



Molecular Characterization of *Staphylococcus aureus* Obtained from Blood Cultures of Paediatric Patients Treated in a Tertiary Care Hospital in Mexico


Guillermo Jose Vazquez-Rosas ^{1,2}

Jocelin Merida-Vieyra ¹

Gerardo Aparicio-Ozores ²

Antonino Lara-Hernandez³

Agustin De Colsa ^{1,4}

Alejandra Aquino-Andrade ¹

¹Molecular Microbiology Laboratory, Instituto Nacional de Pediatría, Mexico City, Mexico; ²Medical Bacteriology Laboratory, Instituto Politécnico Nacional, Mexico City, Mexico;

³Bacteriology Laboratory, Instituto Nacional de Pediatría, Mexico City, Mexico; ⁴Department of Paediatric Infectious Diseases, Instituto Nacional de Pediatría, Mexico City, Mexico

Purpose: *Staphylococcus aureus* is one of the main causative agents of hospital-acquired (HA) infections. In Mexico, information about the characteristics of clinical *S. aureus* isolates is limited. Our aim was to characterize *S. aureus* strains obtained from blood cultures of paediatric patients treated in a tertiary care hospital.

Materials and Methods: We analysed 249 *S. aureus* isolates over the period from 2006 to 2019, and their resistance profiles were determined. The isolates were classified into methicillin-resistant *S. aureus* (MRSA) or methicillin-sensitive *S. aureus* (MSSA). Staphylococcal cassettes chromosome *mec* (SCC*mec*) were detected. Virulence genes (*cna*, *clfA*, *clfB*, *eta*, *etb*, *fnbA*, *fnbB*, *hla*, *pvl*, *sec*, and *tsst*) were amplified, and their clonal relationships were established by pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST) and clonal complex (CC) typing. We reviewed one hundred medical files to collect clinical information.

Results: Thirty-eight percent of the isolates were MRSA and showed an expanded profile of resistance to other non-beta-lactam antibiotics, while MSSA strains presented a reduced resistance profile. SCC*mec*-II was the most frequent element (86.3%). Eight virulence factors were detected in MSSA and six in MRSA. The *pvl* gene was detected in four MRSA-SCC*mec*-IV isolates ($P \leq 0.0001$). MRSA isolates were distributed among 14 clones and were classified into 15 sequence types (ST); the most frequent was ST1011 (17%). The most common CC in MRSA was CC5 (69%, $P \leq 0.0001$), and in MSSA, it was CC30 (30%, $P \leq 0.0001$). Eighty-seven percent of MRSA isolates were HA-MRSA, and 13% were community-acquired MRSA (CA-MRSA). Of 21 HA-MRSA isolates, 17 had SCC*mec*-II, while two CA-MRSA isolates had SCC*mec*-IV. Of MSSA isolates, 77% were derived from HA infections and 23% from CA infections.

Conclusion: MSSA isolates had more virulence factors. MRSA isolates were resistant to more non-beta-lactam antibiotics, and those with SCC*mec*-IV expressed a greater variety of virulence factors. Most *S. aureus* isolates belonged to CC5.

Keywords: MSSA, MRSA, virulence factors, clonal complex, SCC*mec*-II, CC5

Introduction

Among Gram-positive bacteria, *Staphylococcus aureus* is the main causative agent of hospital-acquired (HA) and community-acquired (CA) infections. *S. aureus* can cause mild skin and soft tissue infections and severe infections, including bacteraemia, sepsis, endocarditis, and osteomyelitis.¹ One of the main challenges in the treatment of these infections is antibiotic resistance. Although the worldwide average prevalence of MRSA is 40%,² there are vast differences among different

Correspondence: Alejandra Aquino-Andrade
Molecular Microbiology Laboratory,
Instituto Nacional de Pediatría,
Insurgentes Sur 3700-C, Col. Insurgentes
Cuicuilco, Alcaldía Coyoacán, C.P. 04530,
Mexico City, Mexico
Tel +52 55 1084-0900 Ext. 1859
Email aaquinoa@pediatria.gob.mx

geographical locations: in Latin America, the reported prevalence is between 6–80%,³ in Mexico, 52–57%,⁴ in China, 50%,⁵ and in Europe, 0.9–26.8%.⁶ Molecular characterization of *S. aureus* has become a tool for the investigation and detection of circulating and epidemic clones both in the hospital and in the community. These clones can be typed based on SCCmec, MLST, CC, PFGE and the presence of virulence factors, namely, Panton–Valentine leucocidin (PVL).⁷ HA infections are associated with MRSA clones with SCCmec elements I, II and IV of ST5 and CC5.⁸

In Mexico, there is limited information about the molecular characteristics of MRSA associated with bacteraemia in paediatric patients. Some studies have analysed infections caused by *S. aureus* and comorbidities, such as cancer, and their clinical implications and have also classified the isolates based on their susceptibility profiles.^{9–11} In a tertiary care adult hospital, 444 linezolid (LZD)- and vancomycin (VAN)-sensitive MRSA isolates were studied; all had SCCmec-II.¹² In a report from Latin America, 538 MRSA isolates were typed; 17 isolates from a single hospital in Mexico had SCCmec-II and were classified as USA100 and ST5.³ Our aim was to characterize 249 *S. aureus* isolates obtained from blood cultures of paediatric patients treated in a tertiary care hospital over a 14-year period.

Materials and Methods

Study Setting

Our study was conducted at the Instituto Nacional de Pediatría (INP), which is a tertiary care paediatric hospital with 235 beds, 40 subspecialties and 6981 discharges in 2017.

