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Molecular Epidemiology of Methicillin-Resistant *Staphylococcus aureus* in Cystic Fibrosis Patients from Argentina

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Methicillin-resistant *Staphylococcus aureus* (MRSA) colonization in cystic fibrosis (CF) patients is an increasing problem in many countries. In our Respiratory Center at the Hospital de Niños ''Dr. Ricardo Gutiérrez'', Buenos Aires, Argentina, the prevalence has climbed from 23% in 1995 up to 32% in 2011. Our objective was to analyze the diversity of MRSA isolates recovered from respiratory samples of CF patients attending our center, characterizing their phenotypes and clonal distribution. Therefore, a prospective study was conducted on all CF patients attending the pediatric Respiratory Center between June 2012 and May 2013 to collect MRSA isolates. Antibiotic susceptibility testing, multilocus sequence typing, pulsed-field gel electrophoresis, *spa* typing, and *agr* genotyping were performed on collected isolates. The prevalence of MRSA during this period was 34.2%, and 71.9% of the patients were infected with isolates that carried SCC*mec* IV. High resistance rates were detected for gentamicin, erythromycin, clindamycin, ciprofloxacin, and rifampicin. Strains related to the community-associated MRSA clones, ST5-IV and ST30-IV, were the most frequently recovered. Remarkably, even though most of the isolates were related to these clones, the rate of multi-resistance shown in CF patients was higher than that reported for the same lineages recovered from other infections in our country.

Keywords: MRSA-ST5-IV, MRSA-ST30-IV, multidrug resistance, cystic fibrosis

Introduction

Cystic fibrosis (CF) is one of the most common autosomal inherited conditions in the Caucasian population, with an incidence rate varying across the globe and being around 1/6,100 newborns in Argentina.[1]

CF patients suffer from recurrent pulmonary infections that lead to progressive pulmonary damage and respiratory failure, which are the main causes of morbidity and mortality among these patients.[2] *Staphylococcus aureus* is an early and commonly observed pathogen that is associated with CF lung infection.[3,4]

During chronic infection, *S. aureus* uses multiple strategies to adapt to the airways of CF patients.[5] Of significant importance is the formation of small colony variants (SCVs). The switch to this particular phenotype at a relatively high rate may offer a survival advantage because they can persist intracellularly and evade the host immune system. Moreover, SCVs exhibit more tolerance against treatment while located within host cells.[6]

In the past decades, the number of infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) has increased considerably in the general population. According to the data published by the World Health Organization in 2014, the methicillin resistance rate is more than 50% in our country.[7] This pattern has also been observed in CF patients.[3] In our center, the prevalence of MRSA infection in the CF population has climbed from 23% in 1995 up to 32% in 2011 (Galanternik, personal communication).

Even though MRSA was initially recognized as being acquired from hospitalized patients (HA-MRSA), infections due to community-associated strains (CA-MRSA) have been reported with an increasing frequency since 1990.[8] CA-MRSA strains are genetically distinct from traditional HA-MRSA

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clones. The former are associated with SCC*mec* IV or V, small SCC*mec* that usually do not carry non-β-lactam resistance genes representing a lower fitness cost, and are associated with the carriage of Panton-Valentine leukocidin (PVL); the latter are associated with SCC*mec* I, II, or III and usually carry additional antimicrobial resistance genes.[9] Since the beginning of the 21st century, different studies worldwide have described typical CA-MRSA lineages as a cause of outbreaks in the healthcare setting and they appear to be replacing classical HA-MRSA clones in the hospital environment.[10,11] The evolution of MRSA epidemiology has made the definition of CA-MRSA and HA-MRSA blurry.[12,13]

The information about MRSA lineages associated with CF is scarce in our country; nonetheless, several studies have described MRSA clones related to non-CF patients. Two different studies found MRSA ST5-IV as the prevalent clone in healthy children suffering community-onset MRSA infections.[14,15] In addition, nationwide studies of *S. aureus* infections showed that the two major clones responsible for community-onset infections were ST30-IV and ST5-IV, being the first one found mainly in adults and the second one found in children.[10,16] On the other hand, the majority of the healthcare-associated infections were related to the Cordobes/Chilean clone, ST5-I.[10] This situation makes it reasonable to suppose that these clones could also be recovered from CF patients.

The aim of this study was to analyze the diversity of MRSA isolates recovered from respiratory samples of CF patients in a pediatric center in Argentina, characterizing their phenotypes and clonal distribution.

Materials and Methods

Study design

A prospective observational study was designed to evaluate the prevalence, phenotypic and molecular features of MRSA isolates recovered from respiratory samples from all CF patients up to 18 years of age attending the pediatric Respiratory Center at the Hospital de Niños ''Dr. Ricardo Gutiérrez'' between June 2012 and May 2013. The Respiratory Center of this hospital functions as a national reference center for the treatment of CF patients and currently it is the center with the greatest number of CF patients in Argentina. The study was reviewed and approved by the Research Ethics Committee of the institution. Written informed consent was signed by the parents of the patients to participate in this study.

Patients and isolate selection criteria

Patient inclusion criteria involved confirmed diagnosis (two sweat test positive or two cystic fibrosis transmembrane conductance regulator [CFTR] causing mutations) and at least one positive culture of MRSA during that year. Patients with poor adherence or less than four sputum samples per year were excluded. Data collected included patient demographics, lung function, CFTR mutations, microbiology results, number of hospitalizations during the study period, and CF-related comorbidities.

One MRSA isolate was collected per patient. Additional isolates were collected in those cases in which different phenotypes, considering antibiotic resistance profile, were recovered in the same or consecutive sputum samples.

