinic visits	205	4.23	<b>3.38, 5.08</b>	90	5.44	<b>3.56, 7.31</b>	−0.49	−3.07, 2.10	0.71
≥1 Hospitalization	205	12.7%	<b>4.8, 20.6</b>	90	7.8%	−5.5, 20.0	3.2%	−11.7, 18.0	0.67
Days inpatient per hospitalization	160	0.92	−0.23, 2.08	81	2.19	−0.47, 4.85	−1.82	−4.90, 1.26	0.25

Definition of abbreviations: BMI = body mass index; CF = cystic fibrosis; CI = confidence interval; IV = intravenous; MRSA = methicillin-resistant *Staphylococcus aureus*; *P. aeruginosa* = *Pseudomonas aeruginosa*; SCCmec = staphylococcal cassette chromosome *mec*.

Changes are shown for characteristics 2 years before compared with 2 years after MRSA per SCCmec type. Boldface represents significance at the two-sided 0.05 level.

\*Comparisons by SCCmec type are adjusted for site, sex, age, year of MRSA onset, and *P. aeruginosa* during the 24 months after MRSA acquisition.

<sup>†</sup>Evaluable in n = 160 SCCmec II and n = 81 SCCmec IV with data after 2003.

<sup>‡</sup>Combined inpatient and outpatient intravenous therapy.

significantly different between the two SCCmec types, adjusted for covariates.

In participants with SCCmec IV, changes in clinical parameters in the 2 years before versus 2 years after MRSA acquisition were similar in those with and without the PVL gene and remained similar when adjusted for covariates (data not shown). Whereas those with PVL(−) versus PVL(+) strains had a similar proportion of *P. aeruginosa*-positive cultures, those acquiring PVL(+) isolates had more cultures taken in the 2 years before MRSA detection; the mean (SD) cultures/participant/24 months for those with PVL(−) versus PVL(+) strains was 4.2 (2.9) versus 6.2 (4.5), respectively ( $P = 0.02$ ). The number of cultures increased after MRSA detection in those with either PVL(−) and PVL(+) strains; the mean (SD) cultures/participant/24 months for those with PVL(−) versus PVL(+) strains was 8.9 (3.8) versus 9.2 (3.8), respectively ( $P = 0.67$ ).

## Discussion

In this multicenter study, initial detection of MRSA in children and adolescents with CF occurred at 7 years of age and was similar

among those with different SCCmec types; 20% of participants were less than 3 years of age at the time of their first MRSA-positive culture. The proportion of SCCmec IV strains, both PVL(−) and PVL(+), increased from 1996 to 2010. The increase in SCCmec IV types during this time interval is consistent with the increase in community-associated (SCCmec IV) MRSA infections in the general U.S. population (8, 33). Our prior study in this CF study population has shown that the SCCmec IV isolates are usually USA300 (19). Further, despite some geographic variation of MRSA prevalence within the United States among non-CF patients (34), we found that the distribution of SCCmec II versus SCCmec IV strains did not differ by region (19).

We explored the association of various SCCmec types with CF health status within the 2 years before detection of MRSA and with health care exposures and antibiotic use within the 6 months before detection. These two time intervals were selected to assess various risk factors for MRSA (13, 35, 36); underlying health status often shows slower changes over time in contrast to the short-term health care exposures that could result in acquisition of HA-MRSA,

that is, SCCmec II strains. Furthermore, both exposure and outcome can be measured in this shorter interval as patients with CF are seen at regular intervals and need to produce respiratory cultures frequently. Although CF health status was not associated with acquisition of a specific MRSA type, *P. aeruginosa*-positive cultures and an increased number of CF clinic visits were associated with SCCmec II. Acquisition of *P. aeruginosa* has been associated with more clinic visits in newborn screening studies (37, 38), and *P. aeruginosa* is transmissible from CF patient to CF patient (39). At present, it remains unclear whether CF clinic visits and/or *P. aeruginosa* are risks for acquiring SCCmec II MRSA.

