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Characterization of methicillin-resistant *Staphylococcus aureus* strains colonizing the nostrils of Spanish children

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Abstract

Objective: To characterize the *Staphylococcus aureus* strains colonizing healthy Spanish children.

Methods: Between March and July 2018, 1876 Spanish children younger than 14 years attending primary healthcare centers were recruited from rural and urban areas. *Staphylococcus aureus* colonization of the anterior nostrils was analyzed. MecA and mecC genes, antibiotic susceptibility, and genotyping according to the spa were determined in all strains, and the following toxins were examined: Panton-Valentine leucocidin (pvl), toxic shock syndrome toxin (tst), and exfoliative toxins (eta, etb, etd). Multilocus sequence typing (MLST) and staphylococcal cassette chromosome (SCCmec) typing were performed on methicillin-resistant *Staphylococcus aureus* (MRSA) strains, as well as pulsed-field gel electrophoresis (PFGE).

Results: 619 strains were isolated in 1876 children (33%), and 92% of them were sent for characterization to the Spanish National Centre of Microbiology ($n = 572$). Twenty (3.5%) of these strains were mecA-positive. Several spa types were detected among

MRSA, being t002 the most frequently observed (30%), associating with SCCmec IVc. Among MSSA, 33% were positive for tst, while only 0.73% were positive for pvl. The 20 MRSA strains were negative for pvl, and 6 (30%) harbored the tst gene.

Conclusions: methicillin-resistant *Staphylococcus aureus* nasal colonization in Spanish children is rare, with t002 being the most observed spa type, associated with SCCmec IVc. None of the MRSA strains produced pvl, but up to 30% of *S. aureus* strains were positive for tst.

KEYWORDS
methicillin-resistant, molecular characterization, nasal colonization, *Staphylococcus aureus*

1 | INTRODUCTION

Staphylococcus aureus causes most of the skin and soft tissue infections (SSTIs) in children, such as boils, carbuncles, abscesses (Barrios Lopez et al., 2013), and other more complicated infections, including septic arthritis, osteomyelitis, necrotizing fasciitis, pneumonia, endocarditis, and sepsis (Lowy, 1998; Tong et al., 2015). The nasal cavity is considered the primary anatomical site for *S. aureus* colonization (Kluytmans et al., 1997; Verhoeven et al., 2014), although it can also be found in the axilla, perineum, rectum, and throat. Strains of methicillin-resistant *Staphylococcus aureus* (MRSA) present affinity to human skin and mucous thanks to the phenol-soluble modulin (PSM) peptides PSMα1 and PSMα2 (Joo et al., 2011). In the last decades, an increase in community-associated MRSA infections (CA-MRSA) has been observed, causing SSTIs in healthy individuals, often children (Watkins et al., 2012). In Spain, the first cases were reported in 2006 (Broseta et al., 2006), although very few data regarding these infections in the pediatric population are yet available in many European countries (Del Rosal et al., 2020). Healthy children could constitute an MRSA reservoir in the community, and an elevated rate of colonization increases the risk of future infections (Turner et al., 2019). Methicillin-sensitive *S. aureus* (MSSA) strains producing Panton-Valentine leucocidin (PVL) represent a public health threat, given virulence factors frequently transfer between strains (Rasigade et al., 2010). Data regarding MSSA and MRSA colonization in children are scarce in Spain. We recently studied the prevalence of nasal colonization of MSSA and MRSA in community-dwelling Spanish children, analyzing risk factors for this colonization, in a community nationwide surveillance study: Colonization by *S. aureus* in the Community (COSACO) (Del Rosal et al., 2020). The observed prevalence of *S. aureus* colonization was 33% and of MRSA 1.44%. In this manuscript, we report the characterization of the *S. aureus* strains isolated in the COSACO study.

2 | METHODS

2.1 | Study participants and samples

We conducted a nationwide, observational, cross-sectional multicenter study on children from community settings throughout Spain (the COSACO study; Del Rosal et al., 2020). Between March and July 2018, we recruited patients younger than 14 years attending primary healthcare centers without active infections. Seventy primary healthcare centers from all Spanish regions and in both rural and urban settings took part in the study. According to region features and population density, at least two healthcare centers (from rural, urban, and mixed areas) were selected to take part in the study. Nasal swabs from every patient were collected following the study protocol (Del Rosal et al., 2020). Cultures of nasal swabs, strain isolation, and antimicrobial susceptibility testing of isolates were performed by 27 hospital-based microbiology laboratories.