Biological Material

A total of 249 nonduplicate *S. aureus* isolates obtained from blood cultures of paediatric patients (0 to <18 year-old) with documented bacteraemia from 2006 to 2019 were analysed. The distribution of the isolates by year was as follows: 2006 (n=24), 2007 (n=24), 2008 (n=24), 2009 (n=21), 2010 (n=23), 2011 (n=22), 2012 (n=15), 2013 (n=21), 2014 (n=15), 2015 (n=35), 2016 (n=21), 2018 (n=2), and 2019 (n=2). In 2017, we did not obtain any *S. aureus* isolates. We defined bacteraemia as positive peripheral blood cultures obtained from a patient with signs and symptoms of infection.

Identification

The isolates were identified using a BD Phoenix semiautomated microbiology system (Becton Dickinson, Franklin Lakes, New Jersey, USA). DNA extraction was performed with the QIAmp DNA mini[®] kit (Qiagen, Hilden, North Rhine–Westphalia, Germany). The DNA was eluted and stored at –20 °C until use. Identification as *S. aureus* was corroborated by detection of the *nuc* gene¹³ and by amplification, sequencing, and analysis of the 16S rRNA gene.^{14–16} AmpliTaq Gold[®] 360 Master Mix (Applied Biosystems[™], Foster City, California, USA) was used in all the reaction mixtures.

Resistance Profile

A disk diffusion test was performed for ceftiofloxacin (FOX), gentamicin (GEN), ciprofloxacin (CIP), clindamycin (CLI), erythromycin (ERI), trimethoprim with sulfamethoxazole (SXT) and LZD using sensidiscs (Becton Dickinson, Franklin Lakes, Nueva Jersey, USA), and a broth microdilution test was performed for FOX (Sigma Aldrich, St. Louis, Missouri, USA) following the 2019 guidelines of the Clinical and Laboratory Standards Institute (CLSI).¹⁷

Molecular Characterization of MRSA Isolates

The presence of the *femA* and *mecA* genes was confirmed by PCR.¹⁸ Detection of the *vanA* gene was also performed.¹⁹ SCCmec elements were identified by multiplex PCR (mPCR).²⁰ The presence of genes encoding virulence factors necessary for colonization (*fnbA*, *fnbB*, *clfA*, *clfB* and *cna*), invasion (*hla* and *pvl*), toxins (*sec*, *eta* and *etb*), and superantigen (*tsst*) was detected.^{21–23} The GeneAmp[™] PCR System 9700 was used for all PCRs (Applied Biosystems[™], Foster City, California, USA). We used *S. aureus* ATCC[®] 43300[™] and *Enterococcus faecium* ATCC[®] 29212[™] as positive controls.

PFGE typing was performed using the CHEF Mapper XA System (Bio-Rad, Hercules, California, USA) following the guidelines established in the PulseNet protocol for MRSA from the Centers for Disease Control and Prevention.²⁴ Analysis of clonal relationships was carried out using the Tenover criteria.²⁵ A dendrogram was constructed using the program DendroUPGMA.^{26,27}

ST detection was performed by MLST.²⁸ The sequences obtained were compared with those reported in the *S. aureus* MLST online database from the

University of Oxford.^{29,30} The six most widely distributed CCs were determined using mPCR.³¹

Clinical Data

We reviewed the medical files to collect clinical information, such as age, sex, comorbidity, primary infectious focus (PIF), clinical complications, outcome, length of hospital stay, and antibiotic treatment. An infection was considered HA if the date of the event of the site-specific infection criterion occurred on or after the 3rd calendar day of admission to an inpatient location where the day of admission was calendar day 1.³² We categorized the age as follows: term neonatal (birth-27 days), infant (28 days-12 months), toddler (13 months-2 years of age), early childhood (2-5 years of age), middle childhood (6-11 years of age), and early adolescence (12-<18 years of age).³³ To standardize the duration of treatment in all cases, the day of blood culture collection was taken as day zero.

Statistical Analysis

We compared the overall group infected with MRSA and those infected with MSSA. JPM 11 software (SAS Institute Inc., Cary, NC, USA) was used. The variables were described as frequencies and percentages. Categorical variables were compared using Pearson's χ^2 test. A value of $P < 0.05$ was considered statistically significant.

Results

Detection of the *nuc*, 16S rRNA, *femA*, *mecA* and *vanA* Genes and Identification of the SCCmec Elements

The *nuc* gene was amplified from 245 isolates, while the 16S rRNA gene was sequenced from four isolates, confirming the 249 isolates as *S. aureus*. The *femA* gene was detected in 176 (70.6%) isolates: 91 were MSSA and 85 were MRSA.

The *vanA* gene was not detected in any isolate. The *mecA* gene was amplified in 95 isolates (38.1%), and these isolates were classified as MRSA. Three different SCCmec elements were found: SCCmec-I (3.1%, n=3), SCCmec-II (86.3%, n=82), and SCCmec-IV (9.4%, n=9). In one isolate, it was not possible to determine the SCCmec element with the primers used in this study (Figure 1). Over the years, the MRSA isolates decreased in frequency, while the MSSA isolates increased (Figure 2).

Susceptibility Profile

The 95 MRSA isolates presented the following susceptibility profile: GEN 88.4% (n=84), CIP 5.2% (n=5), ERI 4.2% (n=4), CLI 8.4% (n=8), and SXT 92.6% (n=88); all the isolates were sensitive to LZD. In the 154 isolates classified as MSSA, the following susceptibility profile was obtained: GEN 96% (n=148), CIP 89% (n=138), ERI 57% (n=92), CLI 74% (n=123), SXT 98% (n=152), and LZD 100% (n=154). Twenty-two MSSA isolates with inducible resistance to CLI were detected (Table 1). ERI, CIP and CLI resistance was observed in the MRSA isolates ($P \leq 0.0001$).

