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USA300-related methicillin-resistant *Staphylococcus aureus* clone is the predominant cause of community and hospital MRSA infections in Colombian children

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SUMMARY

Objective: Community-genotype methicillin-resistant *Staphylococcus aureus* (CG-MRSA) isolates are known to be more virulent and clinically aggressive in children. The goal of the present study was characterize the molecular epidemiology of MRSA isolates causing infections in Colombian children.
Methods: An observational and prospective study was conducted between April 2009 and June 2011 at 15 hospitals in Bogotá, Colombia. A detailed epidemiological profile was made of 162 children infected with MRSA. The isolates were subjected to antimicrobial susceptibility testing, molecular characterization including 21 virulence genes, SCC*mec*, *spa* and *agr* typing, multilocus sequence typing (MLST), and pulsed-field gel electrophoresis (PFGE).
Results: Among all isolates included in the study, 85.8% were obtained from patients whose infectious process was initiated in the community; of these, 69,8% occurred in patients without healthcare-associated risk factors. The molecular characterization of the isolates showed a high proportion (95.1%) containing a community-genotype profile with a high prevalence of SCC*mec* type IV, PVL-positives, and also related to CC8. Most CG-MRSA isolates (143, 92.9%) were genetically related to the pandemic clone USA300, differing by the presence of SCC*mec* IVc and the absence of the arginine catabolic mobile element (ACME).
Conclusions: An increase in the frequency of CG-MRSA infections has been reported worldwide. In this study we found that almost all MRSA infections in our pediatric population were caused by community-genotype isolates, supporting the success of the CG-MRSA clones.

1. Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) has become a significant cause of community- and healthcare-associated infections. It is possible to genetically and molecularly differentiate between MRSA isolates associated with the community environment (community-genotype, CG) and those associated with the hospital environment (hospital-genotype, HG).[1] CG-MRSA isolates have been considered more virulent than HG-MRSA, possibly due to the acquisition or overexpression of factors such as Panton–Valentine leukocidin (PVL) and phenol-soluble modulins.[2,3] USA300 is the most widespread CG-MRSA clone on the American continent, although it has also been described in Europe, Asia, and Oceania.[4,5] In the past decade, an increase in both invasive and non-invasive infections in children caused mainly by this clone has been documented, reaching epidemic proportions.[6,7]

Since 2006, the spread of a CG-MRSA clone (ST8-IVc-PVL$^+$) genetically related to the USA300 clone has been described in

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Colombia and other South American countries.[8,9] In 2009, we reported three circulating cases of this clone among Colombian children.[10] Despite recent reports in the Colombian pediatric population,[11,12] information about the molecular characterization of MRSA infections in this population is still limited in our region. Therefore, we performed the molecular characterization of MRSA isolates from pediatric patients with infections treated in 15 separate healthcare institutions in Bogotá, Colombia.

2. Methods

2.1. Study setting

From April 2009 to June 2011, we conducted a prospective study of MRSA infections in pediatric patients from 15 tertiary-level hospitals in Bogotá, which is the largest city in Colombia. The medical records were reviewed to differentiate colonization from true infection in the case of isolates from non-sterile sites like skin (in the absence of clinical significance), catheters, or sputum. Subsequently, only true infections were included in the study. Informed consent was obtained and the study was approved by the institutional review boards of the participating hospitals.

2.2. Genetic and molecular characterization of isolates

The SCC*mec* type and subtype were established as described previously.[13] SCC*mec* IVc was confirmed by differential amplification between IVc and IVE cassettes at specific DNA fragments in the J3 region. The presence of the genes *lukS/F-PV*, *sausa400*, *bsaB*, *etb*, *eta*, *hlg*, *sea*, *seb*, *sec*, *seg*, *seh*, *sei*, *sej*, *sek*, *sel*, *sem*, *sen*, *seo*, *seq*, *arcC*, and *opp* (the final two as markers of the arginine catabolic mobile element, ACME) were also determined, as described previously.[14,15]