2.2 | Methicillin resistance detection and staphylococcal cassette chromosome *mec* typing

For detecting methicillin resistance, a disk diffusion method with cefoxitin (30 μg) was employed, as well as susceptibility testing or disk diffusion (Del Rosal et al., 2020). Also, minimum inhibitory concentrations (MICs) for MRSA strains were studied with E-test® strips (Biomerieux). The methods and antimicrobial susceptibility interpretations were following the European Committee on Antimicrobial Susceptibility Testing guidelines (EUCAST, 2018). When an isolate showed an inhibition zone smaller than 20 mm surrounding the cefoxitin disk (Kateete et al., 2019), we screened the presence of the *mecA* and *mec*C genes using primers by polymerase chain reaction (PCR). *S. aureus* strains were considered MRSA if the *mec*A or *mec*C gene was detected. Cefoxitin-resistant strains without *mecA*/*mec*C detection were excluded from the analysis. MRSA isolates were characterized by pulsed-field gel electrophoresis (PFGE), *spa* typing, multilocus sequence typing (MLST), and staphylococcal cassette chromosome *mec* (*SCCmec*) typing. We screened for the presence of genes that code for the following virulence factors by multiplex PCR reactions: *pvl*, *tst*, *eta*, *etb*, and *etd*. The *mecA* gene was detected by PCR, as described by Geha et al. (1994). The *mecC* gene was studied according to Garcia-Alvarez et al. (2011). SCC*mec* types were determined using a multiplex PCR strategy, which generates an amplification pattern for each *SCCmec* structural type (Oliveira & de Lencastre, 2002). Additional typing of the isolates was performed by 2 different PCR methods to detect the *SCCmec* IV subtypes IVa, IVb, IVc, IVd, and IVh (Milheirico et al., 2007) and *SCCmec* type V (Zhang et al., 2005). PCR-positive controls were used as previously described (Vindel et al., 2009).

FIGURE 1 Map of Spain indicating the location of the twenty *Staphylococcus aureus mecA*-positive strains. The homogeneous distribution of the strains in different areas of Spain is shown: 3.50% (20/572)

2.3 | Pulsed-field gel electrophoresis, *spa* typing, BURP analysis, multilocus sequence typing, and toxin study

All the strains of MSSA and MRSA were characterized for *spa* typing, which is the established method based on sequencing of a single polymorphic variable-number tandem repeat, namely the repeat region of the *S. aureus* protein A gene. This *spa* typing method is highly discriminatory, and it has been used for studying the nature of different outbreaks and for assigning strains to phylogenetic lineages in epidemiological studies. The *spa* type was assigned by employing the Ridom StaphType software, and, besides this, clustering of the isolates was performed by the BURP (Based Upon Repeat Pattern) algorithm implemented in StaphType. Isolates with *spa* types with more than five repeats were clustered into different groups (CCs), with the calculated cost between members of a group being ≤6 (Mellmann et al., 2007).

We determined the bacterial MLST profiles as described in Enright et al., 2000. Allelic profiles and sequence types were assigned using the MLST database (http://www.mlst.net). The presence of genes coding for the virulence factors *pvl*, *tst*, *eta*, *etb*, and *etd* was screened by multiplex PCR reactions following the method described by Vandenesch et al., 2003.

All MRSA isolates and those with frequently occurring *spa* types, like t002-CC002 strains, were genotyped by PFGE after SmaI digestion of chromosomal DNA, prepared using a modification of the protocol described by Cookson et al. (2007). Analysis of the gels was performed according to the criteria of Tenover et al. (1995), and a dendrogram was constructed with Molecular Analyst software (Bio-Rad) using the Dice correlation coefficient and the unweighted pair-group method, with averages having a tolerance position of 0.8%. Positive controls of various PFGE profiles were used as previously described by our group (Vindel et al., 2009). In addition, a prototype USA300 strain kindly provided by Professor Herminia de Lencastre was also used as a PFGE control.

3 | RESULTS

3.1 | Bacterial isolates and patient population

A total of 1876 patients (aged 7.01 ± 4.38 years) were enrolled. The prevalence of *S. aureus* colonization was 33% (95% CI: 30.8–35.1) and of MRSA 1.44% (95% CI: 0.78–2.1) (Del Rosal et al., 2020). A total of 572 *S. aureus* strains (92% of the isolated strains were from the COSACO study) were ultimately sent for molecular characterization to the National Centre of Microbiology (Majadahonda, Spain) (1 sample/child); 20 (3.5%) of them were *mecA*-positive (MRSA) and were isolated from throughout the country (Figure 1). Colonization by *S. aureus* was more frequently observed in older children, males, urban settings, and in those with chronic diseases. In addition, there was a higher MRSA colonization rate among children living in rural environments (Del Rosal et al., 2020).