Virulence Profile

A gene that promotes colonization (*fnbA*) and a gene that favours invasion (*hla*) were the most frequently observed virulence genes. Only four MRSA isolates had the *pvl* gene in which SCCmec-IV was detected (Table 2). The *clfA* and *clfB* genes were not detected in any isolate. The *pvl* gene was more frequent in the MRSA SCCmec-IV ($P \leq 0.0001$).

Determination of Clonality

The 95 MRSA isolates were distributed among 14 clones by PFGE and were assigned letters A to N; 50% of these isolates were grouped into clones A B and C and contained SCCmec-II. Fifteen STs were determined to be distributed among all clones, the most frequent being ST1011 (17%, n=4), ST5 (13%, n=3) and ST5529 (13%, n=3). MRSA-SCCmec-IV belonged to ST8, ST4335, ST544, ST1092, ST4732 and ST30, and the *pvl* gene was amplified in only two ST4335 isolates. Among the total isolates, six CCs were detected, which were distributed as follows: 44.9% (n=112) CC5, 19.6% (n=49) CC30, 10.8% (n=27) CC45, 5.2% (n=13) CC8, 1.6% (n=4) CC22, and 0.8% (n=2) CC1. CCs were not identified in 42 (16.8%) *S. aureus* isolates.

The MRSA isolates (n=95) were grouped mainly into CC5 69.4% (n=66), CC8 8.4% (n=8), CC45 4.2% (n=4), CC30 2.1% (n=2), and CC22 1% (n=1); the type of CC was not classified in 14.7% (n=14) of the isolates (Figure 3). CC5 was statistically significant in the MRSA isolates ($P \leq 0.0001$). The 154 MSSA isolates could not be classified into clones since they presented a great diversity of patterns obtained by PFGE, and the distribution of their CCs was different; CC30 was the most common with 30.5% (n=47), followed by CC5 with 29.8% (n=46),

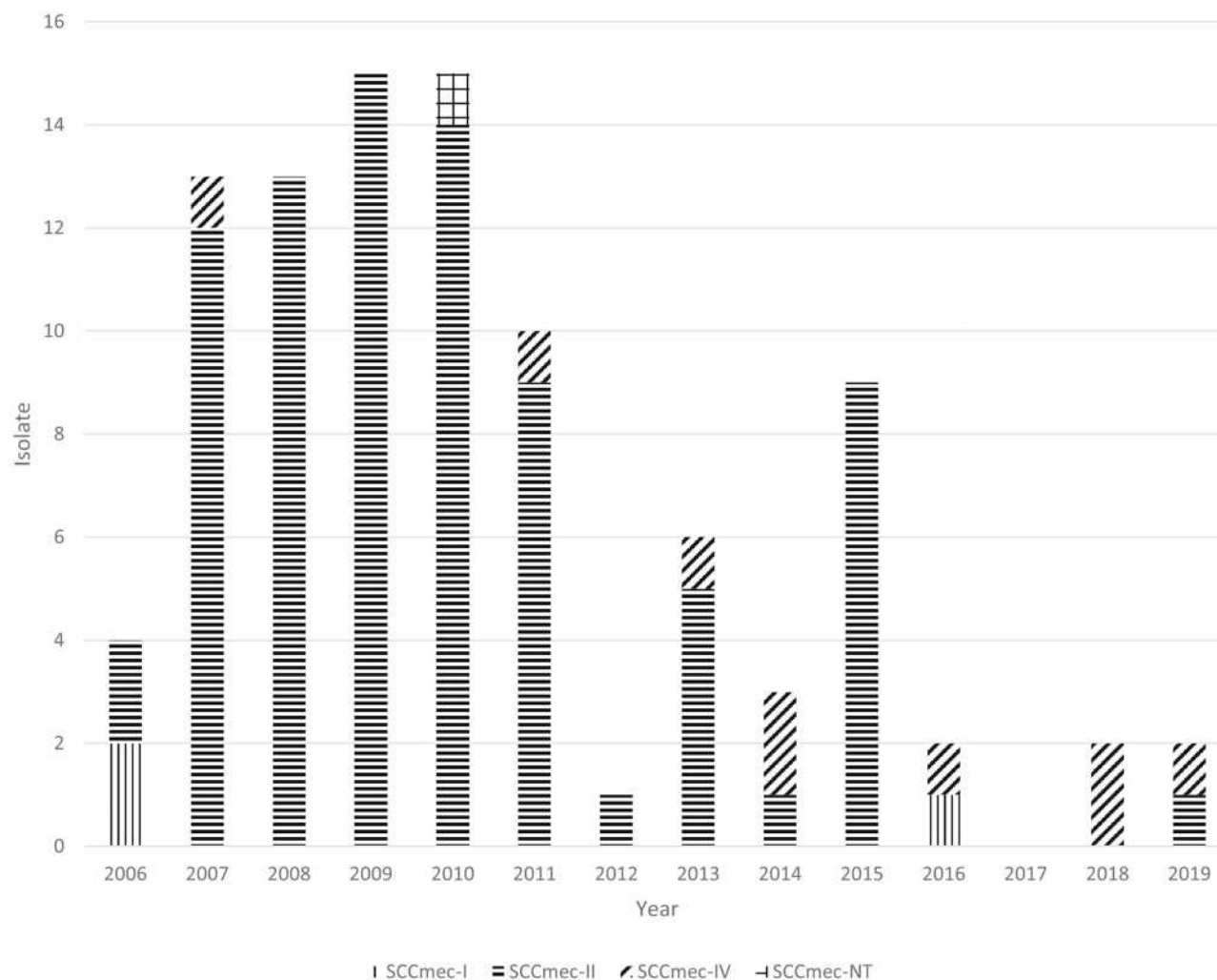


Figure 1 Distribution of SCCmec by year. A predominance of SCCmec-II was observed from 2006 to 2013 and in 2015. The first occurrence of SCCmec-IV was detected in 2007.