2.3. Determination of the genetic relationship between isolates by restriction of housekeeping genes, multilocus sequence typing (MLST), pulsed-field gel electrophoresis (PFGE), agr and spa typing

The relationship of the isolates to clonal complex (CC) 5 or 8 was established by analysis of mutations in the *arcC* and *gmk* genes.[16] The *agr* group was determined as reported previously.[17] The genetic relationship between isolates was determined by PFGE. The pulsotypes were interpreted according to the percentage similarity and the criteria proposed by Tenover et al.[18] GelCompar II program (Applied Maths NV) was used, with a tolerance position of 1.5% and Dice coefficient of 1.0%. MLST and the *spa* type were determined for representative isolates of the main pulsotypes, as reported previously.[19,20]

2.4. Genotypic classification of isolates

MRSA isolates were classified according to their genetic characteristics (genotype), taking into account previous results in our region,[21] including molecular background and the PFGE pulsotype, as follows. Isolates with SCC*mec* type IV (subtype a, b, c, or h) and any of the following characteristics were considered community-genotype (CG-MRSA): the presence of genes *lukS/F-PV*, *seq*, *sek*, or *bsaB* and/or PFGE pulsotype related to the USA300 clone.[1] Isolates with SCC*mec* I or II, the presence of the *sem* and *seo* genes, and/or a PFGE pulsotype related to the Cordobés/Chilean or pediatric clones were considered hospital-genotype (HG-MRSA).[1,22]

2.5. Microbiological characterization of isolates

The susceptibility profile to 11 antibiotics and inducible clindamycin resistance were determined by agar dilution method and the D-test, respectively, as recommended by the Clinical and Laboratory Standards Institute.[23] Isolates found to be non-susceptible or resistant to three or more antimicrobial categories were classified as multidrug-resistant.[24]

2.6. Epidemiological and clinical classification of infections

Community-acquired MRSA (CA-MRSA) infections were classified as those that were detected within the first 48 h of hospital admission, without healthcare-associated risk factors (HARF),[25,26] and hospital-acquired MRSA (HA-MRSA) infections were classified as those detected after the first 48 h of hospital admission.[25] Furthermore, community-onset MRSA infections were classified to be healthcare-associated MRSA (HCA-MRSA) infections based on the presence of healthcare-associated risk factors (HARF).

3. Results

3.1. Genetic and molecular characteristics of MRSA isolates

During the study period, 162 MRSA isolates were collected from pediatric infections (one isolate per patient), of which 154 (95.1%) were SCC*mec* type IV, six (3.7%) were type I, one (0.6%) was type II, and one (0.6%) was non-typeable. *agr I* was the most frequent group (94.4%), followed by *agr II* (4.3%) and *agr III* (1.2%). Restriction analysis of housekeeping genes showed 152 (93.8%) isolates to be related to CC8 and eight (4.9%) to CC5; the other two (4.9%) isolates could not be typed by this method. According to the results of the molecular analyses, 154 (95.1%) isolates were classified as CG-MRSA[22] and the remaining eight (4.9%) were classified as HG-MRSA.

Table 1
Comparison of the genetic backgrounds of MRSA from infected children in Bogotá, Colombia

Characteristics of isolates		CG-MRSA, *n* (%) *n* = 154	HG-MRSA, *n* (%) *n* = 8
Clonal complex	CC5	1 (0.6)	7 (87.5)
	CC8	152 (98.7)	0
	ND	1 (0.6)	1 (12.5)
agr group	I	152 (98.7)	1 (12.5)
	II	0	7 (87.5)
	III	2 (1.3)	0
SCC*mec* type	I	0	6 (75.0)
	II	0	1 (12.5)
	IVa	11 (7.1)	0
	IVb	1 (0.6)	0
	IVc	139 (90.3)	0
	IVh	3 (1.9)	0
	NT	0	1 (12.5)
Toxin gene	*seq*	121 (78.6)	0
	sek	113 (73.4)	0
	eta	92 (59.7)	4 (50.0)
	sej	16 (10.4)	0
	sel	15 (9.7)	1 (12.5)
	seg	12 (7.8)	3 (37.5)
	hlg	10 (6.5)	2 (25.0)
	seh	11 (7.1)	3 (37.5)
	sea	8 (5.2)	1 (12.5)
	see	3 (1.9)	1 (12.5)
	seo	1 (0.6)	8 (100)
	sem	1 (0.6)	8 (100)
	sec	1 (0.6)	4 (50.0)
	seb	1 (0.6)	0
	sei	0	6 (75.0)
	sen	0	5 (62.5)
	etb	0	1 (12.5)
Other virulence factor genes	*lukS/F-PV*	153 (98.7)	0
	bsaB	150 (97.4)	0