3.2 | Population structure of the community-associated methicillin-resistant *Staphylococcus aureus*

Twenty oxacillin-resistant strains were confirmed to carry the *mecA* gene. In general, these strains presented a low level of oxacillin resistance, with only 4 strains having a MIC ≥32 mg/L. The MRSA strains were isolated from 8 girls and 12 boys (Table 1).

3.2.1 | Molecular characterization of methicillin-resistant *Staphylococcus aureus*

Several PFGE profiles and their respective dendrogram were obtained (Table 1; Figure 2). According to Tenover (≥80% similitude) [21], they belonged to 8 types, with the following subtypes: E (7), F (2), G (2), K (1), L (1), N (1), Q (1), and Z (1). The 2 G subtypes were similar to the USA300 profile, with a similitude grade of 84.6%. Thirteen

TABLE 1 Characterization of MRSA strains

Cosaco sample number	*MEC* A	SCC MEC	Subtype IV	Toxins					PFGE
				PVL	TSST	ETA	ETB	ETD	
62	+	IV	IVc	−	−	−	−	−	E1
87	+	IV	IVc	−	−	−	−	−	E1
109	+	IV	IVc	−	−	−	−	−	G1
133	+	IV	IVc	−	−	−	−	−	F1
134	+	IV	IVc	−	−	−	−	−	F1
147	+	IV	IVa	−	+	−	−	−	E6
150	+	IV	IVc	−	−	−	−	−	E7
168	+	IV	IVc	−	−	−	−	−	E5
171	+	IV	IVc	−	−	−	−	−	G2
181	+	IV	IVa	−	−	−	−	−	K
232	+	IV	IVa	−	−	−	−	−	F2
233	+	IV	IVc	−	−	−	−	−	E3
258	+	IV	IVc	−	+	−	−	−	L
320	+	IV	IVc	−	−	−	−	−	E4
385	+	II	ND	−	+	−	−	−	N
443	+	IV	IVc	−	+	−	−	−	E4
453	+	IV	IVc	−	+	+	+	−	E2
464	+	IV	IVa	−	−	−	−	−	O
472	+	ND	ND	−	−	−	−	−	F3
537	+	ND	ND	−	+	−	−	−	Q

Antimicrobials: CM, clindamycin; D, daptomycin; E, erythromycin; L, linezolid; O, oxacillin; SXT, cotrimoxazole; T, teicoplanin; V, vancomycin. *Mec A*: gene *mec*A. MLST: multilocus sequence typing, an unambiguous procedure for characterizing isolates of bacterial species using the sequences of internal fragments of 7 housekeeping genes. ND: not determined. PFGE: pulsed-field gel electrophoresis (E1, E2, E3, E4, different among them in 1, 2, 3 bounds; closely related); (F1 and F2, closely related among themselves); (G1 and G2, 2 different bands, closely related strains); the remainder of the strains have different profiles. SCC MEC: staphylococcal cassette chromosome mec. *Spa*-type: the composition of the variable number of tandem repeats in the 3´ end of the staphylococcal protein A gene (*spa*). Clonal clusters (CCs), according to BURP analysis. Toxins: PVL, Panton-Valentine leucocidin; TSST, toxic shock syndrome toxin; ETA, exfoliative toxin A; ETB, exfoliative toxin B.

different *spa* types were observed in the 20 MRSA strains, belonging to 3 clonal complexes or clonal clusters: CC002 (t002, t067, t2532, t4867), CC012 (t012, t021), CC223 (t223, t790) and 5 singleton (t4407, t20101, t1507, t450) (Table 1). Among the MSSA strains (Table 2), the most prevalent was t012-CC012, with 41 strains (27 harbored the *tst* gene); t021-CC012 had 25 (17 with the *tst* gene); t375-CC375 had 23 (with no *tst* gene); t166-CC166 had 22 (with 18 *tst* genes); t223 had 21 (with 17 *tst* genes); and unknown had 26 (with 10 *tst* genes). Only 1 strain (t355-Singleton) was positive for the *pvl* gene (Table 2). To study a possible relationship among the strains with the same *spa* type, we performed a PFGE of all t002-CC002 strains (6 MRSA and 14 MSSA), given this was the most frequently observed *spa* type in MRSA strains and, besides this, t002 occupies the third place in the list of Ridom SpaServer (MSSA and MRSA) database frequencies. They proved to be different clones (according to Tenover criteria) (Figure 3).

We compared the MRSA strains according to their MLST profiles. The most abundant MLST types were ST5 (4 strains) and ST125 (3 strains). Two of the former 3 strains harbored the *spa* type t067-CC002 and presented a high oxacillin MIC. Seventeen of the 20 MRSA strains harbored *SCCmec* IV (85%); among them, 13 were IVc subtypes.