Microbiology studies

Isolates were identified as *S. aureus* by typical morphology on mannitol salt agar (BioMérieux) and positive results for tube coagulase and DNase tests. Antibiotic susceptibility was analyzed by automatized Vitek 2 (BioMérieux). For those SCVs, rejected by the Vitek system and unable to grow in Mueller Hinton agar or Mueller Hinton agar supplemented with 5% sheep blood, antibiotic susceptibility was evaluated by using the disk diffusion method on Columbia blood agar. Multidrug resistance (MDR) was defined as resistance to three or more antibiotic families.

PCR amplification of mec*A,* lukS/F-PV*,* agr*, and SCC*mec *typing*

After phenol-chloroform extraction of genomic DNA, detection of *mec*A and PVL encoding genes (*lukS/F-PV*) was performed as previously described.[16,17] The *agr* locus was genotyped by multiplex PCR as described elsewhere.[18] SCC*mec* types were also determined by PCR with a simplified version of Kondo's typing system, including M-PCR-1 and M-PCR-2.[19]

Genetic analysis by pulsed-field gel electrophoresis, spa *typing, and multilocus sequence typing*

Genotyping analysis was conducted by using *spa* typing and pulsed-field gel electrophoresis (PFGE) with *Sma*I as previously described.[16] Comparison of the PFGE fingerprints was performed with the BioNumerics software, version 5.1 (Applied Maths, Kortrijk, Belgium). The definition of a PFGE type was based on a similarity cut-off of 75% (Dice coefficient, represented by the unweighted pair group method using arithmetic averages [UPGMA], 1% optimization, and 1% tolerance). HA-MRSA and CA-MRSA clones previously described in Argentina were included in the PFGE pattern analysis.[16,20,21] Representative isolates of the major pulsotypes were studied by multilocus sequence typing (MLST).[22]

Statistical analysis

GraphPad Prism 7 for Mac OS X (v7.0c) was used for statistical analysis. Categorical variables were analyzed by Fisher's exact test. A p value <0.05 was considered statistically significant.

Results

Clinical features

MRSA isolates were recovered from 41 CF patients out of the 120 who attended our center during the study period. The median age at the time of the first MRSA isolate included in the study was 7.0 years (range 29 days–18.3 years). Additional demographic and clinical data are shown in Table 1.

The prevalence of MRSA infection in this population at our center was 34.2%. Fifty-seven MRSA isolates were recovered from 41 CF patients; 22 (53.7%) were chronically infected with MRSA (≥3 positive consecutive cultures in 6 months), and 19 (46.3%) were transiently infected (<3 positive consecutive

Table 1. Clinical and Demographic Characteristics of Cystic Fibrosis Patients Included in the Study

Characteristics	*CF patients (n = 41)*
Age in years, median (range)	7.0 (29 days–18.3 years)
Gender, *n* (%)	
Male	20 (48.8)
CFTR mutation, *n* (%)	
ΔF508 homocygous	17 (41.5)
ΔF508 heterocygous	15 (36.6)
Other mutations	9 (21.9)
Mean FEV1% predicted (SD)[a]	80.9 (21.1)
Coinfections, *n* (%)	
Pseudomonas aeruginosa[b]	14 (45.2)
Other species[b,c]	9 (29.0)
Hospitalizations, *n* (%)	
1	12 (29.3)
>1	13 (31.7)
Total	25 (61)
CF comorbidities, *n* (%)	
CFRD	2 (0.05)
Pancreatic insufficiency	33 (80.5)
CFLD	2 (0.05)

[a]For patients ≥6 years old.
[b]Data available for 31 patients.
[c]Includes *Haemophilus influenzae*, *Stenotrophomonas maltophilia*, *Burkholderia cepacia* complex, or *Aspergillus flavus*. Two patients were coinfected also with *Pseudomonas aeruginosa*.

CF, cystic fibrosis; CFLD, cystic fibrosis-associated liver disease; CFRD, cystic fibrosis-related diabetes; CFTR, cystic fibrosis transmembrane conductance regulator; FEV1, forced expiratory volume in 1 second; SD, standard deviation.

cultures in 6 months) or with a first-time isolate of MRSA. According to the isolate inclusion criteria, more than one isolate was recovered from 11 patients.

Ten SCV isolates were recovered from 7 patients. Five of them were chronically infected with MRSA.

Antimicrobial susceptibility

The rates of resistance to tested antimicrobial agents are shown in Table 2. High resistance rates were detected for gentamicin, erythromycin, clindamycin, ciprofloxacin, and rifampicin. All isolates were susceptible to vancomycin and teicoplanin. Resistance to linezolid was detected in only one isolate.

As mentioned earlier, sequential isolates that differed in their antibiotic resistance profile were detected in 11 patients, changing the overall resistance to all the tested antimicrobial agents, except for vancomycin and teicoplanin.

MDR-MRSA was detected in 37/57 (65%) isolates. A statistically significant association was observed between the isolation of MDR-MRSA and chronically infected patients (Fig. 1).

MDR was not significantly associated with SCV, 9/10 SCV versus 29/47 isolates with the normal morphotype ($p = 0.140$). Table 3 compares the rates of resistance to different antimicrobials for SCV and normal morphotypes.

Genotypic characterization

All isolates were *mec*A positive. SCC*mec* type analysis revealed 41/57 (71.9%) isolates carrying SCC*mec* IV, 5/57 (8.8%) carrying SCC*mec* I, and 2/57 (3.5%) carrying SCC*mec* III. The SCC*mec* type could not be determined in 9/57 (15.8%) isolates (non-typeable, NT).

Among NT isolates, two strains harbored *mec* complex type C, indicating that these isolates carried SCC*mec* V or VII. Further, one isolate presented recombinase A3B3, which, in the context of its antibiotic resistance profile, strongly suggests that it carried SCC*mec* III. The remaining NT isolates presented recombinase A1B1 ($n = 2$) and A2B2 ($n = 4$).