MRSA, methicillin-resistant *Staphylococcus aureus*; CG, community-genotype; HG, hospital-genotype; ND, not determined; NT, non-typeable.

Among the CG-MRSA isolates, 153 (98.7%) were PVL-positive, and the *bsaB*, *seq*, and *sek* genes were detected in 150 (97.4%), 121 (78.6%), and 113 (73.4%) isolates, respectively. In this group, 139 isolates (90.3%) were SCC*mec* IVc, 11 (7.1%) IVa, three (1.9%) IVh, and one (0.6%) IVb subtypes. Only two of the CG-MRSA isolates (1.3%) were *agr* group III, and the remaining 152 (98.7%) were *agr* group I (Table 1). ACME was not found in any isolate.

PFGE analysis revealed that, of the 154 CG-MRSA isolates, 143 (92.9%) were genetically related to the USA300 clone (<6 bands of difference and a similarity >83%) (Figure 1). The U1 pulsotype was the most common and was found in 91 isolates (56.2%) (Table 2). The most frequent enterotoxin profile was *seq–sek*, which was detected in 113 isolates (73.4%), although other enterotoxin profiles were found to a lesser extent. The *eta* gene was detected

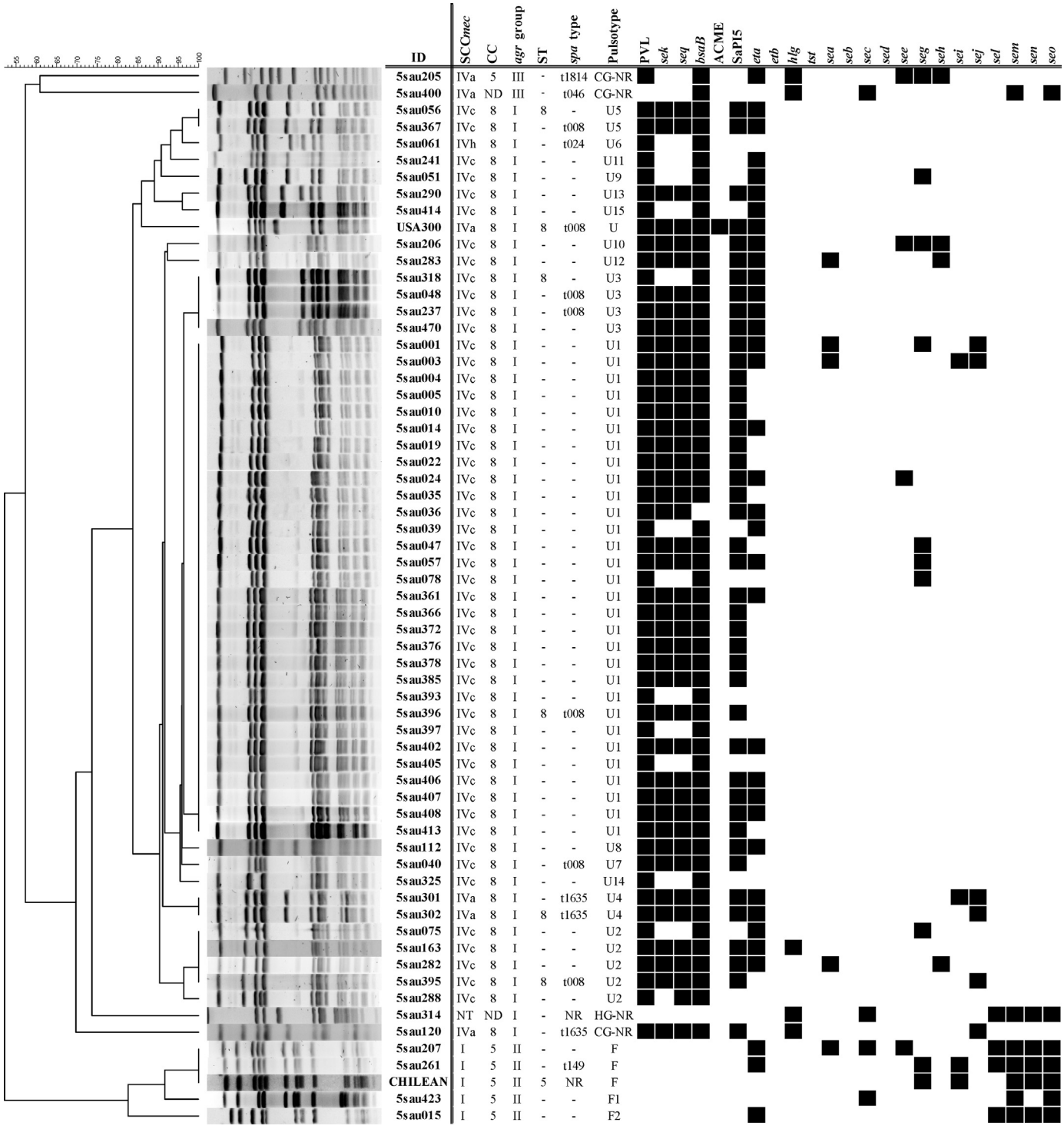

Figure 1. Genetic relationship by PFGE of MRSA isolates from pediatric patients. Isolates representative of the main pulsotypes are shown. GelCompar II program (Applied Maths NV) was used, with a tolerance position of 1.5% and Dice coefficient of 1.0%. PFGE patterns were considered different when they showed a similarity less than 80%. The presence of pathogenicity island 5 (SaPI5) was confirmed by amplification of the *sausa300_0808* gene and specific fragments at the insertion site. NT = not typeable; ND = not