CC45 with 14.9% (n=23), CC8 with 3.2% (n=5), CC22 with 1.9% (n=3), and CC1 with 1.2% (n=2). CCs could not be identified in 18.1% (n=28) of the MSSA isolates. CC30 was statistically significant in the MSSA isolates ($P \leq 0.0001$).

Clinical Data

Of the 249 *S. aureus* isolates, clinical information was obtained for 100 of the patients from whom they were isolated. Twenty-four isolates were MRSA and 76 were MSSA. Seventy-nine percent of the patients with MRSA infections presented with comorbidities; among the most important of which were oncological diseases (16%, n=4), nephropathies (16%, n=4), and neuropathies (16%, n=4). Central venous catheter (CVC) was identified as the main PIF (66%, n=16). Eighty-seven percent (n=21) of the

infections were HA-MRSA, and 13% (n=3) were CA-MRSA. Forty-five percent (n=11) of the patients presented with complications derived from the infection and sepsis was the main complication, with 82% (n=9), followed by septic shock, with 18% (n=2). Twelve percent (n=3) of the patients died, and two deaths were related to the infection. The definitive treatment for infections caused by MRSA was VAN (79%, n=19), followed by teicoplanin (TEC) with 20% (n=5) (Table 3).

On the other hand, of the 76 patients with infections caused by MSSA, 81% (n=62) had a comorbidity, and the most frequent were oncological diseases, with 51% (n=33). As in MRSA, a CVC was the main source of infection (47%, n=36). Seventy-seven percent (n=58) were HA-MSSA, and 23% (n=18) were CA-MSSA. Thirty-eight percent (n=27) of the patients presented with

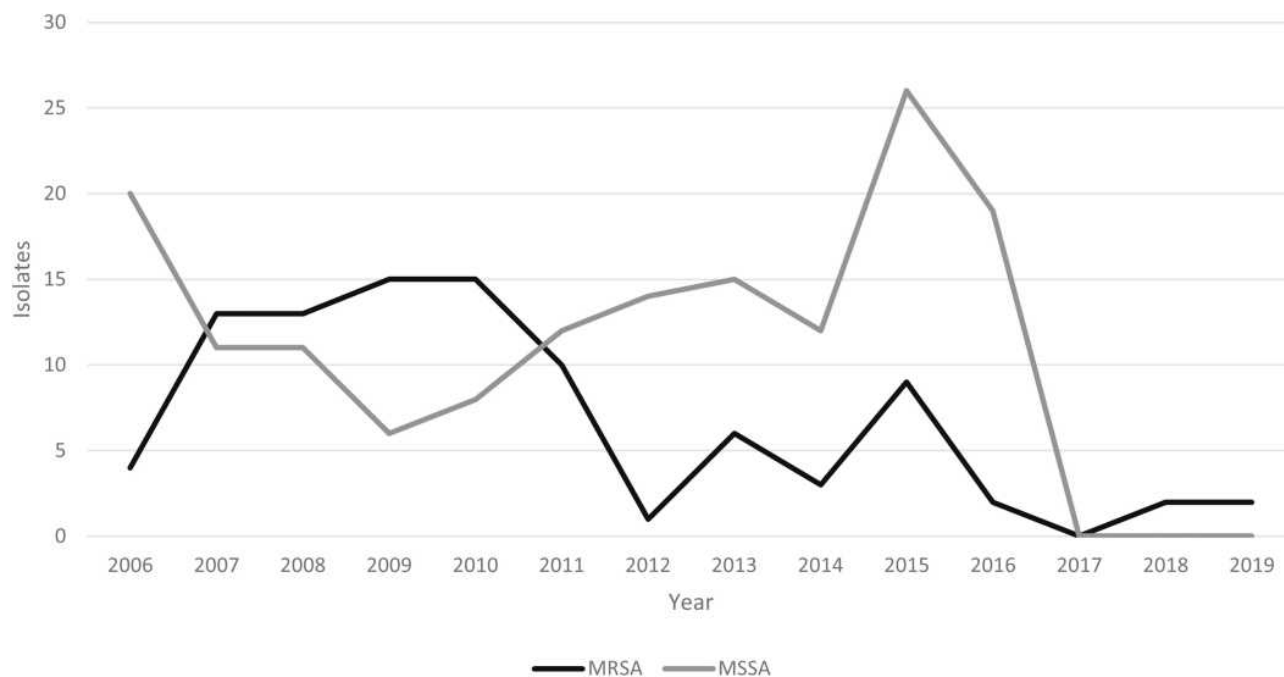


Figure 2 Frequency of MRSA and MSSA. A decrease in the frequency of MRSA can be observed since 2011, while there was an increase in MSSA from 2011 to 2016.

complications derived from the infection, and sepsis was the main complication, with 58% (n=17), followed by septic shock, 20% (n=6). Thirteen percent (n=10) of the patients died; two deaths were associated with the infection. The definitive treatment for infections caused by MSSA was dicloxacillin (DC) in 61% (n=47), followed by VAN in 52% (n=40) and ceftriaxone (CRO) in 27% (n=21) (see [Supplementary material](#)).