The *lukS/F-PV* genes were detected in 18 isolates that were recovered from 15 patients, and 11/15 were chronically infected with MRSA ($p = 0.046$). Seventeen out of 18 PVL-positive isolates harbored SCC*mec* IV.

High clone diversity was observed by PFGE analysis, recognizing more than 15 different pulsotypes (Fig. 2). However, 52.6% (30/57) of MRSA isolates exhibited band patterns closely related to the main CA-MRSA clones described in Argentina: pulsotype A (ST5-IV) and pulsotype C (ST30-IV), 22 and 8 isolates, respectively. Five isolates belonged to ST100-IV, which is related to the Argentinean pediatric clone.[15]

Table 2. Antimicrobial Resistance Rates of Methicillin-Resistant *Staphylococcus aureus* Isolates and Distribution According to SCC*mec* Types

Antimicrobial agent	*Total MRSA,* n = *57 (%)*	*SCC*mec *IV,* n = *41*	*SCC*mec *I,* n = *5*	*SCC*mec *III,* n = *2*	*NT,* n = *9*
Ciprofloxacin	28 (49.1)	17	5	2	4
Clindamycin	23 (40.4)	11	5	2	5
Erythromycin	30 (52.6)	18	5	2	5
Gentamycin	38 (66.7)	26	5	2	5
Rifampicin	16 (28.1)	11	1	2	2
Trimethoprim/ Sulfamethoxazole	8 (14.0)	2	2	2	2
Tetracycline	5 (8.8)	1	1	2	1
Linezolid	1 (1.8)	1	0	0	0
Vancomycin	0	0	0	0	0
Teicoplanin	0	0	0	0	0

MRSA, methicillin-resistant *Staphylococcus aureus*.

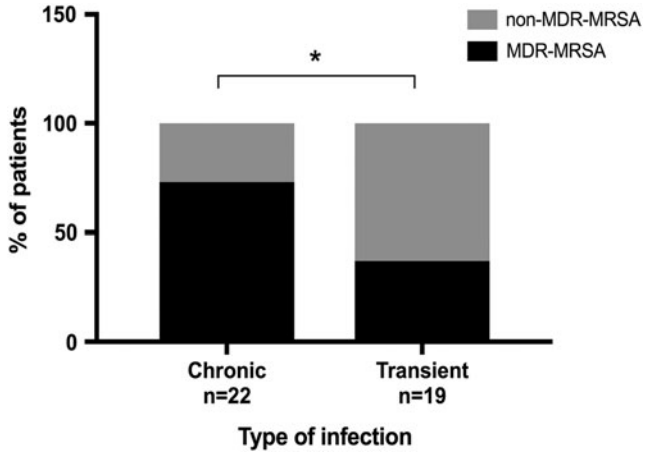


FIG. 1. Association between MDR-MRSA isolation and type of infection. The isolation of MDR-MRSA was significantly associated with chronic infection in CF patients ($p = 0.0296$) compared with the isolation of these isolates from transient infected patients. *$p < 0.05$, Fisher's exact test. Chronic infection was defined as ≥3 positive consecutive cultures in 6 months; transient infection includes patients with <3 positive consecutive cultures in 6 months or a first-time isolate of MRSA.

All patients from whom more than one isolate was recovered presented the same MRSA clone. In five cases, there are differences in the band patterns between the isolates but they still share more than 75% of identity (Fig. 2).

Different *spa* types were recognized, as expected by the PFGE results. The most common *spa* types were t002, t311, and t019, detected in 15, 10, and 7 isolates, respectively. For those isolates related to pulsotype A, *spa* types t002 and t311 represented each 45.5% (10/22) of the isolates. Among the strains clustered in pulsotype C, 75% belonged to *spa* type t019 (6/8). The remaining isolates corresponded to more than 15 additional *spa* types, each of which represented 2–7% of the 57 isolates. Table 4 summarizes the characteristics of MRSA strains detected in CF patients according to SCC*mec* type, PFGE, MLST, *spa* typing, *agr* group, and antimicrobial resistance.

Table 3. Antibiotic Resistance Rates for Small Colony Variant and Normal Morphotype Methicillin-Resistant *Staphylococcus aureus* Isolates

	% resistant isolates		
Antibiotic	*SCV phenotype,* n = *10*	*Normal morphotype,* n = *47*	p[a]
Ciprofloxacin	90	40.4	0.005
Clindamycin	60	40.4	0.308
Erythromycin	80	51.1	0.160
Gentamycin	90	61.7	0.140
Rifampicin	20	29.8	0.709
Trimethoprim/ Sulfamethoxazole	50	6.4	0.003

[a]*p*-Value for Fisher's exact test.

Regarding antibiotic resistance among isolates that harbored SCC*mec* IV, the most frequent SCC*mec* type found in this study, 9/41 (22%) were resistant only to β-lactams, 7/41 (17%) showed resistance to two families of antibiotics, and 25/41 (61%) were MDR isolates, defined as resistant to three or more antibiotic families. Eleven isolates in the latter group showed resistance to β-lactams, gentamicin, erythromycin, and clindamycin.

Considering the two prevalent CA-MRSA clones in Argentina, ST5-IV and ST30-IV, MDR was detected in 18/30 (60%) isolates. Fifteen out of 18 MDR isolates were recovered from chronically infected patients ($p = 0.0086$).

