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Research Article

Molecular Characterization of *Staphylococcus aureus* Isolates Resistant to Antibiotics Obtained from Asymptomatic Carriers from Health Staff and Patients in a Hospital in Colombia

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Abstract

Introduction: Methicillin-resistant *Staphylococcus aureus* (MRSA) is among the bacteria that are currently a motive for global alarm. The aim of this study was to characterize antibiotic-resistant *S. aureus* isolates obtained from personal and patients in a hospital in Colombia.

Materials and Methods: The study selected 381 *S. aureus* strains, 138 from patients with invasive infection and 243 obtained from the health staff. An antibiogram was performed, along with detection of *mecA, pvl* and *hgl* genes, *SCCmec, agr* and *spa* typing and analysis through Restriction Fragment Length Polymorphism (RFLP). Association among the variables was determined through the chi squared test by using the SPSS statistical package v 22.0.

Results: Seven antibodies were determined among the *S. aureus* isolates. The anti-biotype 1 was the most prevalent (19.9%) and found mainly in the health staff. The *pvl* and *hlg* genes were detected in 11.8% and 9.7%, respectively. The *SCCmec* I and III were detected mainly in the health staff (62.5% and 97.2%, respectively), *SCCmec* II in blood (37.5%) and *SCCmec* type IV in deep wounds (43.1%). The *agr* I was the most frequent, especially in deep wounds (45.1%). An RFLP was common in MSSA and MRSA. The *spa* VI was the most frequent (53.7%), mainly in the health staff.

Conclusion: Presence of *S. aureus* was demonstrated and especially the MRSA colonized the health staff working in the hospital. The highest number of MRSA was found in invasive infections with prevalence of CA-MRSA, which evidences that the most pathogenic strains and with greater dissemination power are those being selected.

Keywords: *Staphylococcus aureus* Resistant to Methicillin; Typification; Health Staff; Invasive Infections

Introduction

Antibiotic-resistant *S. aureus* isolates, and especially MRSA, have been considered traditionally as pathogens associated to health care (acquired in health institutions) (HA-MRSA), in patients with established risk factors [1,2] and in the community (CA-MRSA) [3,4].

During early 2000, the Pediatric clone (CC5-ST5-SCCmecIV) and Chilean/Cordobés clone (CC5-ST5-SCCmecI) were reported in Colombia as the principal circulating HA-MRSA strains [4,5]. But these strains started being replaced by MRSA associated to the community (CA-MRSA) since it was reported in 2006 [6] as principal cause of infections in Colombian hospitals [7-9]. These strains are compatible with the USA300 clone (CC8-ST8-SCCmec IV) [7,10]), being more virulent (having genes that code for toxins, like Panton-Valentine leukocidin) and with a resistance pattern to different antibiotics [11-14]. But half the hospital infections due to S. aureus are caused by methicillin-sensitive isolates (MSSA), which suggests that, in spite of not having the gene that codes for resistance to methicillin, it has genetic advantages that favor it [2,15,16].

Epidemiological records indicate that nearly 37% of the population can have this microorganism, mainly in the anterior nostrils, in the skin, armpits, and the groin [15,17,18]. In the community, some groups of people are more prone to being colonized, like health care staff [19-21]. Other groups are insulin-dependent diabetics, patients in chronic hemodialysis, people with dermatological diseases, and intravenous drug addicts [22,23]. Likewise, it can survive on surfaces of diverse environments, especially, hospital environments [24,25]. These strains frequently register the highest rates of resistance to antimicrobial agents and are associated to the most severe pathologies in hospital centers in the city of Cali. Due to the aforementioned, it is important to consider the presence of these strains of community and intra-hospital origin to elaborate strategies for their control and correct treatment.

The aim of this study was to characterize molecularly *S. aureus* isolates with resistance to antibiotics obtained from the health staff colonized and from samples from patients with invasive infections in a university hospital from the city of Cali, Colombia.

Materials and Methods

This work selected 381 *S. aureus* strains obtained from 138 isolates of patients with invasive disease due to *S. aureus* who were in the intensive care unit (ICU) in Hospital Universitario San Juan de Dios in the city of Cali between 2014 and 2015. Invasive disease due to *S. aureus* is defined as the isolation of the bacteria from a site normally sterile in a patient with clinical signs and symptoms consistent with the bacterial infection. Fifty six isolates were obtained from blood, 53 from pus samples from deep wounds, 27 from sputum, and the other two samples were obtained from urine.

The 243 remaining *S. aureus* strains were isolated from asymptomatic carriers from the health staff (220 medical students and 23 health care workers) from whom a nasal swab was obtained. The asymptomatic carriers included in the study signed the informed consent, selecting those without respiratory disease and who had not received antibiotic therapy in the last three months.

Obtaining the bacterial isolates

The bacterial isolates were obtained by culturing the samples in phenol red mannitol salt agar (Oxoid Ltd., Hampshire, United Kingdom) and incubating for 24 to 48h at 37°C. Colonies identified as probable *S. aureus* were confirmed by observing the presence of Gram positive cocci in clusters, from a direct extended with Gram stain and with positive coagulase and DNAse tests.

Antibiotic sensitivity tests

The antimicrobial sensitivity test was performed on paired samples by using the agar diffusion method with discs. The isolates were classified as sensitive, intermediate sensitivity, or resistant according to the recommendations from the Clinical and Laboratory Standards Institute (CLSI) [26].