Discussion

This study describes the main characteristics of a collection of *S. aureus* isolates obtained from blood cultures over 14 years in a tertiary care paediatric hospital in Mexico; 38.1% of these isolates were MRSA. The average frequency of MRSA worldwide is 40%,² but this frequency can vary among different regions. In the US, the frequencies of infections caused by MRSA range from 23.7–45%;³⁴ in Europe, 0.9–26.8%;^{6,35} in Asia, 39.6–56.6%;³⁶ in Latin America, 6–80%;³ and in Mexico, a frequency of 52–57% has been reported.⁴ A decrease in the frequency of MRSA worldwide from 45–40% has been observed,² which may be due to different factors, for instance, the type of hospital, the origin of the isolates and the patient characteristics. In addition, this decrease may be related to the implementation of surveillance programmes that in some countries are very well structured, including the search and destroy policy of

carriers in the Netherlands, the enhanced mandatory surveillance programme in the United Kingdom, and the nationwide MRSA Prevention Initiative in the U.S.³⁷ However, there is not enough evidence to suggest that these programmes are solely responsible for this phenomenon.^{37–39} The common factor among the different studies is that control measures should always be accompanied by a programme for compliance with hand hygiene.⁴⁰ Increases in the hospital frequency of MRSA could be related to the presence of outbreaks, such as the event that occurred in a cancer hospital in Mexico, where an increase in MRSA isolates from 4–20.4% was observed in 2014 due to an outbreak, which was controlled by a programme that reinforced hand hygiene.⁹

In our study, a decrease in the frequency of MRSA was observed in recent years (2011–2019). It is important to mention that this could be the result of the implementation of a permanent monitoring programme for adequate hand-washing in 2013.⁴¹ However, there is no surveillance and eradication programme for carriers of this pathogen.

The decrease in the frequency of MRSA and the increase in MSSA, as well as other *Staphylococcus* spp. that are causative agents of bacteraemia, has been associated with a greater diversity of virulence factors in these groups, favouring colonization and invasion.^{42,43} Our study focused on isolates from blood cultures of paediatric patients, and we observed a decrease in the presentation of

Table 1 Susceptibility Profile of *S. aureus* Isolates

Isolates	Agent															
	FOX (%)		GEN (%)		ERI (%)		CLI (%)		CIP (%)		STX (%)					
	S	R	S	R	S	I	R	S	I	R	S	I	R			
<i>S. aureus</i> n=249	154(61.8)	95(38.1)	232(93.1)	17(6.8)	92(36.9)	17(6.8)	140(56.2)	123(49.3)	6(2.4)	120(48.1)*	143(57.4)	10(4)	96(38.5)	240(96.3)	6(2.4)	3(1.2)
MSSA n=154	154(61.8)	0	148(59.4)	6(2.4)	88(35.3)	17(6.8)	49(19.6)	115(46.1)	6(2.4)	33(13.2)	138(55.4)	9(3.6)	7(2.8)	152(61)	1(0.4)	1(0.4)
MRSA n=95	0	95(38.1)	84(33.7)	11(4.4)	4(1.6)	0	91(36.5)	8(3.2)	0	87(34.9)	5(2)	1(0.4)	89(35.7)	88(35.3)	5(2)	2(0.8)
SCCmec-I n=3	0	3(1.2)	3(1.2)	0	1(0.4)	0	2(0.8)	3(1.2)	0	0	1(0.4)	0	0	3(1.2)	0	0
SCCmec-II n=82	0	82(32.9)	74(29.7)	8(3.2)	0	0	82(32.9)	0	0	82(32.9)	1(0.4)	0	81(32.5)	77(30.9)	5(2)	0
SCCmec-IV n=9	0	9(3.6)	7(2.8)	2(0.8)	3(1.2)	0	6(2.4)	5(2)	0	4(1.6)	3(1.2)	1(0.4)	5(2)	8(3.2)	0	1(0.4)
SCCmec-ND n=1	0	1(0.4)	0	1(0.4)	0	0	1(0.4)	0	0	1(0.4)	0	0	0	0	0	1(0.4)

Notes: *Twenty-two *S. aureus* isolates with inducible resistance to CLI were detected; all of them were MSSA. Abbreviations: S, susceptible; I, intermediate; R, resistant; FOX, cefoxitin; GEN, gentamicin; ERI, erythromycin; CLI, clindamycin; CIP, ciprofloxacin; LZD, linezolid; STX, trimethoprim with sulfamethoxazole; MSSA, methicillin-sensitive *Staphylococcus aureus*; MRSA, methicillin-resistant *Staphylococcus aureus*; SCCmec, staphylococcal cassette chromosome *mec*.

MRSA, similar to the global trend, so it is important to continue studying and monitoring this pathogen.

The treatment of choice for MRSA infections should focus on the susceptibility profile, age group, PIF and comorbidities. Although resistance to beta-lactams in *S. aureus*, which is mediated by *mecA*, limits some therapeutic options, it has been observed in different studies that there are still alternatives for the treatment of bacteraemia, such as LZD, daptomycin (DAP) and SXT.^{3,44} Our results also indicate that there is a high susceptibility to antibiotics, including oxazolidinones (LZD 100%) folate inhibitors (SXT 96.3%) and glycopeptides (VAN 100%). In turn, MRSA remains susceptible to lincosamides, which are second-line treatments for MRSA, at levels close to 50% of susceptibility.^{45,46} Although VAN is the first-choice antimicrobial for MRSA bacteraemia,⁴⁶ there are other treatment alternatives, such as LZD, SXT and CLI, in monotherapy or in combination with DAP or ceftaroline (CPT).⁴⁵⁻⁴⁸ Therefore, interpretive reading of the antibiograms and the usage of first-choice antimicrobials are essential to reduce the risk of therapeutic failure and increase resistance rates.