Discussion

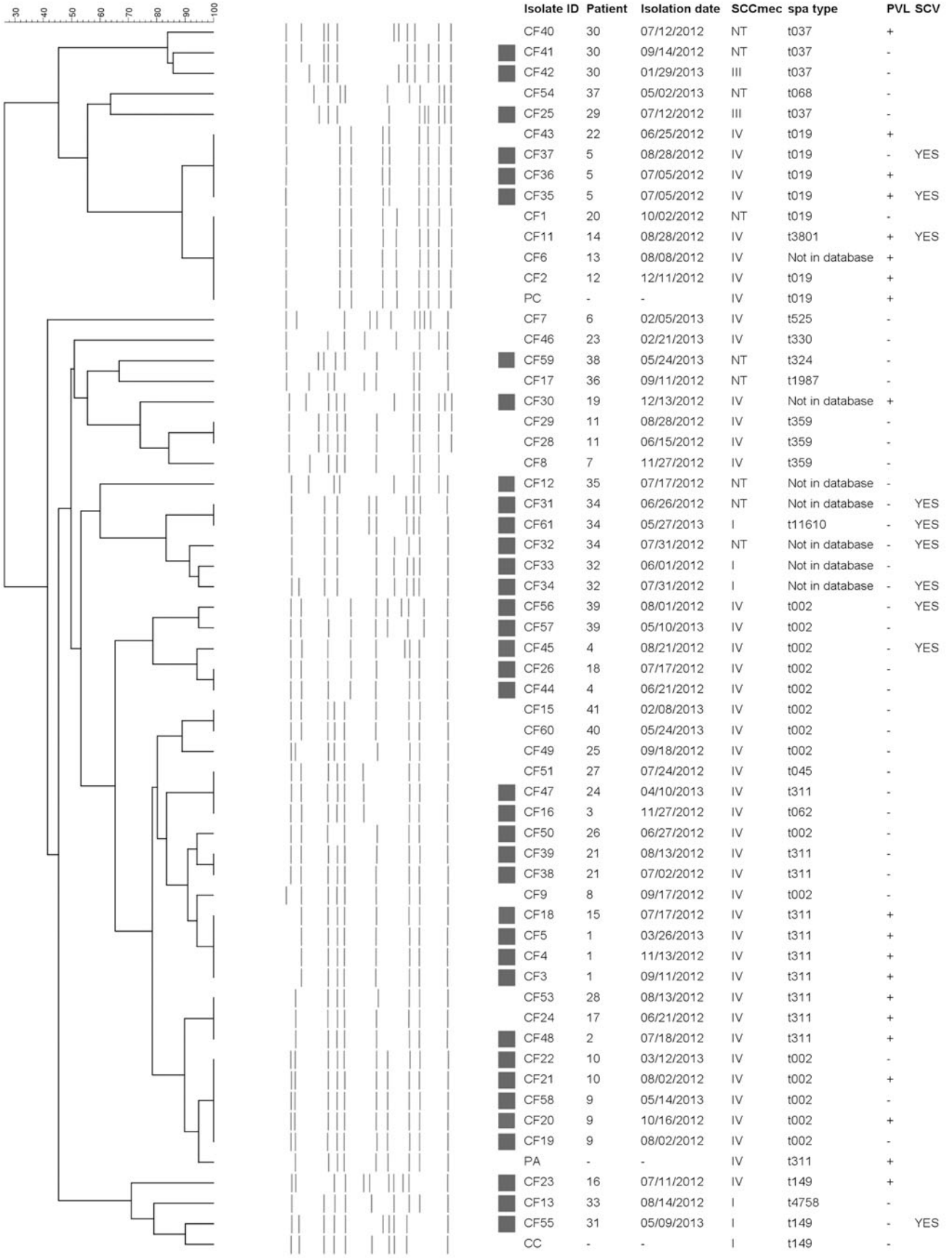
For the first time, this study provides information about the prevalence and genetic background of MRSA isolates recovered from CF patients attending a pediatric hospital of Buenos Aires, Argentina, during 1 year. The prevalence of MRSA in the center has climbed from 23% in 1995 (Galanternik, personal communication) up to 34.2% in the study period. This observation is consistent with the increasing trend observed worldwide.[3,23] The prevalence of MRSA in CF patients varies according to the region and the center where it is studied. The rate reported herein is higher to that one reported by other studies. The Cystic Fibrosis Foundation Patient Registry reported 25.8% in 2010 and 2014 in the United States.[3] In Canada and Europe, the prevalence of MRSA in the CF population is even lower, 3–15%.[23–26]

The median age of our study population at the time of the first MRSA isolate included in the study was 7.0 years (29 days–18.3 years). Importantly, the number of patients included in the study who were hospitalized at least once in a year (25/41, 61%, Table 1) was higher than the mean expected for the general CF population in our center (39% of all the patients attending the center are hospitalized at least once during a year) (Lubovich, personal communication).

Most CF MRSA described herein carried SCC*mec* IV, consistent with what was found by Lima *et al.* in a hospital in Brazil,[27] but it differs with what was reported between 2004 and 2011 in other regions where SCC*mec* II or I were the most frequently SCC*mec* types associated with CF MRSA isolates.[25,28,29] Nonetheless, a shift from SCC*mec* II to predominance of SCC*mec* IV type was recently reported in the United States, with USA300 being the predominant clone.[30]

In this study, 52.6% of MRSA isolates analyzed exhibited band patterns related to the main CA-MRSA clones circulating in Argentina: ST30-IV and ST5-IV,[10] showing that, in agreement with our hypothesis, these lineages are also present in the CF population. The majority of the isolates presented *spa* types related to those of the two prevalent clones: t002, t311, and t019. Other genotypes were also found, showing that in addition to ST30 and ST5 multiple MRSA lineages were present in the CF population.