To conduct this test, a standardized amount (Mc Farland 0.5 standard) of S. aureus in Mueller-Hinton agar medium (Scharlau Chemie S.A.) and the sensi-disks were placed: oxacillin (OXA, 1 ìg), Cefoxitin (FOX, 30 ìg), cephalexin (CEF, 30 µg), gentamicin (GEN, 10 ìg), ciprofloxacin (CIP, 5 ìg), erythromycin (ERI, 15 ìg), clindamycin (CLI, 2 ìg), trimethoprim/sulfamethoxazole (SXT 1.25/23.75 ìg), tetracycline (TCY, 30 ìg), chloramphenicol (CHL, 30 ìg), vancomycin (VAN, 30 ìg), imipenem (IMP, 10 µg), and ampicillin (AMP, 10U) (Oxoid).

Methicillin resistance (MRSA) was screened with the antibiotic Cefoxitin (30 ìg), considering as resistant those strains whose inhibition halo was below or equal to 21 mm and sensitive above or equal to 22 mm. The ATCC® 25923 (halo 23 - 29 mm) *S. aureus* strain was used as control.

The HA-MRSA was identified in the test for sensitivity to antibiotics with a phenotypic multi-resistant to antibiotics, which included simultaneous resistance to â-lactam antibiotics (ampicillin, oxacillin, Cefoxitin, cephalexin, and imipenem), macrolides, aminoglycosides, and quinolones. The CA-MRSA was identified through resistance to â-lactam antibiotics and simultaneous sensitivity to erythromycin, trimethoprim/sulfamethoxazole, and clindamycin.

DNA Isolation and Extraction

DNA extraction was carried out from a bacterial culture in broth (1.5 ml) of "Luria-Bertani" Lysogeny through the night, using the genomic DNA extraction kit (MO BIO Laboratories Inc.).

Detection of *mecA, pvl* and *hlg* genes

Confirmation of MRSA isolates was performed through *mecA* gene detection via PCR, using MR1 and MR2 primers according to the conditions established by Tokue., *et al* [27]. *S. aureus* ATCC® 43300 and ATCC® 25923 strains were used as positive and negative controls, respectively.

To establish the presence of pathogenicity determinants, *pvl* (Panton-Valentine leukocidin) and *hlg* (ã-hemolysin) genes were amplified according to the protocol described by Lina G., *et al* [28]. The *S. aureus* ATCC 49775 strain was used as positive control.

SCCmec, agr and *spa* typing

Determination of SCCmec types was conducted with the specific primers for each subtype shown in table 1 [29].

Primer name	Sequence (5´-3´)	Primer specificity
MR1	GTG GAA TTG GCC AAT ACA GG	*mecA*: 478-497
MR2	TGA GTT CTG CAG TAC CGG AT	*mecA*: 1816-1797
IS5	AACGCCACTCATAACATATG-GAA	*SCCmec Type I*
Ma6	TATACCAAACCCGACCAAC	*SCCmec Type I*
mA1	TGC TAT CCA CCC TCA AAC AGG	*SCCmec Type II*
mA2	AAC GTT GTA ACC ACC CCA AGA	*SCCmec Type II*
mcR2	CGC TCA GAA ATT TGT TGT GC	*SCCmec Type III*
mcR5	CAG GGA ATG AAA ATT ATT GGA	*SCCmec Type III*
4a1	TTT GAA TGC CCT CCA TGA ATA AAA	*SCCmec Type IVa*
4a2	T AGA AAA GAT AGA AGT TCG AAA	*SCCmec Type IVa*
4b1	GA AGTACATTTATCTTTGCGTA	*SCCmec Type IVb*
4b2	AGTCATCTTCAATATCGAGA-AAGTA	*SCCmec Type IVb*
Pan-agr	GTC ACA AGT ACT ATA AGC TGC GAT	*agr* group I, II, III, and IV
agrI	GTA TTA CTA ATT GAA AAG TGC CAT AGC	*agr* group I
agrII	GTA TTA CTA ATT GAA AAG TGC CAT AGC	*agr* group II
agrIII	CTG TTG AAA AAG TCA ACT AAA AGC TC	*agr* group III
agrIV	CGA TAA TGC CGT AAT ACC CG	*agr* group IV
S1	ATGGTTATTAAGTTGGGATGG	*1.1 kb containing hld, the P2 and P3 promoters, and agrB*
S2	CAGCGGGTACTTTAGGTT	*1.1 kb containing hld, the P2 and P3 promoters, and agrB*

luk-PV-1	ATC ATT AGG TAA AAT GTC TGG ACA TGA TCC A	*Pvl*
luk-PV-2	GCA TCA AST GTA TTG GAT AGC AAA AGC	*Pvl*
hlg1	GCC AAT CCG TTA TTA GAA AAT GC	*hemolisina-ã*
hlg-2	CCA TAG ACG TAG CAA CGG AT	*hemolisina-ã*
1113F	TGTAAAACGACGGCCAGTTA-AAGACGATCCTTCGGTGAGC	*Variable X region, spa gene*
1514R	CAGGAAACAGCTATGACCCAG-CAGTAGTGCCGTTTGCTT	*Variable X region, spa gene*
spaF	AGCACCAAAAGAGGAAGACAA	*Variable X region, spa gene*
spaR	GTTTAACGACATGTACTCCGT	*Variable X region, spa gene*

Table 1: Primers used in this study.