The *SCCmec* elements allow to classify the MRSA strains into HA and CA.⁴⁹ A change was observed in the distribution of the *SCCmec* elements in our hospital. Type II decreased, while type IV was detected more frequently in recent years. In some regions of the world, there has been a decrease in cassettes I, II and III, historically associated with HA infections, and an increase in cassettes IV and V (associated with CA infections). This exchange has been widely described in the U.S.,⁵⁰ Iran⁵¹ and South Africa.⁵²

Currently, the detection of *SCCmec* elements and their classification is not sufficient to determine the best treatment, since the search for virulence factors is also important. In several studies, MRSA isolates have a greater variety of virulence genes, among which the presence of *pvl*, *tsst* and *sea* stand out, and these are mainly associated with MRSA isolates with *SCCmec-IV*.⁵³⁻⁵⁶

The frequency of *pvl* in MRSA varies from 9-30%; in Iran (9.7%), South Africa (14%), the US (26%), and China (30%).^{50-52,55} In the current study, we found the *pvl* gene in four isolates (1.6%), all with *SCCmec-IV*. In 2016, the first MRSA *SCCmec-IV* isolate with *pvl* was detected. It is important to monitor the change in the distribution of *SCCmec* elements because in other countries, in particular the US, MRSA *SCCmec-IV* isolates reach 28%, China 61%, South Africa 48% and Japan 52.3%.^{50-53,55} PVL is

Table 2 Virulence Profile of *S. aureus* Isolates

Isolates	Virulence Factors								
	Colonization			Invasion		Toxins			Superantigen
	<i>fnbA</i> (%)	<i>fnbB</i> (%)	<i>cna</i> (%)	<i>hla</i> (%)	<i>pvl</i> (%)	<i>sec</i> (%)	<i>eta</i> (%)	<i>etb</i> (%)	<i>tsst</i> (%)
<i>S. aureus</i> n=249	207(83.1)	18(7.2)	16(6.4)	186(74.6)	4(1.6)	7(2.8)	5(2)	1(0.4)	38(15.2)
MSSA n=154	135(54.2)	11(4.4)	16(6.4)	113(45.3)	0	6(2.4)	5(2)	1(0.4)	37(14.8)
MRSA n=95	72(28.9)	7(2.8)	0	73(29.3)	4(1.6)	1(0.4)	0	0	1(0.4)
SCCmec-I n=3	3(1.2)	2(0.8)	0	3(1.2)	0	0	0	0	0
SCCmec-II n=82	63(25.3)	0	0	64(25.7)	0	1(0.4)	0	0	0
SCCmec-IV n=9	6(2.4)	5(2)	0	5(2)	4(1.6)	0	0	0	1(0.4)
SCCmec-ND n=1	0	0	0	1(0.4)	0	0	0	0	0

Notes: None of isolates harbored *dfA* and *dfB*.

Abbreviations: MSSA, methicillin-sensitive *Staphylococcus aureus*; MRSA, methicillin-resistant *Staphylococcus aureus*; SCCmec, staphylococcal cassette chromosome mec; ND, not determined.

one of the main virulence factors that complicate the clinical features and the therapeutic approach, since prolonged treatment of MRSA infections, producing this

toxin, with VAN can lead to therapeutic failure, so it is necessary to use combinations, such as LZD and CLI, to inhibit the production of PVL.^{57,58}