We analyzed the distribution of the *S. aureus* strains according to toxin production. None of the MRSA strains was *pvl*-positive, 6 were *tst* positive and only 1 harbored *eta* and *etb* genes. On the contrary, 4 of the MSSA strains were positive for *pvl* (1 harbored *spa* type t355-Singleton and 3 the t1445-Singleton type), 180 were positive for the *tst* gene (28 harbored *spa* type t012-CC012; 18 type t166-CC166; 17 type t021-CC012 and t223-CC223; and 2 the t002-CC002 type), among the more representative *spa* types (Table 3).

3.2.2 | Resistance profile (antimicrobial susceptibility)

All the MRSA strains were sensitive to vancomycin, teicoplanin, linezolid, daptomycin, clindamycin, and trimethoprim-sulfamethoxazole, although four were resistant to erythromycin, according to the

SPA-type clonal clusters	MLST	Antimicrobials MIC (mg/l)							
		O	V	T	L	D	E	CM	SXT
t002-CC002	Undefined	8	1	1	1.50	0.12	0.12	0.023	0.023
t002-CC002	ST5115	4	1	1.50	2	0.12	0.12	0,094	0.032
t4100-Singleton	ST72	32	1.50	1.50	1.50	0.25	0.12	0.094	0.032
t012-CC012	ST30	6	1	0.75	1.5	0.19	0.12	0.064	0.047
t012-CC012	ST30	4	1	0.75	1.5	0.12	0.12	0.094	0.047
t002-CC002	ST5	6	1	1	2	0.19	0.12	0.094	0.032
t002-CC002	ST5	12	1	1	2	0.12	0.12	0.094	0.032
t067-CC002	ST125	>256	1.50	1.50	2	0.19	0.12	0.094	0.047
t20101-Singleton	ST72	16	1.50	1.50	2	0.19	0.12	0.125	0.047
t4407- Singleton	ST6	24	1	1.50	2	0.19	24	0.094	0.032
t1507-Singleton	ST30	16	1	1	0.75	0.12	0.25	0.125	0.032
t450-Singleton	ST5115	3	1	1	2	0.19	0.12	0.064	0.032
t2532-CC002	ST125	1	1	0.50	1.50	0.12	12	0.094	0.032
t067-CC002	ST125	64	1	1	1.5	0.19	12	0.094	0.032
t223-CC223	ST22	1.50	1	0.75	1	0.12	0.12	0.094	0.032
t002-CC002	ST5	4	1	1.50	2	0.19	0.12	0.094	0.023
t002-CC002	ST5	12	1	1	2	0.19	12	0.190	0.032
t790-CC223	ST22	1.50	1	0.75	1.50	0.19	0.09	0.094	0.047
t4867-CC002	ST34	1.50	1	0.75	0.75	0.12	0.25	0.094	0.094
t021-CC012	Undefined	48	1	1	1.50	0.25	0.19	0.094	0.047

European Committee on Antimicrobial Susceptibility Testing [15]. Two of these strains were ST125 and 1 ST6, and the other was ST5.

4 | DISCUSSION

In our COSACO study, the MRSA prevalence in Spanish children was 1.4%. This prevalence was higher among younger children and in those living in rural areas (Del Rosal et al., 2020). In this manuscript, we have focused on molecular characterization and toxin production from 572 strains, 92% of the *S. aureus* strains isolated in the COSACO study. Regarding MRSA strains, t002-CC002 was the most frequently observed *spa* type, associated with the *SCCmec* IVc type. In MSSA, the most prevalent *spa* types were t012-CC012, t021-CC012, and t375-CC375. Up to 30% of the MSSA and MRSA strains were positive for *tst*. The detection of *pvl* was exceptionally in MSSA strains; none of the MRSA strains were positive for the pvl gene. In our study, we did not detect *mec*A or *mec*C in 7 of 27 cefoxitin-resistant strains. Some cefoxitin-resistant *S. aureus* strains are not linked to the presence of *mec* genes, being the resistance caused by hyperproduction of β-lactamase or mutations in the penicillin-binding protein 4 promoter or other genes (Argudín et al., 2018). Given we have focused on the characterization of *mec*A/*mec*C-positive MRSA, these 7 strains were excluded from the analysis. A strong evolutionary relationship has previously been revealed between clinical and nasal colonization isolates (Lamers et al., 2011). In addition, nasal decolonization of MRSA has been reported to reduce the progression to infection (van Rijen et al., 2008). For this reason, the performance of surveillance studies like ours is relevant.