The *agr* groups were established between MSSA and MRSA isolates through independent amplification of the 440 bp, 572 bp, 406 bp, and 588 pb fragments, using the *Pan-agr* and *agr* I, *agr* II, *agr* III, and *agr* IV primer set, respectively [30].

In addition, a 1.1 kb PCR product of the locus *agr*, which contains *hld*, P2 and P3 promoters, and *agr* B was generated with S1 and S2 primers; thereafter, it was purified for RFLP analysis through digestion with the *Alu*I enzyme (Promega), according to Sakoulas., *et al* [31]. The sequence of the primers used for the amplifications is shown in table 1. All the PCR reactions took place in 50 µl (final volume) in a GeneAmp PCR System 2400 thermocycler (Perkin-Elmer Instruments®, Norwalk, Conn).

The PCR products were visualized through agarose gel electrophoresis at 2% (100 V, 45 minutes). Band sizes were verified by running in parallel a 100-pb molecular weight marker (Bioline, UK) and visualized by using a UV trans-illuminator after staining with Sybr Green.

Calculation of the variable number of spa gene tandem repeats

The variable X region of the spa gene was amplified with 1113F and 1514R [32] and spa F and spa R [33] primers.

With the amplifiers of the X region of the gen spa, the variable number of tandem repeats (VNTR) or spa types was calculated according to that described by Sabat., *et al* [34]. In this case, the 263 bp amplifier was used as reference, which contains nine repetitions in the X region and the following approximations were conducted:

- Size of simple repetitions in the X region = 24 bp
- Size of nine repetitions in the X region = 24 bp x 9 repetitions = 216 bp
- Size of nine repetitions in the X region (18) = 263 bp
- Extra region 263 – 216 = 47 bp
- Size of the amplifier in this = X
- Size of the amplifier used to calculate the repeats = X-47 = Y
- Number of repetitions determined in the SAMR strains = Y/24 = R repetitions

Statistical analyses

The unit of analysis was the bacterial isolate from which the microbiological and molecular characteristics were registered. The microbiological variable corresponded to the degree of sensitivity or resistance to each antibiotic evaluated and was categorized into different degrees, thus: resistance (1), intermediate sensitivity (2), and sensitivity (3), according to the standards for each antibiotic established for *S. aureus* through the CLSI [26]. The molecular variables corresponded to the presence of amplified bands specific for *mecA, pvl, hgl* genes and the *sccrmec, agr* and *spa* variants.

The association among the variables and among the groups established (type of clinical sample, health care worker, student) was determined through statistical analysis, using the chi-squared test. Statistical significance was assigned for p values <0.05, considering 95%CI (alpha) and 5% error (beta). Statistical analyses were performed by using the SPSS statistical package v. 22.0 (Chicago, Inc.).

Results

Antimicrobial susceptibility

According to the analysis of the test for sensitivity to antibiotics registered in table 2, it was found that the highest number of *S. aureus* isolates with resistance to antibiotics was obtained from isolates that colonized the health staff, with resistance especially to tetracycline (32.8%), erythromycin (26.8%), trimethoprim/sulfamethoxazole (18.1%), and clindamycin (18.9%). The highest number of isolates with resistance to Oxacillin (22.6%) and Cefoxitin (22.3%) was obtained from clinical samples. All the isolates were sensitive to vancomycin linezolid and tigecycline.

Origin	Antibiogram[a]										
	CEF	CIP	CLI	ERI	GEN	OXA	AMP	FOX	TCY	SXT	IMP
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Clinical	87	34	48	50	38	86	132	85	54	17	43
samples	(22.8)	(8.9)	(12.6)	(13.1)	(10.0)	(22.6)	(34.6)	(22.3)	(14.2)	(4.5)	(11.3)
Health	94	45	72	102	44	36	159	50	125	69	50
Personnel	(24.7)	(11.8)	(18.9)	(26.8)	(11.5)	(9.4)	(41.7)	(13.1)	(32.8)	(18.1)	(13.1)
Total	181	79	120	152	82	122	291	135	179	86	Total
	(47.5)	(20.7)	(31.5)	(39.9)	(21.5)	(32)	(76.4)	(35.4)	(47)	(22.6)	

Table 2: Profile of sensitivity to antibiotics of isolates of Staphylococcus aureus obtained from patients and health personnel. N = 381.

PEN: Penicillin; CLI: Clindamycin; FOX: Cefoxitin; SXT: Trimethoprim/Sulfamethoxazole; GEN: Gentamicin; ERI: Erythromycin; CIP: Ciprofloxacin; TCY: Tetracycline; OXA: Oxacillin

From this sensitivity analysis, seven profiles or anti-biotypes were determined among the *S. aureus* isolate. Table 3 shows the antibiotic sensitivity and resistance profile for each anti-biotype. Anti-biotype 2 presents the profile with the highest number of resistance to antibiotics, while anti-biotype 7 was only resistant to trimethoprim/sulfamethoxazole.