Regarding antibiotic susceptibility, high resistance rates were detected for gentamicin, erythromycin, clindamycin, ciprofloxacin, and rifampicin among MRSA isolates. Increasing resistance to multiple antibiotics among different CF respiratory bacteria has been previously described.[31] In this study, MDR-MRSA accounted for 65% of the isolates recovered.



Isolate ID	Patient	Isolation date	SCCmec	spa type	PVL	SCV
CF40	30	07/12/2012	NT	t037	+	
CF41	30	09/14/2012	NT	t037	-	
CF42	30	01/29/2013	III	t037	-	
CF54	37	05/02/2013	NT	t068	-	
CF25	29	07/12/2012	III	t037	-	
CF43	22	06/25/2012	IV	t019	+	
CF37	5	08/28/2012	IV	t019	-	YES
CF36	5	07/05/2012	IV	t019	+	
CF35	5	07/05/2012	IV	t019	+	YES
CF1	20	10/02/2012	NT	t019	-	
CF11	14	08/28/2012	IV	t3801	+	YES
CF6	13	08/08/2012	IV	Not in database	+	
CF2	12	12/11/2012	IV	t019	+	
PC	-	-	IV	t019	+	
CF7	6	02/05/2013	IV	t525	-	
CF46	23	02/21/2013	IV	t330	-	
CF59	38	05/24/2013	NT	t324	-	
CF17	36	09/11/2012	NT	t1987	-	
CF30	19	12/13/2012	IV	Not in database	+	
CF29	11	08/28/2012	IV	t359	-	
CF28	11	06/15/2012	IV	t359	-	
CF8	7	11/27/2012	IV	t359	-	
CF12	35	07/17/2012	NT	Not in database	-	
CF31	34	06/26/2012	NT	Not in database	-	YES
CF61	34	05/27/2013	I	t11610	-	YES
CF32	34	07/31/2012	NT	Not in database	-	YES
CF33	32	06/01/2012	I	Not in database	-	
CF34	32	07/31/2012	I	Not in database	-	YES
CF56	39	08/01/2012	IV	t002	-	YES
CF57	39	05/10/2013	IV	t002	-	
CF45	4	08/21/2012	IV	t002	-	YES
CF26	18	07/17/2012	IV	t002	-	
CF44	4	06/21/2012	IV	t002	-	
CF15	41	02/08/2013	IV	t002	-	
CF60	40	05/24/2013	IV	t002	-	
CF49	25	09/18/2012	IV	t002	-	
CF51	27	07/24/2012	IV	t045	-	
CF47	24	04/10/2013	IV	t311	-	
CF16	3	11/27/2012	IV	t062	-	
CF50	26	06/27/2012	IV	t002	-	
CF39	21	08/13/2012	IV	t311	-	
CF38	21	07/02/2012	IV	t311	-	
CF9	8	09/17/2012	IV	t002	-	
CF18	15	07/17/2012	IV	t311	+	
CF5	1	03/26/2013	IV	t311	+	
CF4	1	11/13/2012	IV	t311	+	
CF3	1	09/11/2012	IV	t311	+	
CF53	28	08/13/2012	IV	t311	+	
CF24	17	06/21/2012	IV	t311	+	
CF48	2	07/18/2012	IV	t311	+	
CF22	10	03/12/2013	IV	t002	-	
CF21	10	08/02/2012	IV	t002	+	
CF58	9	05/14/2013	IV	t002	-	
CF20	9	10/16/2012	IV	t002	+	
CF19	9	08/02/2012	IV	t002	-	
PA	-	-	IV	t311	+	
CF23	16	07/11/2012	IV	t149	+	
CF13	33	08/14/2012	I	t4758	-	
CF55	31	05/09/2013	I	t149	-	YES
CC	-	-	I	t149	-	

FIG. 2. Dendrogram of MRSA isolates recovered from CF patients. PFGE pattern analysis for 57 MRSA isolates recovered from respiratory samples from pediatric CF patients from June 2012 to May 2013. Squares represent multidrug resistant isolates, defined as resistant to three or more antibiotic families. CC, cordobes clone (ST5-I-t149); PA, pulsotype A (ST5-IV-t311); PC, pulsotype C (ST30-IV-t019).

Table 4. Molecular Typing and Antimicrobial Resistance of Cystic Fibrosis Methicillin-Resistant *Staphylococcus aureus* Strains

*SCC*mec	*MRSA isolates* (n)	*PFGE pulsotypes*	*MLST (ST/CC)*	spa *types* (n)[a]	agr *group* (n)[a]	*Drug resistance to non-β-lactams,* n *(%)*[b]
IV	22	A	5/5	t311 (10), t002 (10), t045, t062	II (22)	GEN 12 (54.5); ERY 12 (54.5); CIP 7 (31.8); CLI 10 (45.5); RIF 6 (27.3)
	8	C	30/30	t019 (6), t3801, not described *spa* type	III (8)	GEN 4 (50); ERY 3 (37.5); CIP 4 (50); CLI 1 (12.5); STX 1 (12.5)
	5	D	100/5	t002 (5)	II (5)	GEN 5; ERY 2; CIP 4; RIF 4; STX 1
	7	Others	ND	t149, t330, t359 (3), t525, not described *spa* type	I (5); II; III	GEN 3; ERY 4;: CLI 2; RIF 1; LNZ 1
III	2	Others	ND	t037 (2)	I (2)	GEN 2; ERY 2; CIP 2; CLI 2; RIF 2; STX 2
I	5	Others	ND	t149, t4758, t11610, not described *spa* types (2)	II (5)	GEN 5; ERY 5; CIP 5; CLI 5; RIF 1; STX 2
NT	1	C	30	t019	III	
	8	Others	ND	t037 (2), t068, t324, t1987, not described *spa* types (3)	I (6); II (2)	GEN 5; ERY 5; CIP 4; CLI 5; RIF 2; STX 2

[a]N is not expressed when there is only one isolate with that characteristic.
[b]% of resistance to non-β-lactams expressed only for strains related to the two main CA-MRSA clones in Argentina.
CC, clonal complex; ND, not determined; NT, non-typeable; MLST, multilocus sequence typing; PFGE, pulsed-field gel electrophoresis; ST, sequence type.