Children present a higher risk of infection from a certain type of *SCCmec*, such as *SCCmec* type IV, compared to adults (David & Daum, 2010). In Israel, Rokney et al. found that the clone *ST5-t002-CC002-IV-pvl+* was the main cause of community-associated staphylococcal infections in the pediatric population (Rokney et al., 2019). Some 17.8% of these strains were non-susceptible to erythromycin and clindamycin. Similarly, we observed that 85% of the MRSA strains in our study were *SCCmec* type IV; among these, 35% were t002-CC002. However, only 1 of our strains was erythromycin-resistant, and none

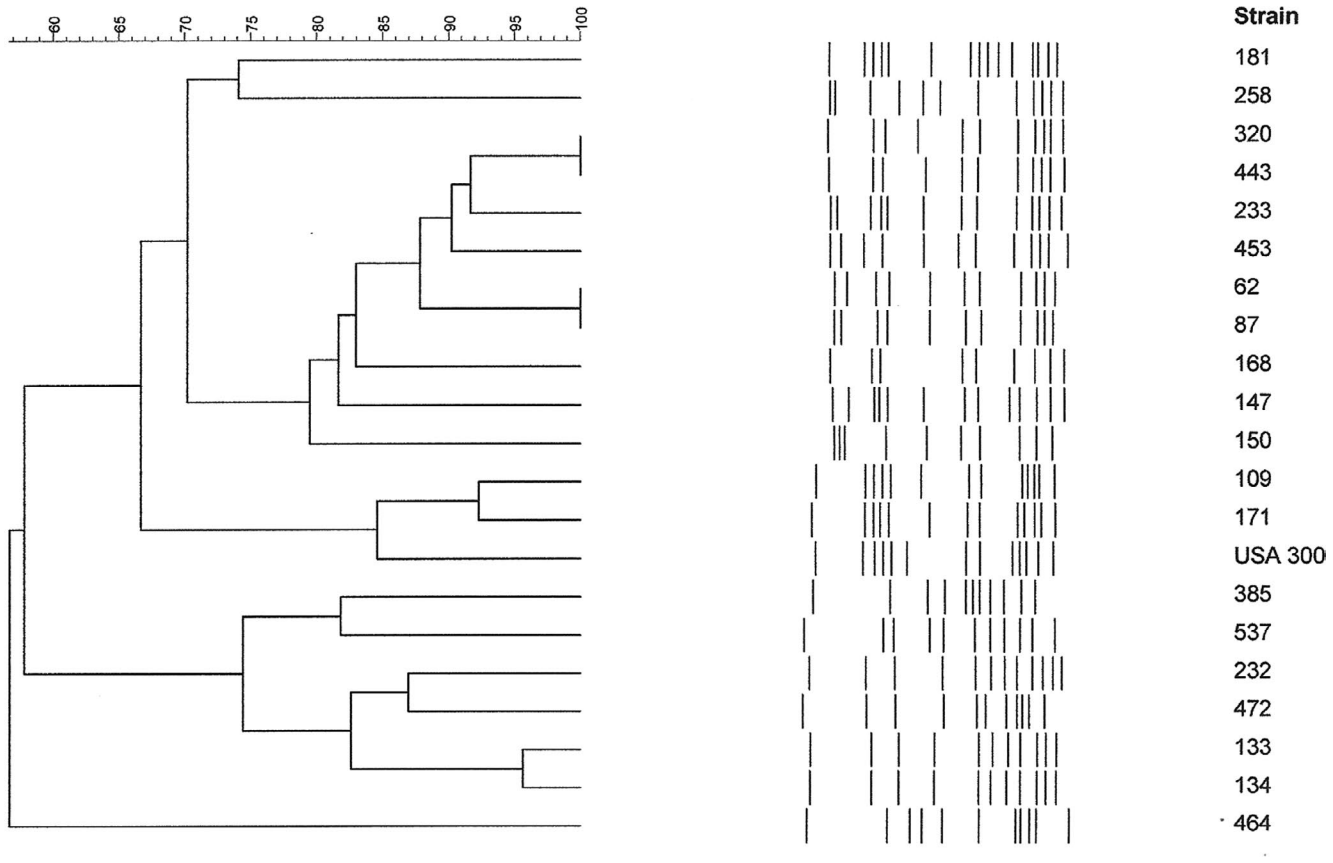

FIGURE 2 PFGE pattern of MRSA isolates. Dendrogram resulting from the comparison of PFGE band restriction patterns of the 20 MRSAs (*mecA* +) with the USA300 clone band pattern

TABLE 2 Frequency of *spa*-types/clonal complexes among isolated *Staphylococcus aureus*