		Sample Site									
Antibio-type	**Isolates n (%)**	**Nt n (%)**	**Bl n (%)**	**Wd n (%)**	**Ur n (%)**	**Sp n (%)**	**SAS M**	**MRS A**	**HA- MRS A**	**CA- MRS A**	**Susceptibility profile**
1	76 (19.9)	75 (16.7)	-	-	-	1 (0.3)	76 (19.9)	-	-	-	AMP, OXA, CEF, FOX, SXT, ERI, TCY, CLI. IMP, VAN
2	69 (18.1)	34 (8.9)	13 (3.4)	19 (5.0)	-	3 (0.8)	-	69 (18.1)	48 (12.6)	21 (5.5)	FOX, VAN
3	40 (10.5)	-	20 (5.2)	20 (5.2)	-	-	-	40 (10.5)	9 (2.4)	31 (8.4)	AMP, FOX, OXA, VAN
4	35 (9.2)	24 (6.3)	4 (1.0)	1 (0.3)	-	6 (1.6)	-	35 (2.1)	34 (8.9)	1 (0.3)	AMP, FOX, OXA, ERI, VAN
5	74 (19.4)	65 (17)	3 (0,8)	4 (1.0)	1 (0.3)	2 (0.5)	74 (19.4)	-		-	AMP, FOX, OXA, ERI, TCY, CEF, GEN, IMP, VAN
6	43 (11.3)	27 (7.1)	3 (0,8)	3 (0,8)	-	10 (2.6)	43 (11.3)	-	-	-	AMP, FOX, OXA, ERI, TCY, CEF, SXT, IMP, VAN
7	44 (11.5)	18 (4.7)	13 (3,4)	6 (1.6)	1 (0.3)	6 (1.6)	44 (11.5)	-	-	-	AMP, FOX, OXA, ERI, TCY, CLI, CIP, GEN CEF, IMP, VAN
Total	381 (100)	243 (63.7)	56 (14.7)	53 (13.9)	2 (0.5)	28 (7.4)	237 (62.2)	144 (37.8)	91 (23.9)	53 (13.9)	

Table 3: Distribution of antibiotypes by sample site, type of *S. aureus* and their respective antibiotic resistance profiles. n = 381. Abbreviations: MSSA: Methicillin-Susceptible *S aureus*; MRSA: Methicillin-Resistant *Staphylococcus aureus;* CA-MRSA: Community-Acquired Methicillin-Resistant *Staphylococcus aureus*, HA-MRSA: Hospital-Acquired Methicillin-Resistant *Staphylococcus aureus.* Clinical samples obtained from patients in the ICU: Bl: Blood; Wd: Wound; Ur: Urine; Sp: Sputum; Nt: Nasal Trace (obtained from health personnel); P values $p < 0,05$.

Anti-biotype 1, with a profile of resistance to gentamicin and ciprofloxacin was present in samples from the respiratory tract: in nasal isolates from the health staff (16.7%) and in the sputum sample from a patient with nosocomial pneumonia (0.3%).

Anti-biotypes 2, 3, 4, and 6 were detected in nasal swab isolates from the health staff and in clinical samples, except in urine.

Anti-biotype 3 was only detected in isolates from blood clinical samples and deep wounds, while anti-biotype 7 was detected in all the samples.

The antibiotic sensitivity test also served to detect MRSA isolates, which represented 37.8%; 55.6% was detected in clinical samples, mainly in blood samples (27.1%) and in deep wounds (16.4%).

Isolates determined as HA-MRSA corresponded to 23.9% and the CA-MRSA to 13.9%, and the in clinical samples these were found mainly in blood with 18.7% and 41.5%, respectively (Table 4).

Sample Site	***Staphylococcus aureus* isolates**				***SCCmec* type *n=135***					***agr* group**			
												pvl	***hlg***
	MS SA n (%)	MR SA n (%)	HA- MR n (%)	CA- MR n (%)	I n (%)	II n (%)	III n (%)	IV n (%)	I n (%)	II n (%)	III n (%)	n (%)	n (%)
Nt	179	64	54	10	15	10	35	4	5	18	18	17	21
	(75.5)	(44.4)	(59.3)	(18.9)	(62.5)	(20.8)	(97.2)	(7.8)	(9.8)	(48.6)	(75)	(37.8)	(56.8)
Bl	17	39	17	22	16.7	18	-	18	17	9	2	10	7

	(7.2)	(27.1)	(18.7)	(41.5)	(3)	(37.5)		(35.3)	(33.3)	(24.3)	(8.3)	(22.2)	(18.9)
Wd	29	24	7	17	8.3	7	1	22	23	1	1	11	4
	(12.20	(16.7)	(7.7)	(32.1)	(0.5)	(14.6)	(2.7)	(43.1)	(45.1)	(2.7)	(4.2)	(24.4)	(10.8)
Ur	1	1	1	-	-	1	-	2	-	-	-	1	1
	(0.4)	(0.7)	(1.1)			(2.0)		(3.9)				(2.2)	(2.7)
Sp	11	16	12	7.5	12.5	12	-	7	6	9	3	6	4
	(4.6)	(11.1)	(13.2)	(1.0)	(0.8)	(25)		(13.7)	(11.8)	(24.3)	(12.5)	(13.3)	(10.8)
Total	237	144	91	53	24	48	36	51	51	37	24	45	37
	(62.2)	(37.8)	(23.9)	(13.9)	(6.3)	(12.6)	(9.4)	(37.8)	(13.4)	(9.7)	(6.3)	(11.8)	(9.7)

Table 4: Molecular characteristics of *S. aureus* isolates.