Of note, 61% of the isolates harboring SCC*mec* IV, the most frequent SCC*mec* recovered in this study, were MDR. These high rates of resistance to non-β-lactam antibiotics agree with those reported by Cocchi *et al.* for CF MRSA isolates harboring SCC*mec* IV.[25]

Of great concern is that multi-resistance was detected in 60% of the isolates that were related to the prevalent CA-MRSA clones in our country. These results demonstrate a key difference compared with reports from non-CF patients in Argentina where these CA-MRSA clones were not associated with MDR.[10,16]

Moreover, isolation of MDR-MRSA was significantly associated with chronic infection. *S. aureus* has a spectacular capacity to adapt to its host during chronic infection. Specifically, in CF airways, *S. aureus* is subjected to numerous selective pressures resulting from antibiotic interventions, the host immune system, and coinfection of the airways with other microorganisms.[32] To survive in this hostile niche, *S. aureus* has developed several adaptive mechanisms to cope with changing selective pressures: the emergence of SCV,[6] changes in the protein A gene repeat region,[33] biofilm formation,[34] immune evasion,[35] and changes in antibiotic resistance.[36] The high rate of multi-resistance observed herein could be related to the frequent and complex antibiotic therapy to which chronic CF patients are exposed to and to other selective pressures present in the CF airways that promote *S. aureus* adaptation.

Even though we did not find an association between MDR and SCV, this phenotype was significantly associated with higher rates of resistance to ciprofloxacin and trimethoprim/sulfamethoxazole. Trimethoprim/sulfamethoxazole-resistant SCV have been isolated from the airways of CF patients and persist for extended periods.[37] Eight out of 10 SCV isolates were recovered from chronically infected patients, and they did not belong to a specific SCC*mec* type nor to a defined pulsotype. Their appearance in chronically infected patients is consistent with the idea that the change in morphology may be related to *S. aureus*'s adaptation to CF patients' airways and to the persistence phenomena.[5]

Persistence of the initial strain was demonstrated in those patients from whom sequential MRSA isolates were recovered, as described by Al-Zubeidi *et al.*[28] In some cases, PFGE band pattern differences were detected among the persistent strains within the same patient but they still belonged to the same PFGE type. As previously mentioned, these genetic changes might be related to microevolutionary events occurring in this particular ecological niche.

Furthermore, PVL-positive isolates also appear to be associated with chronically infected patients. Seventeen out of 18 PVL-positive isolates harbored SCC*mec* IV. This differs with what was previously described in an Italian study in which CF MRSA isolates harboring SCC*mec* IV did not express PVL.[25] Nonetheless, PVL-positive isolates have been described in CF and are associated with the development of invasive lung infections, including lung abscesses.[38] The finding of MRSA-ST5-IV and MRSA-ST30-IV clones harboring PVL coding genes in CF patients is of great concern because these clones have been previously described in our country and have been causing invasive infections.[20,39,40]

As a limitation of this study, it should be mentioned that isolates belonged to one center and they were recovered only from children, noting that the current median predicted survival age for individuals with CF is 41.6 years.

To conclude, MRSA prevalence has increased in our hospital and most isolates carried SCC*mec* IV. Strains related to the main CA-MRSA clones circulating in Argentina were also recovered from CF patients, but the rates of antibiotic resistance were higher than those reported for non-CF patients. This difference was associated with chronic infection and may be related to complex antimicrobial therapies received by CF patients. These strains could represent an emerging health threat not only for CF patients but also for the general community, as they seem to have originated from CA-MRSA lineages, which are highly transmissible. Further studies should be done to elucidate the mechanisms involved in the evolution of antibiotic resistance during antibiotic treatment in CF.

This study provides an overview of the epidemiology of MRSA in CF disease in our country. The development of a prospective multicenter national study would be relevant to thoroughly describe the implications of MRSA in the CF pathology.

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Disclosure Statement

No competing financial interests exist.