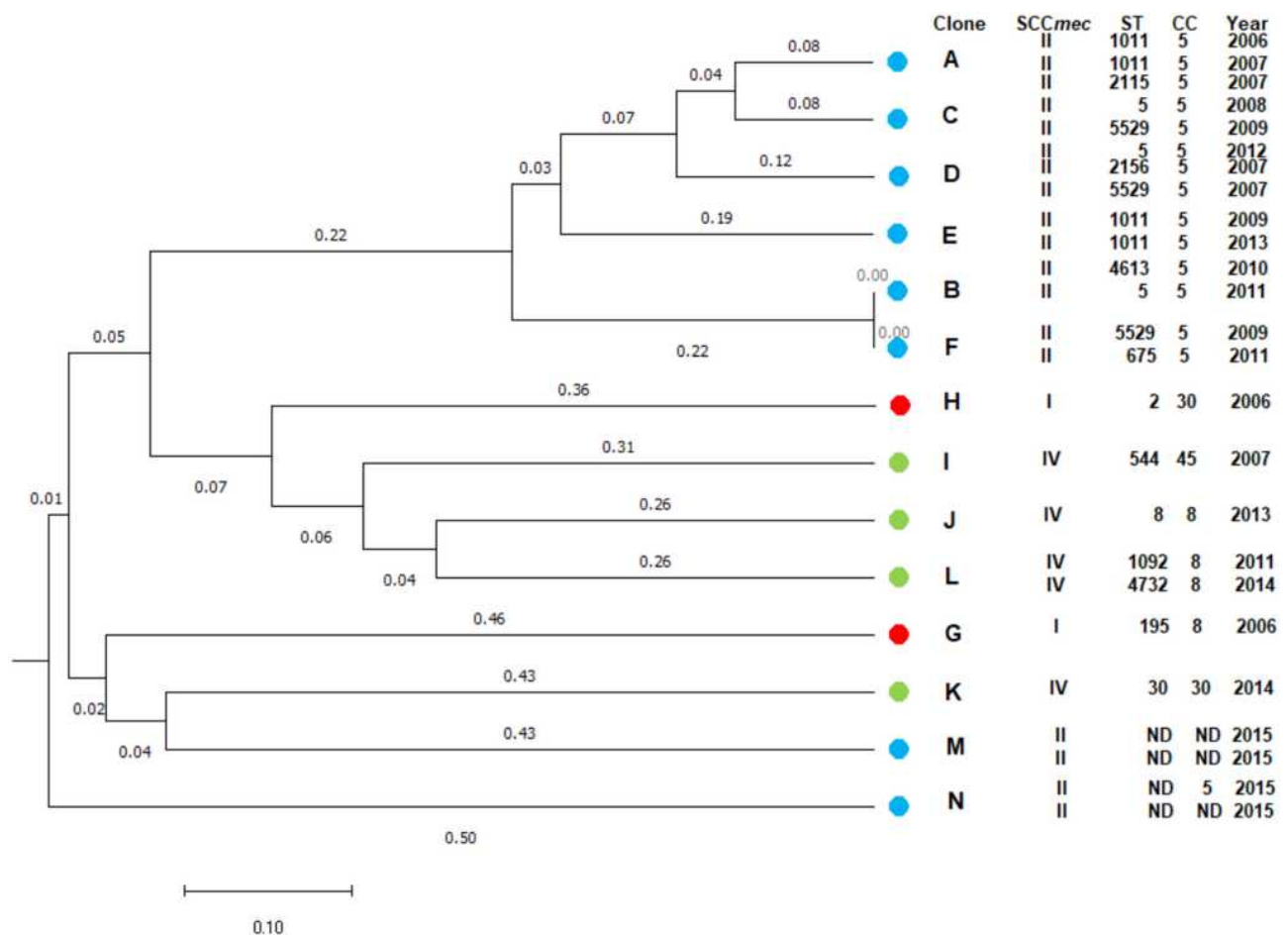


Figure 3 Characterization of the main *S. aureus* clones. SCCmec-II was found in eight of the 14 main clones, followed by SCCmec-IV in four of the 14 clones. Between 2016 and 2019, we obtained six MRSA isolates: two in 2016 (CC45 and CC8), two in 2018 (CC8 and ND) and two in 2019 (both CC45). None of these isolates clustered in a clone by PFGE.

Abbreviations: SCCmec, staphylococcal cassette chromosome mec; ST, sequence type; CC, clonal complex; ND, not determined.

Table 3 Clinical Data of MRSA Isolates

Isolate	Age	Sex	Year	SCCmec	Comorbidity	PIF	Acquired	Complication	Outcome	LHS	Treatment						
											DC	CRO	CTX	CLI	LZD	VAN	TEC
Sa-125	Toddler	F	2011	II	Neurological disorder	CVC	HA	Sepsis	Alive	105						0 to +24	0 to +26
Sa-142	Infant	M	2011	II	Hematological disorder	CVC	HA	Sepsis	Alive	30						0 to +24	
Sa-158	Infant	M	2012	II	Congenital disease	CVC	HA	Sepsis	Alive	42						0 to +10	
Sa-174	Middle childhood	M	2013	II	Immunological disorder	CVC	HA	Sepsis	Alive	86						+3 to +16	
Sa-177	Infant	F	2013	II	Neurological disorder	CVC	HA	None	Alive	101						0 to +21	
Sa-178	Infant	M	2013	II	Gastrointestinal disorder	CVC	HA	None	Alive	53						-1 to +9	
Sa-187	Infant	F	2013	II	Gastrointestinal disorder	CVC	HA	Sepsis	Death	40						0 to +10	
Sa-189	Early adolescence	M	2013	IV	Nephropathy	CVC	HA	Sepsis	Alive	9							0 to +7
Sa-190	Infant	M	2013	II	Oncological disorder	CVC	HA	Sepsis	Alive	64	0 to +1		+2 to +5			+3 to +21	
Sa-197	Middle childhood	M	2014	IV	Hematological disorder	CVC	HA	Sepsis	Alive	63						-3 to +33	
Sa-206	Early adolescence	F	2014	II	Oncological disorder	CVC	HA	Sepsis	Alive	31						-3 to +10	
Sa-218	Middle childhood	F	2015	II	Nephropathy	OC	HA	None	Alive	27							+6 to +20
Sa-221	Middle childhood	F	2015	II	Nephropathy	OC	HA	None	Alive	27							+1 to +15
Sa-222	Middle childhood	F	2015	II	Nephropathy	OC	HA	None	Alive	27							+1 to +15
Sa-226	Infant	M	2015	II	Oncological disorder	OC	HA	None	Alive	33						0 to +14	
Sa-241	Early childhood	M	2015	II	None	Surgical wound	HA	Septic shock	Death	44						0 to +22	
Sa-249	Early childhood	F	2015	II	Neurological disorder	CVC	HA	None	Alive	26		0 to +13				0 to +13	
Sa-250	Middle childhood	M	2015	II	Hematological disorder	CVC	HA	None	Death	80						+6 to +14	
Sa-252	Toddler	F	2015	II	Neurological disorder	CVC	HA	None	Alive	164		+2 to +23				+2 to +23	
Sa-272	Term neonatal	F	2016	IV	None	CVC	HA	None	Alive	28	+1		+6 to +16			+2 to +16	
Sa-276	Early adolescence	M	2018	IV	None	Bones	CA	Septic shock	Alive	44	0					+2 to +8	
Sa-279	Middle childhood	M	2018	IV	None	SSTI	CA	None	Alive	7						+1 to +19	+8 to +44
																+2 to +6	

Sa-280	Toddler	M	2019	IV	None	CSF	HA	None	Alive	48							+1 to +41
Sa-281	Early childhood	M	2019	II	Oncological disorder	CVC	CA	None	Alive	64							+1 to +20

Notes: Isolates with *pvl* gene are indicated in bold. Deaths associated with infection are indicated in italics. The day of blood culture collection was considered day zero.
Abbreviations: PIF, primary infectious focus; SCCmec, staphylococcal cassette chromosome mec; M, male; F, female; CVC, central venous catheter; OC, other catheter; CSF, cerebrospinal fluid; SSTI, skin and soft tissue infection; HA, hospital acquired; CA, community acquired; LHS, length of hospital stay; DC, dicloxacillin; CRO, ceftriaxone; CTX, cefotaxime; CLI, clindamycin; LZD, linezolid; VAN, vancomycin; TEC, teicoplanin.