MRSA: Methicillin Resistant *Staphylococcus aureus*; CA-MRSA: Community-Acquired Methicillin-Resistant *Staphylococcus aureus;* MSSA: Methicillin Sensitive *Staphylococcus aureus*, SCCmec: *Staphylococcal* Cassette mec

The anti-biotypes determined in MRSA were: anti-biotype 2, anti-biotype 3, and anti-biotype 4, with anti-biotype 2 (resistance to all the antibiotics assessed) being the most frequent at 18.1% prevalence. This anti-biotype was also the most frequent in HA-MRSA isolates (12.6%), while anti-biotype 4 was the most prevalent among the CA-MRSA isolates (8.9%) (Table 3).

In MSSA isolates, the anti-biotypes determined were: anti-biotype 1 (19.9%), anti-biotype 5 (19.4%), anti-biotype 6 (11.3%), and anti-biotype 6 (11.5%).

Molecular analysis of *S. aureus* isolates

All the MRSA isolates amplified a 1400-pb fragment of the *mecA* gene, confirming resistance to methicillin among these isolates.

The pathogenic *pvl* and *hlg* genes were detected in 11.8% and 9.7% of the cases, respectively. In isolates obtained from the nasal tracking of the health staff, the frequency of these genes was 37.8% and 56.8%, respectively, and in clinical isolates, the *pvl* gene was most frequent in deep wounds (24.4%) and the hlg gene in blood (18.9%) (Table 4). These pathogenic genes were detected among the MRSA isolates, 33 isolates with the *pvl* gene (22.9%) and 25 with the *hgl* gene (17.4%). In the HA-MRSA isolates, the *pvl* gene was present in 20.9% of the cases and the *hgl* gene in 16.5%, and among CA-MRSA isolates the prevalence was 24.5% and 17%, respectively.

In the MSSA isolates, each pathogenic gene was detected in 5.1% of the cases.

Table 4 also shows the analysis of the typing of the *SCCmec* complex and the *agr* groups found. The *SCCmec* types I and III were detected mainly in nasal tracking samples from the health staff with a prevalence of 62.5% and 97.2%, respectively. The *SCCmec* type II was detected mainly in clinical isolates of blood samples (37.5%) and the *SCCmec* type IV in isolates from deep wounds (43.1%). Five MRSA isolates present in the health staff did not amplify with the specific primers used to detect *SCCmec* types I to IV genes.

For the agr groups agr, these were distributed in the following manner: agr I was the most frequent in deep wounds with 45.1% and agr groups II and III in isolates from samples of nasal tracking from the health staff (48.6% and 75%, respectively).

The RFLP generated in the amplified region in the *agr* locus is shown in figure 1; the MSSA isolates obtained from clinical samples had a profile composed of eight bands, while the MSSA isolates that colonized the nasal mucus of the health staff had four profiles with six, four, eight, and seven bands.

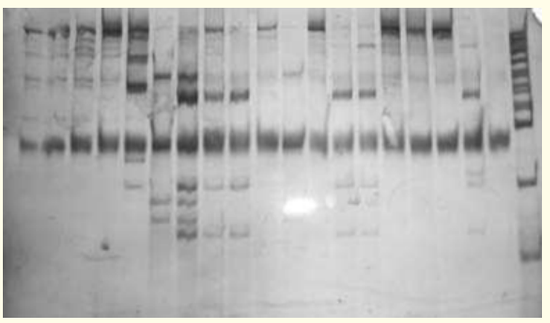

Figure 1: Genetic variability of the *S. aureus* isolates evaluated through digestion with the AluI restriction enzyme of the agr locus. The MSSA isolate from clinical samples (rows1-4), MSSA isolate from asymptomatic carriers from the health staff (rows 5-9), MRSA isolate from clinical samples (rows 10-14), MRSA isolate from asymptomatic carriers from the health staff (rows 15-19), MW marker, 100 pb, row 20.

The MRSA isolates from clinical samples and from the health staff had two profiles of four and seven bands. A common RFLP profile (composed of four bands) was present in MSSA and MRSA isolates.

Table 5 shows the distribution of the eight VNTR or *spa* types obtained from the analysis of the X variable region of the *spa* gene amplified through PCR in 339 (88.9%) *S. aureus* isolates, 217 (56.9%) isolates were health staff and 122 (32%) isolates were clinical. The allele with six repetitions was denominated *spa* I and the allele with seven repetitions, *spa* II. The *spa* VI and *spa* VII were found in the clinical samples and in the nasal tracking from the health staff, with spa VI being the most frequent (53.7%).

Spa type	No. of Repetition	Size (bp)	MSS A n (%)	MRS A n (%)	HA- MRS A n (%)	CA- MRS A n (%)	Sample Site				
							Nt	Bl	Wd	Ur	Sp
I	6	200	12 (3.5)	-	-	-	9 (2.7)	3 (0.9)	-	-	-
II	7	225	6 (1.8)	-	-	-	5 (1.5)	-	-	-	1 (0.3)
III	8	250	17 (5)	-	-	-	14 (4.1)	-	3 (0.9)	-	-
IV	10	275	19 (5.6)	-	-	-	10 (2.9)	2 (0.6	6 (1.8	-	1 (0.3
V	11	300	24 (7)	49 (14.5)	8 (2.4)	41 (12.1)	23 (6.8)	22 (6.5)	21 (6.2)	-	7 (2.1
VI	15	400	120 (35.4)	62 (18.3)	50 (14.7)	10 (2.9)	142 (41.9)	17 (5)	14 (4.1	1 (0.3)	8 (2.4)
VII	17	450	2 (0.6)	23 (6.8)	21 (6.2)	2 (0.6)	7 (2.1)	8 (2.4)	4 (1.2)	4 (1.2)	5 (1.5)
VIII	19	500	-	5 (1.5)	5 (1.5)	-	-	1 (0.3)	-	-	4 (1.2)
Total			200 (59)	139 (41)	84 (24.8)	53 (15.6)	-	-	-	-	-

Table 5: Distribution of spa types in MSSA, MRSA, CA-MRSA, HA-MSRA isolates and sample site. n = 339. P > 0,05.