References

1. Sociedad Argentina de Pediatría. 2008. Consenso Nacional de Fibrosis Quística. Arch. Argent. Pediatr. 106:e01–e52.
2. O'Sullivan, B.P., and S.D. Freedman. 2009. Cystic fibrosis. Lancet. 373:1891–1904.
3. Cystic Fibrosis Foundation. 2015. Cystic Fibrosis Patient Registry 2015. Bethesda, MD: Cystic Fibrosis Foundation.
4. Lopes, S.P., N.F. Azevedo, and M.O. Pereira. 2015. Microbiome in cystic fibrosis: shaping polymicrobial interactions for advances in antibiotic therapy. Crit. Rev. Microbiol. 41: 353–365.
5. Hirschhausen, N., D. Block, I. Bianconi, A. Bragonzi, J. Birtel, J.C. Lee, A. Dubbers, P. Kuster, J. Kahl, G. Peters, and B.C. Kahl. 2013. Extended *Staphylococcus aureus* persistence in cystic fibrosis is associated with bacterial adaptation. Int. J. Med. Microbiol. 303:685–692.
6. Kahl, B.C., K. Becker, and B. Loffler. 2016. Clinical significance and pathogenesis of staphylococcal small colony variants in persistent infections. Clin. Microbiol. Rev. 29: 401–427.
7. World Health Organization (WHO). 2014. Antimicrobial resistance: global report on surveillance. WHO, Geneva, Switzerland.
8. Chatterjee, S.S., and M. Otto. 2013. Improved understanding of factors driving methicillin-resistant *Staphylococcus aureus* epidemic waves. Clin. Epidemiol. 5:205–217.
9. Naimi, T.S., K.H. LeDell, K. Como-Sabetti, S.M. Borchardt, D.J. Boxrud, J. Etienne, S.K. Johnson, F. Vandenesch, S. Fridkin, C. O'Boyle, R.N. Danila, and R. Lynfield. 2003. Comparison of community- and health care-associated methicillin-resistant *Staphylococcus aureus* infection. JAMA. 290:2976–2984.
10. Egea, A.L., P. Gagetti, R. Lamberghini, D. Faccone, C. Lucero, A. Vindel, D. Tosoroni, A. Garnero, H.A. Saka, M. Galas, J.L. Bocco, A. Corso, and C. Sola. 2014. New patterns of methicillin-resistant *Staphylococcus aureus* (MRSA) clones, community-associated MRSA genotypes behave like healthcare-associated MRSA genotypes within hospitals, Argentina. Int. J. Med. Microbiol. 304:1086–1099.
11. Otter, J.A., and G.L. French. 2011. Community-associated meticillin-resistant *Staphylococcus aureus* strains as a cause of healthcare-associated infection. J. Hosp. Infect. 79: 189–193.
12. Bal, A.M., G.W. Coombs, M.T. Holden, J.A. Lindsay, G.R. Nimmo, P. Tattevin, and R.L. Skov. 2016. Genomic insights into the emergence and spread of international clones of healthcare-, community- and livestock-associated meticillin-resistant *Staphylococcus aureus*: blurring of the traditional definitions. J. Glob. Antimicrob. Resist. 6:95–101.
13. Otter, J.A., and G.L. French. 2012. Community-associated meticillin-resistant *Staphylococcus aureus*: the case for a genotypic definition. J. Hosp. Infect. 81:143–148.
14. Gardella, N., S. Murzicato, S. Di Gregorio, A. Cuirolo, J. Desse, F. Crudo, G. Gutkind, and M. Mollerach. 2011. Prevalence and characterization of methicillin-resistant *Staphylococcus aureus* among healthy children in a city of Argentina. Infect. Genet. Evol. 11:1066–1071.
15. Sola, C., H. Paganini, A.L. Egea, A.J. Moyano, A. Garnero, I. Kevric, C. Culasso, A. Vindel, H. Lopardo, and J.L. Bocco. 2012. Spread of epidemic MRSA-ST5-IV clone encoding PVL as a major cause of community onset staphylococcal infections in Argentinean Children. PLoS One. 7:e30487.
16. Fernandez, S., L. de Vedia, M.J. Lopez Furst, N. Gardella, S. Di Gregorio, M.C. Ganaha, S. Prieto, E. Carbone, N. Lista, F. Rotrying, M.E. Stryjewski, and M. Mollerach. 2013. Methicillin-resistant *Staphylococcus aureus* ST30-SCC*mec* IVc clone as the major cause of community-acquired invasive infections in Argentina. Infect. Genet. Evol. 14:401–405.
17. Lina, G., Y. Piemont, F. Godail-Gamot, M. Bes, M.O. Peter, V. Gauduchon, F. Vandenesch, and J. Etienne. 1999. Involvement of Panton-Valentine leukocidin-producing *Staphylococcus aureus* in primary skin infections and pneumonia. Clin. Infect. Dis. 29:1128–1132.
18. Gilot, P., G. Lina, T. Cochard, and B. Poutrel. 2002. Analysis of the genetic variability of genes encoding the RNA III-activating components *agr* and TRAP in a population of *Staphylococcus aureus* strains isolated from cows with mastitis. J. Clin. Microbiol. 40:4060–4067.
19. Kondo, Y., T. Ito, X.X. Ma, S. Watanabe, B.N. Kreiswirth, J. Etienne, and K. Hiramatsu. 2007. Combination of multiplex PCRs for staphylococcal cassette chromosome *mec* type assignment: rapid identification system for *mec*, *ccr*, and major differences in junkyard regions. Antimicrob. Agents Chemother. 51:264–274.
20. Gardella, N., M. von Specht, A. Cuirolo, A. Rosato, G. Gutkind, and M. Mollerach. 2008. Community-associated