Clonal complexes	*spa*-type	MRSA	MSSA
		F	F
CC015	t069, t073, t1170, t1268, t1523, t1618, t1985, t2536, t2869, t3085, t3537, t620, t630, t6606, t6704, t7669, t798	0	1
	t031, t302, t728	0	2
	t050	0	3
	t230	0	4
	t1451	0	8
	t571	0	12
	t015	0	15
CC012	t093, t1626, t1654, t2147, t253, t2557, t2579, t297, t318, t3382, t363, t6511, t7085, t822, t840, t932	0	1
	t037, t1070, t7785	0	2
	t018, t122	0	4
	t019	0	10
	t021**	1	25
	t012*	2	41
CC002	t1107, t1277, t151, t2066, t2168, t2264, t242, t509, t653, t8348	0	1
	t045, t1094	0	2
	t2167	0	3
	t2532, t4867	1	0
	t067	2	1
	t002	6	12

(Continues)

TABLE 2 (Continued)

Clonal complexes	*spa*-type	MRSA	MSSA
		F	F
CC166	t3905, t3909, t6390, t864, t884	0	1
	t136, t352, t862	0	2
	t369, t3906	0	3
	t1057	0	5
	t240	0	11
	t166***	0	21
CC084	t1875, t1957, t228, t2325, t279, t368, t393, t803	0	1
	t094, t1877	0	2
	t085	0	3
	t084	0	15
CC159	t1425, t1688, t1994, t269, t3204, t3454	0	1
	t272, t645	0	3
	t159	0	5
CC223	t1433, t309, t4565	0	1
	t712, t233	0	2
	t9606	0	3
	t005	0	4
	t790	1	0
	t223****	1	22
CC330/065	t130, t330, t560, t706	0	1
	t4545, t880	0	2
	t065	0	12
CC081	t056, t081, t1315, t472, t7475	0	1
	t078 ψ	0	4
CC616	t1722, t616, t825	0	1
	t1406	0	2
	t364	0	5
	t493	0	19
CC008	t068, t8163	0	1
	t024	0	2
	t701	0	4
	t008	0	8
CC375	t1691, t525	0	1
	t375	0	23
Singleton	t1194, t1328, t1639, t1721, t20074 #, t20077, t20078 #, t20079, t20080 Ω, t20081, t20082 Φ, t20083 #, t20088 #, t20094, t20095, t20096 #, t3072, t355 p, t4463, t4600, t818, t937	0	1
	t1507, t20101, t4100, t4407, t450	1	0
	t267, t20076	0	2
	t127, t189	0	3
	t20073	0	4
	t209 Φ Φ	0	6
No founder	t091, t10234, t10389, t1152, t11791, t1192, t12198, t12228, t12238, t12818, t12831, t13748, t1456, t1537, t1617, t1642, t1784, t18809, t1977, t2088, t2435, t248, t2637, t275, t287, t3272, t333, t339, t3638, t399, t4523, t515, t529, t5891, t638, t693, t777, t8025, t809, t929, t949, t9931	0	1
	t026, t20076, t4446, t458, t528, t 5738, t748, t779	0	2
	t1445 p p p	0	3
	t148	0	4

F, Frequency, the number of times that the *spa* lineage occurred in 545 MSSA and 20 MRSA. # 1 toxic shock syndrome toxin (TSST) positive; *27 TSST positives; **17 TSST positives; *** 18 TSST positives; **** 17 TSST positives; Φ 1 exfoliative toxin A (ETA) positive; Φ Φ 5 ETA positives; Ω 1 exfoliative toxin D (ETD) positive; ψ 3 ETD positives; p 1 PVL positive; p p p 3 PVL positives