The *spa* I (3.5%), *spa* II (1.8%), *spa* III (5%), and *spa* IV (5.6%) types were only determined in MSSA isolates, while *spa* VIII (1.5%) was only present in MRSA isolates. The *spa* VI and *spa* VII were found in in MSSA and MRSA isolates.

The HA-MRSA isolates presented *spa* V (2.4%), *spa* VI (14.7%), *spa* VII (6.2%), and *spa* VIII (1.5%) types and the CA-MRSA isolates presented *spa* V (12.1%), *spa* VI (2.9%), and *spa* VII (0.6%) types.

Discussion

Although the *S. aureus* resistance percentage in ICU has improved over time, evidencing a decreased resistance percentage from 2001 to 2009 for marker antibiotics [35], our study still evidences a high percentage of *S. aureus* with antibiotic resistance with prevalence, for example, of 47% to tetracycline, 39.9% to erythromycin, 31.5% to clindamycin, 22.6% to trimethoprim/sulfamethoxazole, as shown in table 2.

What is cause for concern is that these resistant isolates were also found in nasal carriers from the health staff. It has been proposed that one of the factors that permits the presence of these isolates in asymptomatic carriers is due to the use of antibiotics that enhances the overgrowth of the organisms of the skin and the mucus surface [17,18]. It has been demonstrated the penicillin use is associated to acquiring MRSA, just like the use of fluoroquinolones, macrolides, and cephalosporins increase the risk of having these strains [19,21]. In agreement with these affirmations, our study found that all the anti-biotypes determined were present in the *S. aureus* isolates that colonized the health staff. The presence of these isolates is quite relevant in the transmission of the microorganism in hospitals; in many cases, it has been determined that the nasal colonization of health care workers and patients normally precedes intra-hospital infection due to this bacteria [22,23].

Further, it is important to highlight that the MSSA isolates have a variable profile of antibiotic resistance, not only having the anti-biotype 7 with a resistance profile against a single antibiotic, anti-biotypes 1 (19.9%), anti-biotype 5 (19.4%), and anti-biotype 6 (11.3%) with resistance profiles to two and three antibiotics also prevailed, which is why these isolates could be potential MRSA strain reservoirs, as established by several studies that suggest that the appearance of "epidemic" MRSA clones was, in part, the result of the successful horizontal transference of the *mec* gene in an MSSA clone ecologically adequate and efficient in the transmission [36,37].

In this study, 5.1% of the MSSA isolates had the pathogenic *pvl* and *hgl* genes, agreeing with reports by Oosthuysen., *et al.* who consider that this MSSA population could act as potential reservoir for CA-MRSA clones in acquiring *SCCmec* elements, which leads to increasing CA-MRSA clones positive for *pvl* [38]. The presence of MSSA among asymptomatic carriers from the health staff can be an important factor for the establishment of community MRSA in our country, like the USA 300 clone that has a proven high dissemination power and is highly virulent with capacity to cause more aggressive infections [6,7], favored, perhaps, by its origin from MSSA isolates found in asymptomatic carriers; this may be evidenced by the greater variability observed in the MSSA isolates from the health staff with four RFLP profiles, with those from clinical samples with a single RFLP profile. This variability was also determined with the analysis of the X region of the spa gene, the MSSA isolates had seven *spa* types of the eight determined in this study.

In relation to the MRSA isolates, these had three anti-biotypes, whose profiles are composed by the simultaneous resistance to more than seven antibiotics. However, the RFLP analysis evidences the presence of only two profiles present in the clinical isolates and in those colonizing the health staff, which evidences that resistance to antibiotics in MRSA is directly related to the successful diffusion of specific clones. In Colombia, traditionally, the prevalence of few epidemic clones has been reported; only one multi-resistant clonal type was initially reported, corresponding to the pediatric clone circulating in hospitals during early 1990 [39]. It was then replaced by the Pediatric and Chilean/Cordobés clones with multi-resistance profile (resistant to â-lactams, macrolides, lincosamides, aminoglycosides, and quinolones) among hospital isolates [4,5]. Currently, hospital clones are being replaced by community clones [6,7,13]. In this study, phenotypically determined isolates, like HA-MRSA, corresponded to 23.9% and the CA-MRSA isolates to 13.9%.

Likewise, the analysis of the *SCCmec* complex permitted establishing isolates compatible with a hospital or community origin, thus, the presence of the *SSCmec* types I and III was determined mainly in the health staff and *SSCmec* type II in clinical isolates from blood. These three types have been traditionally related to a hospital origin [29,36], while the *SSCmec* type IV was determined mainly in isolates from abscesses of deep wounds and is related to a community origin, agreeing with the literature that relates the CA-MRSA with skin infection and infection of deep wounds [1,3]. The results from the analysis of the *agr* groups placed *agr* I with greater frequency in deep wounds (45.1%); the molecular characteristic of the CA-MRSA isolates that circulate in Colombia is the presence of pathogenic genes belonging to the *agr* I group [7]. Association has been reported between specific *agr* groups and the type of infection, *agr* I has been related with invasive infections, *agr* II with endocarditis, and *agr* III with toxigenic strains [30].