determined; NR= not reported *spa* type number (for Cordobés/Chilean clone and 5sau314 isolate the repeats in *spa* typing were TIMEMDMGMGMK and A2DKBEMBB, respectively); CG-NR = isolated community-genotype MRSA with pulsotype not related to USA300 clone; HG-NR = isolated hospital-genotype MRSA with pulsotype not related to Cordobés/Chilean clone.

Table 2
Molecular classification and antimicrobial susceptibility of MRSA isolated from infected children

Characteristics of isolates	All subjects (*N* = 162)	CO-MRSA (*n* = 139)		HA-MRSA (*n* = 23)
		CA (*n* = 97)	HCA (*n* = 42)	
Type of isolate				
CG-MRSA	154 (95.1)	97 (100)	41 (97.6)	16 (69.6)
HG-MRSA	8 (4.9)	0	1 (2.4)	7 (30.4)
Pulsed-field pulsotype[a,b]				
U1	91 (56.2)	54 (55.7)	27 (64.3)	10 (43.5)
U2	16 (9.9)	12 (12.4)	3 (7.1)	1 (4.3)
U3	12 (7.4)	7 (7.2)	4 (9.5)	1 (4.3)
U4	8 (4.9)	6 (6.2)	0	2 (8.7)
U5	7 (4.3)	5 (5.2)	1 (2.4)	1 (4.3)
U6	4 (2.5)	2 (2.1)	2 (4.8)	0
U7	4 (2.5)	3 (3.1)	1 (2.4)	0
U8	2 (1.2)	1 (1.0)	1 (2.4)	0
U9 to U15	7 (4.3)	5 (5.2)	2 (4.8)	0
CG-NR	3 (1.9)	2 (2.1)	0	1 (4.3)
F	6 (3.7)	0	1 (2.4)	5 (21.7)
HG-NR	2 (1.2)	0	0	2 (8.7)
Antimicrobial susceptibility profile				
OXA	84 (51.9)	54 (55.7)	20 (47.6)	10 (43.4)
OXA, TET	53 (32.7)	29 (29.9)	16 (38.1)	8 (34.8)
OXA, ERY	8 (4.9)	5 (5.2)	3 (7.1)	0
OXA, ERY, TET	6 (3.7)	6 (6.2)	0	0
OXA, GEN, CIP, ERY, CLI	4 (2.5)	0	1 (2.4)	3 (13.0)
OXA, GEN, CIP, ERY, TET, CLI	2 (1.2)	1 (1.0)	0	1 (4.4)
OXA, GEN, CIP, TET, CLI	1 (0.6)	0	0	1 (4.4)
OXA, GEN, ERY, CLI	1 (0.6)	0	1 (2.4)	0
OXA, GEN, ERY, TET	1 (0.6)	1 (1.0)	0	0
OXA, GEN	1 (0.6)	1 (1.0)	0	0
OXA, TET, CLI	1 (0.6)	0	1 (2.4)	0