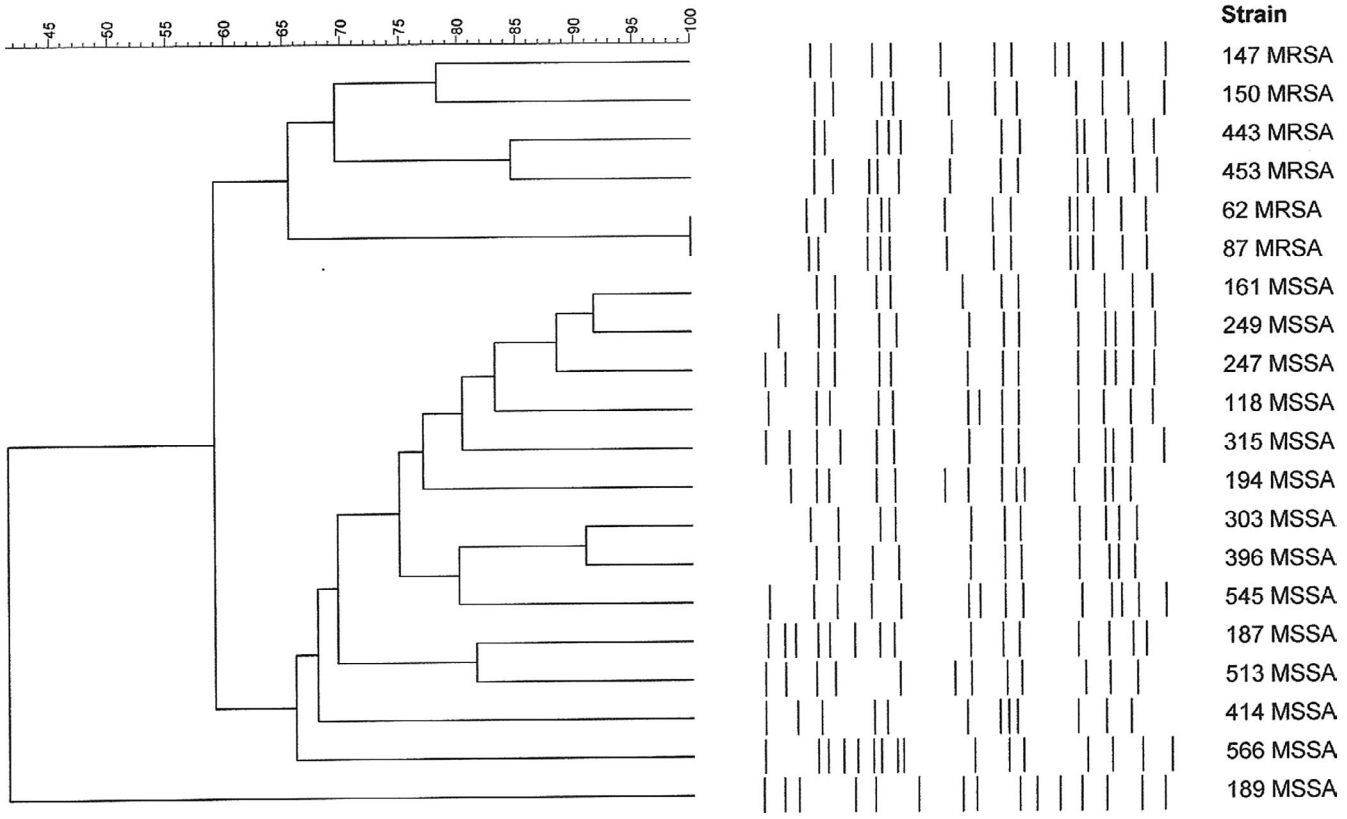

FIGURE 3 PFGE pattern of t002 *S. aureus* strains. Dendrogram resulting from the comparison of PFGE band restriction patterns of the 6 MRSAs with 14 MSSA isolates; both groups harbor the t002 *spa*-type. PFGE clusters were defined based on a similarity of 80% or higher

TABLE 3 Distribution of toxins among all the *Staphylococcus aureus* strains[a]

	*mec*A strains (−) (*n* = 545)		*mec*A strains (+) (*n* = 20)	
	(+)	(-)	(+)	(−)
PVL	4 (0.73%)	541 (99.27%)	0	20 (100%)
TSST	180 (33.03%)	365 (66.97%)	6 (30%)	14 (70%)
ETA	23 (4.22%)	522 (95.78%)	1 (5%)	19 (95%)
ETB	8 (1.47%)	537 (98.53%)	1 (5%)	19 (95%)
ETA+ETB	4 (0.72%)	541 (99.27%)	0	20(100%)
ETD	10 (1.83%)	535 (98.17%)	0	20 (100%)

ETA, exfoliative toxin A; ETB, exfoliative toxin B; ETD, exfoliative toxin D; PVL, Panton-Valentine leucocidin; TSST, toxic shock syndrome toxin.

[a]Seven more *mecA* (−) and *mecC* (−) strains are not included because they have decreased sensitivity to oxacillin that should be more widely studied. Two of these strains were TSST (+) and 1 ETD (+).

of them were *pvl*-positive or clindamycin-resistant. In a study performed in Colombian children, the authors observed that MSSA strains presented more diverse and frequent virulence genes than MRSA strains. In addition, *SCCmec* type IVc was the most commonly observed one among MRSA strains (Jiménez et al., 2011). In our study, we observed a similar distribution.

As for toxins, we must pay attention to the *tst* gene, which is present in up to 30% of the *mecA*-negative and *mecA*-positive strains that colonized the children in our study. Eight PFGE profiles were obtained among the 20 MRSA *mecA*-positive strains. It is necessary to point out that 2 G subtypes were similar to the USA300 profile; although this is a low number, it is necessary to keep in mind.