Outcomes after MRSA acquisition showed a general intensification of care among all participants as evidenced by an increased number of clinic visits and use of CF medications. Of note, SCCmec type and PVL status were not known to the treating physicians in this study, and thus MRSA type did not influence treatment decisions. However, the use of inhaled antibiotics and more IV antibiotic days increased only in participants with SCCmec II strains within 6 months of MRSA acquisition. Both of

these observations may reflect the higher prevalence of *P. aeruginosa* in this group, and the latter could be explained by the higher rate of antibiotic resistance genes in SCCmec II isolates requiring IV antibiotics. Overall, similar trends in care intensification were noted when the observation time was extended to 2 years. We can only speculate whether intensification of care reflects a response to progression of CF lung disease as evidenced in the decline in lung function observed in both groups, or is related to patient and clinician concerns after detection of MRSA. Others have reported initiation of CF therapies not directly targeting MRSA, use of inhaled tobramycin, was higher in subjects with MRSA compared with MSSA despite similar prevalence of *P. aeruginosa* (40, 41).

Our findings that participants with PVL(-) isolates had more hospital admissions within the 6 months after MRSA detection than those with PVL(+) isolates are somewhat at odds with prior reports of acute necrotizing pneumonia in children with CF (17) or previously healthy people (15, 16).

In the current analyses we lack information on the use of oral antibiotics targeting MRSA as these data have been available in the CF Patient Registry only since 2012. However, we previously demonstrated that those participants with longstanding SCCmec II MRSA infection had more exacerbations and increased use of oral antibiotics compared with those with SCCmec IV MRSA (25). We suggest that differences in study design may explain the seemingly different results in the current study compared with our prior study that prospectively evaluated exacerbations in the same participants. These differences include the duration of

MRSA infections, that is, 3–4 years in the prior study versus the maximum 2 years in the current study and the outcome measures assessed, that is, symptoms and use of oral antibiotics versus the variables available in the CF Patient Registry. Future multicenter, prospective studies would be necessary to further evaluate the association of MRSA-specific factors, underlying disease severity, and clinical care practices with patient outcomes.

Although we cannot prove causality in this epidemiologic study, the higher rate of clinic visits and *P. aeruginosa*, which is also transmissible, could imply a health care-related source of infection for those with SCCmec II and thus argue for strict infection control policies. Combined with our prior observation of more exacerbations in subjects with chronic SCCmec II MRSA compared with those with SCCmec IV, knowledge of SCCmec status may be beneficial both regarding epidemiologic/risk assessment and subsequent treatment decisions. Again, firm conclusions should be made cautiously, based solely on epidemiologic studies; however, the higher use of antibiotics seen in our prior study and other risks may indicate that MRSA-specific therapies should be intensified in patients acquiring SCCmec II isolates.

There are limitations to this study. Although this is a multicenter study, it is unknown whether our findings are generalizable to all patients with CF, including adults, or to those with transient MRSA. Lung function is not reliably tested in children less than 6 years of age, but the proportion of participants without lung function data was similar across MRSA types. Hospitalizations and intravenous antibiotic treatment were not recorded before 2003. These missing data affect

power, but are unlikely to cause bias. Although the ability to use data in the Registry since 1996 can enhance participant inclusion and follow-up, it is likely that changes in medical management that occurred over time can influence outcomes. Finally, being an exploratory study, adjustment for multiple testing was not performed, and therefore spurious findings cannot be ruled out.

In conclusion, we demonstrate that first detection of MRSA occurred in early childhood among those who go on to have persistent infection. Presence of any *P. aeruginosa*-positive culture and an increased number of CF clinic visits 6 months before initial MRSA detection adjusted for *P. aeruginosa* status were potential risk factors associated with acquisition of SCCmec II MRSA implicating patient and care-related risks. There was no increased morbidity in those with PVL(+) isolates. Short-term changes after initial MRSA detection showed increases in multiple chronic medications and clinic visits for all participants and, when adjusting for covariates, was similar among the various SCCmec types. To better delineate changes that occur during transition from early to chronic MRSA infection, translational and ideally prospective studies that study bacterial changes and host factors will be necessary. ■

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