MRSA, methicillin-resistant *Staphylococcus aureus*; CO-MRSA, community-onset MRSA infection; CA, community-acquired; HCA, healthcare-associated; HA-MRSA, hospital-acquired MRSA infection; CG-MRSA, community-genotype MRSA; HG-MRSA, hospital-genotype MRSA; OXA, oxacillin; TET, tetracycline; ERY, erythromycin; GEN, gentamicin; CIP, ciprofloxacin; CLI, clindamycin.
[a] The pulsotypes related to USA300 were appointed as U and the pulsotypes related to Cordobés/Chilean clone were appointed as F.
[b] CG-NR = isolated CG-MRSA with pulsotype not related to USA300 clone; HG-NR = isolated HG-MRSA with pulsotype not related to Cordobés/Chilean clone.

in 92 CG-MRSA isolates (59.7%). The majority of the CG-MRSA isolates were related to CC8 (98.7%), and five representative isolates of the main pulsotypes belonged to sequence type (ST) 8. The *spa* type was determined for 20 isolates (selected from the main PFGE pulsotypes; Figure 1). Of these, seven selected isolates of pulsotypes U1, U2, U3, U4, and U6 were *spa* type t008 and four isolates with pulsotype U5 were *spa* type t024. Among the 11 CG-MRSA isolates not related to USA300 (CG-NR), the *spa* types found were: t024, t1814, and t1635 in one, one, and nine isolates, respectively. The *spa* type t1635 was found in eight isolates that belonged to a new emergent clone.[22]

Molecular characterization of the eight HG-MRSA isolates revealed that six isolates (75.0%) were SCC*mec* type I, one (12.5%) was type II, and one (12.5%) was non-typeable. All eight isolates had *sem* and *seo* genes, seven were *agr* group II and one was *agr* group I. Seven (87.5%) isolates were related to CC5; the clonal complex of the remaining isolate was not determined (Table 2). The restriction and PFGE analyses showed that five (62.5%) isolates were genetically related to the Cordobés/Chilean clone and the three remaining isolates (37.5%) produced pulsotypes unrelated to the Cordobés/Chilean, Pediatric, Brazilian, or New York/Japan clones, which frequently circulate in South American hospitals (Table 2). A representative isolate of pulsotype F harbored *spa* type t149 (TO2MEMDMGMGMK) related to that of the Cordobés/Chilean clone (TIMEMDMGMGMK).

3.2. Microbiological characteristics of MRSA isolates

Although most of the CG-MRSA isolates (83, 53.9%) were only resistant to oxacillin (not resistant to non-β-lactam antibiotics), resistance to tetracycline, erythromycin, gentamicin, clindamycin, and ciprofloxacin was also found in 61 (39.6%), 17 (11.0%), four (2.6%), three (1.9%), and one (0.6%) isolates, respectively. Resistance to trimethoprim–sulfamethoxazole, vancomycin, linezolid, chloramphenicol, and rifampin was not found. Three CG-MRSA isolates (1.9%) were multidrug-resistant (resistant to more than three antibiotics). Of the 16 (10.4%) CG-MRSA isolates resistant to erythromycin, 11 (7.1%), three (1.9%), and two (1.2%) had M, constitutive, and inducible MLS_B phenotype, respectively. Six (75.0%) of the eight HG-MRSA isolates were multidrug-resistant.