methicillin-resistant *Staphylococcus aureus*, eastern Argentina. Diagn. Microbiol. Infect. Dis. 62:343–347.
21. Sola, C., G. Gribaudo, A. Vindel, L. Patrito, and J.L. Bocco. 2002. Identification of a novel methicillin-resistant *Staphylococcus aureus* epidemic clone in Cordoba, Argentina, involved in nosocomial infections. J. Clin. Microbiol. 40:1427–1435.
22. Enright, M.C., K. Knox, D. Griffiths, D.W. Crook, and B.G. Spratt. 2000. Molecular typing of bacteria directly from cerebrospinal fluid. Eur. J. Clin. Microbiol. Infect. Dis. 19: 627–630.
23. Raidt, L., E.A. Idelevich, A. Dubbers, P. Kuster, P. Drevinek, G. Peters, and B.C. Kahl. 2015. Increased prevalence and resistance of important pathogens recovered from respiratory specimens of cystic fibrosis patients during a decade. Pediatr. Infect. Dis. J. 34:700–705.
24. Cystic Fibrosis Canada. 2014. The Canadian Cystic Fibrosis Registry: 2014 Annual Report. Cystic Fibrosis Canada, Toronto, ON.
25. Cocchi, P., L. Cariani, F. Favari, A. Lambiase, E. Fiscarelli, F.V. Gioffre, A. d'Aprile, E. Manso, G. Taccetti, C. Braggion, G. Doring, M. de Martino, and S. Campana. 2011. Molecular epidemiology of meticillin-resistant *Staphylococcus aureus* in Italian cystic fibrosis patients: a national overview. J. Cyst. Fibros. 10:407–411.
26. Tkadlec, J., E. Varekova, R. Pantucek, J. Doskar, V. Ruzickova, T. Botka, L. Fila, and O. Melter. 2015. Characterization of *Staphylococcus aureus* strains isolated from Czech Cystic Fibrosis Patients: high rate of ribosomal mutation conferring resistance to MLS(B) antibiotics as a result of long-term and low-dose Azithromycin treatment. Microb. Drug Resist. 21:416–423.
27. Lima, D.F., N.B Brazão, T.W. Folescu, F.P. Neves, A.G. Ferreira, E.A. Santos, E.A. Marques, and R.S. Leão. 2014. Panton-Valentine leukocidin (PVL) gene carriage among *Staphylococcus aureus* strains with SCC*mec* types I, III, IV, and V recovered from cystic fibrosis pediatric patients in Brazil. Diagn. Microbiol. Infect. Dis. 78:59–62.
28. Al-Zubeidi, D., P.G. Hogan, M. Boyle, C.A. Burnham, and S.A. Fritz. 2014. Molecular epidemiology of methicillin-resistant *Staphylococcus aureus* isolated in serial cultures from the respiratory tract of children with cystic fibrosis. Pediatr. Infect. Dis. J. 33:549–553.
29. Muhlebach, M.S., S.L. Heltshe, E.B. Popowitch, M.B. Miller, V. Thompson, M. Kloster, T. Ferkol, W.C. Hoover, M.S. Schechter, and L. Saiman. 2015. Multicenter observational study on factors and outcomes associated with various methicillin-resistant *Staphylococcus aureus* types in children with cystic fibrosis. Ann. Am. Thorac. Soc. 12:864–871.
30. Zemanick, E.T., T. Ong, C.L. Daines, E.P. Dellon, M.S. Muhlebach, and C.R. Esther, Jr. 2016. Highlights from the 2015 North American Cystic Fibrosis Conference. Pediatr. Pulmonol. 51:650–657.
31. Rutter, W.C., D.R. Burgess, and D.S. Burgess. 2017. Increasing incidence of multidrug resistance among cystic fibrosis respiratory bacterial isolates. Microb. Drug Resist. 23:51–55.
32. Cullen, L., and S. McClean. 2015. Bacterial adaptation during chronic respiratory infections. Pathogens. 4:66–89.
33. Garofalo, A., C. Giai, S. Lattar, N. Gardella, M. Mollerach, B.C. Kahl, K. Becker, A.S. Prince, D.O. Sordelli, and M.I. Gomez. 2012. The length of the *Staphylococcus aureus* protein A polymorphic region regulates inflammation: impact on acute and chronic infection. J. Infect. Dis. 206:81–90.
34. Schwartbeck, B., J. Birtel, J. Treffon, L. Langhanki, A. Mellmann, D. Kale, J. Kahl, N. Hirschhausen, C. Neumann, J.C. Lee, F. Gotz, H. Rohde, H. Henke, P. Kuster, G. Peters, and B.C. Kahl. 2016. Dynamic *in vivo* mutations within the *ica* operon during persistence of *Staphylococcus aureus* in the airways of cystic fibrosis patients. PLoS Pathog. 12: e1006024.
35. Windmuller, N., A. Witten, D. Block, B. Bunk, C. Sproer, B.C. Kahl, and A. Mellmann. 2015. Transcriptional adaptations during long-term persistence of *Staphylococcus aureus* in the airways of a cystic fibrosis patient. Int. J. Med. Microbiol. 305:38–46.
36. McAdam, P.R., A. Holmes, K.E. Templeton, and J.R. Fitzgerald. 2011. Adaptive evolution of *Staphylococcus aureus* during chronic endobronchial infection of a cystic fibrosis patient. PLoS One. 6:e24301.
37. Chatterjee, I., A. Kriegeskorte, A. Fischer, S. Deiwick, N. Theimann, R.A. Proctor, G. Peters, M. Herrmann, and B.C. Kahl. 2008. *In vivo* mutations of thymidylate synthase (encoded by *thyA*) are responsible for thymidine dependency in clinical small-colony variants of *Staphylococcus aureus*. J. Bacteriol. 190:834–842.
38. Elizur, A., R.C. Orscheln, T.W. Ferkol, J.J. Atkinson, W.M. Dunne, Jr., R.S. Buller, J.R. Armstrong, E.R. Mardis, G.A. Storch, and C.L. Cannon. 2007. Panton-valentine leukocidin-positive methicillin-resistant *Staphylococcus aureus* lung infection in patients with cystic fibrosis. Chest. 131:1718–1725.
39. Fernandez, S., C. Ledo, S. Lattar, M. Noto Llana, A.M. Bertelli, S. Di Gregorio, D.O. Sordelli, M.I. Gomez, and M.E. Mollerach. 2017. High virulence of methicillin resistant *Staphylococcus aureus* ST30-SCC*mec*IVc-spat019, the dominant community-associated clone in Argentina. Int. J. Med. Microbiol. 307:191–199.
40. Fernandez, S., S. Murzicato, O. Sandoval, L. Fernandez-Canigia, and M. Mollerach. 2015. Community-acquired necrotizing pneumonia caused by methicillin-resistant *Staphylococcus aureus* ST30-SCC*mec*IVc-spat019-PVL positive in San Antonio de Areco, Argentina. Rev. Argent Microbiol. 47:50–53.

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