Characterization of the *spa* types permitted establishing that the *spa* VI type (15 repetitions) had the highest frequency (53.7%), found mainly in the health staff and in clinical isolates; similarly, it was more frequent among MSSA (35.4%) and MRSA (18.3%) isolates, which evidences that the MSSA isolates and those colonizing the health staff are potential MRSA reservoirs and of nosocomial infections.

Conclusion

This study demonstrated the presence of antibiotic-resistant *S. aureus* and, especially, the MRSA colonizing the health staff who works in the hospital. The highest number of MRSA isolates was causing invasive infections (blood and deep wounds) with prevalence of CA-MRSA, which evidences that the most pathogenic strains and with greater dissemination power are those being selected.

These strains are phenotypically diverse (presence of seven anti-biotypes) and are compatible with community clones, which are highly diverse with great capacity for dissemination in the community. This advantage likely permits their dominating over hospital clones. The condition of asymptomatic carrier is a risk factor for developing infections acquired in the hospital. The constant mobility of the health staff between wards in the same hospital and, probably, among hospitals, poses the need to generate strategies to prevent institutional and network dissemination of MRSA and MSSA.

Bibliography

1. Chambers HF and DeLeo FR. "Waves of Resistance: Staphylococcus aureus in the Antibiotic Era". *Nature Reviews Microbiology* 7.9 (2009): 629-641.

2. Deurenberg RH and Stobberingh EE. "The evolution of Staphylococcus aureus, Infection". *Genetics and Evolution* 8.6 (2008): 747-763.

3. Tenover FC and Goering RV. "Methicillin-resistant Staphylococcus aureus strain USA300: Origin and epidemiology". *Journal of Antimicrobial Chemotherapy* 64.3 (2009): 441-446.

4. Guzmán-Blanco M., *et al*. "Epidemiology of methicillin-resistant Staphylococcus aureus (MRSA) in Latin America". *International Journal of Antimicrobial Agents* 34.4 (2009): 304-308.

5. Sola C., *et al*. "Evolution and molecular characterization of methicillin resistant Staphylococcus aureus epidemic and sporadic clones in Cordoba, Argentina". *Journal of Clinical Microbiology* 44.1 (2006): 192-200.

6. Álvarez C, *et al*. "Community-associated methicillin-resistant Staphylococcus aureus, Colombia". *Emerging Infectious Diseases* 12.12 (2006): 2000-2001.

7. Jiménez JN., *et al*. "CC8 MRSA strains harboring SCCmec type IVC are predominant in Colombian hospitals". *PLOS ONE* 7.6 (2012): e38576.

8. Machuca MA., *et al*. "Molecular typing and virulence characteristic of methicillin-resistant Staphylococcus aureus isolates from pediatric patients in Bucaramanga, Colombia". *PLoS ONE* 8.8 (2013): e73434.

9. Reyes J., *et al*. "Dissemination of Methicillin-Resistant Staphylococcus aureus USA300 Sequence Type 8 Lineage in Latin America". *Clinical Infectious Diseases* 49.12 (2009): 1861-1867.

10. Arias C., *et al*. "MRSA USA300 clone and vancomycin-resistant Enterococcus faecalis: a United States.- Colombian connection?" *New England Journal of Medicine* 359.20 (2008): 2177-2179.

11. David MZ., *et al*. "What is Community-associated methicillin-resistant Staphylococcus aureus?" *Journal of Infectious Diseases* 197.9 (2008): 1235-1243.

12. Popovich KJ., *et al*. "Are community-associated methicillin-resistant Staphylococcus aureus (MRSA) strains replacing traditional nosocomial MRSA strains?" *Clinical Infectious Diseases* 46.6 (2008): 787-794.

13. Portillo BC., *et al*. "Molecular epidemiology and characterization of virulence genes of community-acquired and hospital-acquired methicillin-resistant Staphylococcus aureus isolates in Colombia". *International Journal of Infectious Diseases* 17.9 (2013): e744-e749.

14. Thurlow LR., *et al*. "Virulence strategies of the dominant USA300 lineage of community-associated methicillin-resistant Staphylococcus aureus (CA-MRSA)". *FEMS Immunology and Medical Microbiology* 65.1 (2012): 5-22.

15. de la Gandara MP., *et al*. "Molecular Types of Methicillin-Resistant Staphylococcus aureus and Methicillin-Sensitive S. aureus Strains Causing Skin and Soft Tissue Infections and Nasal Colonization, Identified in Community Health Centers in New York City". *Journal of Clinical Microbiology* 53.8 (2015): 2648-2658.

16. Lim K-T, *et al*. "Characterisation of the Virulence Factors and Genetic Types of Methicillin Susceptible Staphylococcus aureus from Patients and Healthy Individuals". *Indian Journal of Microbiology* 52.4 (2012): 593-600.

17. Niven D., *et al*. "Epidemiology of Staphylococcus aureus nasal colonization and influence on outcome in the critically ill". *Journal of Critical Care* 24.4 (2009): 583-589.