We also used PFGE to compare strains with the same *spa* type. For this reason, we chose t002-CC002 strains (6 MRSA and 14 MSSA), given this was the most abundant *spa*-type observed among the MRSA strains and the third in relative global frequencies of *spa*-type occurrences in the Ridom SpaServer (MSSA and MRSA). We compared the *spa* types of the COSACO MRSA strains with the MRSA strains received from various geographic areas around the country in the National Reference Laboratory of Staphylococci, from the National Centre of Microbiology (Majadahonda, Spain). As in the COSACO study, we also found t002-CC002 and t067-CC002 *spa* types among the strains received in the center during the same study period (data not shown); *spa* types t012-CC012, t021-CC012,

and t223-CC223 were represented in this group of strains, as we had observed in the COSACO MRSA strains. Most of these strains were isolated in adults in both in-hospital and community-based populations. Other authors have also reported that t067-CC002 and t002-CC002 are dominant among MRSA strains isolated in Spain, in contrast to the relatively low frequency of these *spa* types in other European countries (Vindel et al., 2009).

One of our limitations is that we have only sampled the anterior nares, not studying other sites of colonization. Therefore, we could have underestimated the prevalence of *S. aureus* colonization in our population. Also, we have studied only one isolate from each patient. *Le* et al. studied whether one isolate is enough for identifying individuals who are *S. aureus* colonized in the community (Le et al., 2018). Among their participants, 89% intranasally carried a single *S. aureus spa* type, 81.4% when they analyzed *mecA*-positive strains. According to this, our results could have slightly underestimated the prevalence.

It has been suggested that older children are more prone to colonization with *S. aureus* (Lamaro-Cardoso et al., 2009). Other authors have studied younger children, including only children younger than 5 years of age (Kateete et al., 2019; Tavares et al., 2010). In our case, we included patients up to 14 years of age recruited during a short period (4 months who lived in both rural and urban settings throughout the country. This approach has lead to a better understanding of the strain flow and allowed us to demonstrate that the prevalence of MRSA nasal carriage among children was low in the country. Our study suggests the need for permanent active surveillance of the mentioned carrier strains.

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CONFLICT OF INTEREST

None declared.

AUTHOR CONTRIBUTIONS

Federico Roman: Formal analysis (lead); Investigation (equal); Supervision (equal); Writing-original draft (equal). **Ana Mendez-Echevarria:** Conceptualization (lead); Funding acquisition (lead); Investigation (equal); Methodology (equal); Supervision (equal); Writing-original draft (supporting); Writing-review & editing (lead). **Teresa Del Rosal:** Conceptualization (supporting); Funding acquisition (supporting); Investigation (equal); Supervision (equal); Writing-review & editing (equal). **Cesar Garcia-Vera:** Conceptualization (equal); Funding acquisition (supporting); Investigation (supporting); Methodology (equal); Validation (supporting); Writing-review & editing (supporting). **Luis Escosa-Garcia:** Conceptualization (supporting); Data curation (supporting); Funding acquisition (supporting); Methodology (supporting); Writing-review & editing (equal). **Martin Agud:** Data curation (equal); Investigation (equal); Writing-review & editing (equal). **Fernando Chaves:** Conceptualization (equal); Methodology (equal); Writing-original draft (supporting); Writing-review & editing (equal). **Jose Gutierrez-Fernandez:** Data curation (equal); Investigation (equal); Writing-review & editing (equal). **Enrique Ruiz de Gopegui:** Data curation (equal); Investigation (equal); Validation (equal); Writing-review & editing (equal). **Guillermo Ruiz-Carrascoso:** Data curation (equal); Investigation (equal); Validation (equal); Writing-review & editing (equal). **Maria del Carmen Ruiz-Gallego:** Data curation (equal); Validation (equal); Writing-review & editing (equal). **Albert Bernet:** Data curation (equal); Investigation (equal); Validation (equal); Writing-review & editing (equal). **Sara Maria Quevedo:** Data curation (equal); Investigation (equal); Validation (equal); Writing-review & editing (equal). **Ana Maria Fernandez-Verdugo:** Data curation (equal); Investigation (equal); Writing-review & editing (equal). **Talia Sainz:** Conceptualization (equal); Methodology (equal); Writing-review & editing (equal). **Cristina Calvo:** Conceptualization (equal); Data curation (equal); Investigation (equal); Methodology (equal); Writing-review & editing (equal).

ETHICS STATEMENT

Informed consent was obtained from the parents or guardians of all the children before their inclusion, as well as from those patients aged 12 years or older. The study was approved by the Clinical Research Ethics Committee at La Paz University Hospital, Madrid (Ref.: PI18/00372).

DATA AVAILABILITY STATEMENT

All data are provided in full in this paper with the exception, of the study protocol and the main demographic data of the screened population, which are available at: https://doi.org/10.2147/IDR.S282880.

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