3.3. Clinical and epidemiological classification

It was found that of the 162 patients included in the study, infection had initiated in the community (with or without HARF) in 139 of the patients (85.8%) and infection had initiated in the hospital (HA-MRSA) in 23 of the patients (14.2%). Among community-onset MRSA infections, 97 (69.7%) occurred in patients 69,8% who had no HARF (CA-MRSA) and 42 (30.2%) were associated with healthcare (HCA-MRSA). According to the molecular classification, 97 (100%) CA-MRSA, 41 (97.6%) HCA-MRSA, and 16 (69.6%) HA-MRSA infections were caused by CG-MRSA isolates (Table 2).

4. Discussion

In Colombia, the first two cases of CG-MRSA infection in adult patients were reported in 2006,[8] and in 2009, the circulation of this USA300 variant (ST8-MRSA-IVc-PVL^+-ACME-negative) was found in 31% of adult patients.[9] Likewise, in 2010, we reported that 26.4% of MRSA infections in Colombian adults were caused by CG-MRSA.[27] The findings from US hospitals suggest similar frequencies among CG- and HG-MRSA isolates in adult patients;[1,28] in

pediatric patients, there is a slightly greater proportion of CG-MRSA.[29] However, our results showed that almost all infections in the Colombian pediatric population were generated by CG-MRSA isolates. These results show that MRSA infections in children are quite different in our country. This may be due to an improved competitive advantage for the CG-MRSA USA300-related clone in this population.

The significance of PVL and ACME in the increased virulence and pathogenicity of USA300 is controversial. Kreisel et al. found that 97% of USA300 isolates causing bacteremia in adult patients had PVL and suggested its role as a virulence factor.[30] In the case of infections in our pediatric population, the almost ubiquitous presence of PVL in CG-MRSA isolates could favor its successful spread, although it would be necessary to conduct further studies to clarify the determinants of this high proportion.

In contrast to the USA300 clone circulating in the USA, the CG-MRSA clones circulating in our region harbor SCC*mec* type IVc (3.1.2) and do not carry ACME. It was previously believed that ACME was contributing to the ease of spread of USA300 by skin contact.[31] However, ACME-negative USA300 strains are frequently found in Australia, Colombia, and other South American countries.[8,9,27,31] Although Australian isolates differ by the presence of the *mer* operon at this position, we did not find DNA insertions upstream or downstream of SCC*mec* in the Colombian strains (data not shown).

In a study of the epidemiological distribution of ACME in *S. aureus* with pulsotypes closely related to the USA300 clone, all SCC*mec* type IVa isolates were found to carry ACME, in contrast to isolates with SCC*mec* IVc or IVb, or in non-typeable isolates in which ACME was not detected.[32] These results are in agreement with our current results. However, in our case, ACME was also not detected in the isolates with SCC*mec* IVa. It is possible that the Colombian isolates possess another type of gene or mobile genetic element that compensates for the absence of ACME. Therefore, further analysis to determine differences in our isolates would be enlightening.

The microbiological analyses showed that a high percentage of isolates were susceptible to most non-β-lactam antibiotics, a result that is in agreement with the previous reports for CG-MRSA isolates. However, these isolates were also detected in high proportions in hospital infections and in patients with HARF, who are frequently treated with antibiotics, which generates the danger of the emergence of multiple resistance in strains with community genotypes that are usually more virulent.

The high frequency of CG-MRSA infections in children in the Colombian pediatric population in community and hospital settings should be noted. Furthermore, molecular analyses revealed genetic differences between clones related to the USA300 strains circulating in Colombia and those circulating in the USA and elsewhere in the world, raising new questions about the features that are shared or that differentiate these clones contributing to their successful propagation.

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