18. Stenehjem E and Rimland d. "MRSA nasal colonization burden and risk of MRSA infection". *American Journal of Infection Control* 41.5 (2013): 405-410.

19. Saito G., *et al*. "Methicillin-resistant Staphylococcus aureus colonization among health care workers in a downtown emergency department in Toronto, Ontario". *Canadian Journal of Infectious Diseases and Medical Microbiology* 24.3 (2013): e57-e60.

20. Collazos LF., *et al*. "Characterization of Staphylococcus aureus Isolates That Colonize Medical Students in a Hospital of the City of Cali, Colombia". *International Journal of Microbiology* (2015).

21. Pathare NA., *et al*. "Prevalence of methicillin resistant Staphylococcus aureus [MRSA] colonization or carriage among health-care workers". *Journal of Infection and Public Health* 9.5 (2016): 571-576.

22. Safdar N and Bradley EA. "The risk of infection after nasal colonization with Staphylococcus aureus". *American Journal of Medicine* 121.4 (2008): 310-315.

23. Wertheim HF., *et al*. "The role of nasal carriage in Staphylococcus aureus infections". *Lancet Infectious Diseases* 5.12 (2005): 751-762.

24. Fritz SA., *et al*. "Staphylococcus aureus Contamination of Environmental Surfaces in Households with Children Infected with Methicillin-Resistant S. aureus". *Journal of the American Medical Association Pediatrics* 168.11 (2014): 1030-1038.

25. Uhlemann AC., *et al*. "The Environment as an Unrecognized Reservoir for Community-Associated Methicillin Resistant Staphylococcus aureus USA300: A Case-Control Study". *PLOS ONE* 6.7 (2011): e22407.

26. Clinical and Laboratory Standards Institute. "Performance Standards for Antimicrobial Susceptibility Testing: Twenty-Fifth Informational Supplement". M100-S25. CLSI, Wayne, PA, USA (2015).

27. Tokue Y., *et al*. "Comparison of a Polymerase Chain Reaction Assay and a Conventional Microbiological Method for Detection of Methicillin-Resistant Staphylococcus aureus". *Antimicrobial Agents and Chemotherapy* 36.1 (1992): 6-9.

28. Lina G., *et al*. "Involvement of Panton-Valentine leukocidin-producing Staphylococcus aureus in primary skin infections and pneumonia". *Clinical Infectious Diseases* 29.5 (1999): 1128-1132.

29. Zhang K., *et al*. "Novel Multiplex PCR Assay for Characterization and Concomitant Subtyping of Staphylococcal Cassette Chromosome mec Types I to V in Methicillin-Resistant Staphylococcus aureus". *Journal of Clinical Microbiology* 43.10 (2005): 5026-5033.

30. Azimian A., *et al*. "Ocurrence of Methicillin Resistant Staphylococcus aureus (MRSA) among clinical samples in Tehran - Iran and its correlation with polymorphism of specific accessory gene regulator (agr) groups". *Brazilian Journal of Microbiology* 43.2 (2012): 779-785.

31. Sakoulas G., *et al*. "Accessory Gene Regulator (agr) Locus in Geographically Diverse Staphylococcus aureus Isolates with Reduced Susceptibility to Vancomycin". *Antimicrobial Agents and Chemotherapy* 46.5 (2002): 1492-1502.

32. Harmsen DH., *et al*. "Typing of methicillin-resistant Staphylococcus aureus in a university hospital setting by using novel software for spa repeat determination and database management". *Journal of Clinical Microbiology* 41.12 (2003): 5442-5448.

33. Oliveira DC., *et al*. "Comparison of DNA sequencing of the protein A gene polymorphic region with other molecular typing techniques for typing two epidemiologically diverse collections of methicillin-resistant Staphylococcus aureus". *Journal of Clinical Microbiology* 39.2 (2001): 574-580.

34. Sabat A., *et al*. "New method for typing Staphylococcus aureus strains: multiple locus variable number tandem repeat analysis of polymorphism and genetic relationships of clinical isolates". *Journal of Clinical Microbiology* 41.4 (2003): 1801-1804.

35. Castillo JS., *et al*. "Mortality among critically ill patients with methicillin-resistant Staphylococcus aureus bacteremia: a multicenter cohort study in Colombia". *Revista Panamericana de Salud Pública* 32.5 (2012): 343-350.

36. Enright MC., *et al*. "The evolutionary history of methicillin-resistant Staphylococcus aureus (MRSA)". *Proceedings of the National Academy of Sciences of the United States of America* 99.11 (2002): 7687-7692.

37. Rasigade JP., *et al*. "Global distribution and evolution of panton-valentine leukocidin-positive methicillin-susceptible Staphylococcus aureus, 1981-2007". *Journal of Infectious Diseases* 201.10 (2010): 1589-1597.

38. Oosthuysen WF., *et al*. "Population structure analyses of Staphylococcus aureus at Tygerberg Hospital, South Africa, reveals a diverse population, a high prevalence of Panton-Valentine leukocidin genes, and unique local methicillin-resistant S. aureus clones". *Clinical Microbiology and Infection* 20 (2014): 652-659.

39. Cruz C., *et al*. "Tracking methicillin-resistant Staphylococcus aureus clones in Colombian hospitals over 7 years (1996-2003): emergence of a new dominant clone". *International Journal of Antimicrobial Agents* 26.6 (2005): 457-462.

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