The MSSA isolates showed a greater diversity of virulence factor genes compared to MRSA, and it has been shown that MSSA, by presenting a greater number of virulence genes and acquiring resistance to antibiotics such as macrolides and lincosamides, complicates the clinical management of patients.⁵⁹

The MRSA isolates with SCCmec-IV were more susceptible to antibiotics and several virulence genes were found, among which *pvl*, *fnbA* and *fnbB* stand out. The SCCmec-II isolates mainly harboured invasion genes, such as *hla* and *sec*. It is important to determine the presence of virulence genes in isolates, regardless of whether they are MSSA or MRSA, since it allows for a better therapeutic approach and consideration of the possible clinical complications that the patient may experience, including fulminant pneumonia, endocarditis, or sepsis.⁵⁷

The genetic characteristics of *S. aureus* have shown that each geographic region can have its own clonal distribution.⁶⁰⁻⁶² This discrimination cannot be solely achieved with the PFGE method, and other tools such as MLST and CC typing are needed. According to several studies, CC5 is the main CC detected in the Americas and Asia.^{55,63-65} We observed the same trend, since of the 14 clones, 50% were grouped into CC5 (69%), followed by CC8 (8.4%). CC5 is more common in MRSA with SCCmec-II, and CC8 has been associated with MRSA-SCCmec-IV isolates with PVL, which leads to more serious clinical conditions, in particular fulminant pneumonia or deep vein thrombosis.⁵⁸ In our study, 14.7% of the MRSA and 18% of the MSSA isolates could not be grouped into any CC because the method used only detected the six most common CCs distributed around the world.³¹ PFGE, MLST, CC, *spa* typing, SCCmec, CC, and virulence factor detection are methods used to determine the MRSA epidemiology, and this information can impact the treatments applied to patients. ST5 was one of the most common in our collection, which coincides with that reported in other Latin American countries, such as Brazil (89%) and Guatemala (95%), but differs from that reported in Colombia (79%) and Ecuador (72%), where ST8 occurs more frequently.³

To control the dispersion of MRSA in our hospital, we must implement a permanent surveillance programme to study its spread and continue to monitor the different genetic characteristics of MRSA.

Conclusion

The MRSA isolates were grouped into clones, while the MSSA did not have a clonal relationship; however, most

of the *S. aureus* isolates belonged to CC5, and the interpretation of the susceptibility profiles of the isolates showed that there are still first-line therapeutic options for the management of *S. aureus* infections in our hospital to control and prevent the emergence of new resistance strains.

MRSA was detected in 38.1% of the isolates from our hospital. The frequency of MRSA decreased over the years, while an increase in the number of MSSA was observed. SCCmec-II was the most common among the studied isolates; however, starting in 2016, the frequency of SCCmec-IV increased.

MRSA strains containing SCCmec-IV exhibited a greater variety of virulence genes related to colonization or invasion than those containing SCCmec-II. However, the *pvl* gene was only detected in 1.6% of the isolates.

Abbreviations

CA, Community-acquired; CA-MRSA, Community-acquired methicillin-resistant *Staphylococcus aureus*; CC, Clonal complex; CIP, Ciprofloxacin; CLI, Clindamycin; CLSI, Clinical and Laboratory Standards institute; CPT, Ceftaroline; CRO, Ceftriaxone; CVC, Central venous catheter; DAP, Daptomycin; DC, Dicloxacillin; ERI, Erythromycin; FOX, Cefoxitin; GEN, Gentamicin; HA, Hospital-acquired; HA-MRSA, Hospital-acquired methicillin-resistant *Staphylococcus aureus*; INP, Instituto Nacional de Pediatría; LHS, Length of hospital stay; LZD, Linezolid; MLST, Multilocus sequence typing; mPCR, Multiplex PCR; MRSA, Methicillin-resistant *Staphylococcus aureus*; MSSA, Methicillin-sensitive *Staphylococcus aureus*; PIF, Primary infectious focus; PFGE, Pulsed-field gel electrophoresis; PVL, Pantone-Valentine leucocidin; SCCmec, Staphylococcal cassette chromosome *mec*; ST, Sequence type; SXT, Trimethoprim with sulfamethoxazole; TEC, Teicoplanin; VAN, Vancomycin.

Data Sharing Statement

We confirm the data patient were deidentified. All the data generated or analysed during this study are included in this published article.

Ethics Approval and Informed Consent

This study was approved by the research, ethics, and biosafety committees of Instituto Nacional de Pediatría

(IRB: 00008064 and IRB: 00008065) under registration INP 2018/17. The ethics committee did not require informed consent because the samples obtained were part of the standard care for hospitalized patients, and the isolates were obtained retrospectively. The patient data were deidentified.

Author Contributions

All authors contributed to data analysis, drafting or revising the article, have agreed on the journal to which the article will be submitted, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no competing interests.

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