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Prevalence of methicillin-resistant *Staphylococcus aureus* colonization among healthcare workers at a tertiary care hospital in northeastern Brazil

Lucas S.C. da Silva [a,b,1], Yasmin M.F.S. Andrade [a,b,1], Arianne C. Oliveira [b], Beatriz C. Cunha [b], Eliana G. Oliveira [b], Tamara S. Cunha [b], Suzelle S. Mafra [b], Jéssica B. Almeida [a,b], Suzi P. Carvalho [a], Flávia S. Nascimento [b], Manoel Neres Santos Junior [a,b], Raiane C. Chamon [c,d], Kátia R.N. Santos [c], Guilherme B. Campos [b], Lucas M. Marques [a,b,*]

[a] *State University of Santa Cruz (UESC), Campus Soane Nazaré de Andrade, Ilhéus, Brazil*
[b] *Multidisciplinary Institute of Health, Federal University of Bahia (UFBA), Vitória da Conquista, Brazil*
[c] *Institute of Microbiology Paulo de Góes, Federal University of Rio de Janeiro (UFRJ), Rio de Janeiro, Brazil*
[d] *Pathology Department, Medicine Faculty, Fluminense Federal University (UFF), Niterói, Brazil*

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SUMMARY

Background: *Staphylococcus aureus* is a human pathogen of clinical importance related to a variety of infections.
Aim: The objective of this study was to analyze the molecular and epidemiological characteristics of *S. aureus* obtained from healthcare professionals (HCP) of a hospital in southwestern Bahia, Brazil.
Methods: Samples were collected from hands, nasal cavity, and laboratory coats of 80 HCP. The bacterial isolates recovered from 240 samples were identified as *S. aureus*, and then analyzed for their antimicrobial resistance profile, genotypic characterization, and pathogenicity.
Findings: 178 isolates were identified as *S. aureus*, being mostly isolated from the nasal cavity. Thirty isolates (16.8%) were characterized as MRSA. The virulence gene frequency varied according to isolate source. All virulence genes were identified in at least one hand isolate. Isolates from laboratory coats did not show *seb* and *pvl*. Isolates from the nasal cavity did not exhibit *pvl*. The *SCCmec* type I was identified in 56.7% of MRSA isolates. Among MRSA isolates, 14 PFGE pulsotypes were characterized, with profile A being predominant (nine isolates). Clonal complexes CC5, CC45, and CC398 were found. MRSA isolates induced cytokine gene expression in macrophages, with IL-10 and IL-17 being expressed more often.

* Corresponding author. Multidisciplinary Institute of Health: Rua Rio de Contas, 58 - Quadra 17 - Lote 58, Bairro Candeias, Vitória da Conquista, CEP: 45.029-094, BA, Brazil. Tel.: +55 77 3429 2710; fax: +55 77 3429 2710.
E-mail address: lucasm@ufba.br (L.M. Marques).
[1] LSCS. and YMFSA contributed equally to this paper.

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Conclusion: We found a high colonization rate for *S. aureus* among HCP. Moreover, we observed that MRSA strains presented different virulence factors and could induce cytokine gene expression, indicating an urgent need to control colonization rates of HCP by MRSA isolates in order to protect hospital patients and the general public.

Introduction

Staphylococcus aureus is a gram-positive and coagulase-positive bacterium. This microorganism comprises human and animal microbiota and is associated with opportunistic infections, such as pneumonia, skin infections, osteomyelitis and endocarditis [1]. Transmission of this microorganism in a hospital environment is a high-risk factor for infection, since healthcare professionals (HCP) can act as vectors for hospitalized patients [2]. Although there are studies that evaluate the presence of this microorganism in patients [3,4], there is still a lack of data on this pathogen in health professionals in Brazilian hospitals and cities.

The pathogenicity of *S. aureus* could be attributed to their ability to form biofilm on biotic and abiotic surfaces. Consequently infections associated with biofilm formation by *S. aureus* are difficult to treat, as these sites function as protection against the action of antibiotics [5]. Moreover, the impact of these infections is even more severe due to the involvement of methicillin-resistant *S. aureus* (MRSA) [6], which are endemic strains in many hospitals worldwide, especially in developing countries such as Brazil [7]. The essential factor for resistance is the acquisition of a genetic mobile element, Staphylococcal chromosome cassette (*SCCmec*). This contains the *mecA* gene, which codifies for PBP2a, which has a low affinity for β-lactam antibiotics [1]. Over the years, the development of molecular techniques has allowed epidemiological analyses of these infections and has been important for the screening of infectious outbreaks and virulent clones [8]. Therefore, the present study aimed to evaluate phenotypic and molecular characteristics of MRSA strains, establishing an epidemiological profile and associating this profile with practices and habits of health professionals in a hospital in southwest Bahia.

Methods

Study population

We selected 80 HCP from a public hospital in southwestern Bahia, among them, doctors, nurses, nursing technicians, nutritionists, administrative employees and general services assistants. The sample collections were carried out from November 2012 to March 2013. The study was approved by the Ethics Committee in Research with people from the State University of Southwestern Bahia (UESB). Before the collection, the professionals answered a questionnaire to evaluate their professional practice habits related to transmission of microorganisms. Nasal (N), hand (H) and laboratory coat (LC) samples were collected using a swab and inoculated in sterile Tryptic Soy Broth (TSB) (Kasvi®).

Isolation and characterization of the microorganisms of interest

After 24 hours of incubation, 100 μl of the suspension was seeded onto Mannitol Salt agar (Kasvi®), incubated at 37 °C for 24 hours. Gram staining (Prolab®), coagulase (Centelab®) and catalase (Labsynth®) tests were performed to identify possible *S. aureus* isolates.

Phenotypic and genotypic characterization of antimicrobial resistance

The sensitivity of the isolates to oxacillin and vancomycin was evaluated by microdilution in Muller-Hinton broth (Kasvi®) [9]. The presence of a *mecA* gene was evaluated for amongst oxacillin-resistant strains by conventional PCR [10]. Susceptibility to amoxicillin, erythromycin, ciprofloxacin, and clindamycin was assessed by the disc diffusion method [9]. Isolates resistant to two or more different antibiotics were considered multidrug resistant.

Evaluation of biofilm production

Bacteria were cultured in glycosylated TSB (Kasvi®) (1%) under agitation at 37 °C. The samples were then diluted in TSB medium (1%) (Kasvi®) and added to microplates (Prolab®) and incubated at 37 °C. After washing with 200μl of PBS solution, 50μl of violet crystal (Synth®) was added. The plates were then washed with 200μl of PBS distilled water, and then subjected to optical density determination at 492 nm using a Microplate Reader Model 550 (Bio-Rad) ELISA reader. Strains of *Streptococcus pyogenes* were used as negative controls. The biofilm unit was established by dividing the optical density of the biofilm produced by each *S. aureus* sample by the optical density of *S. pyogenes* [11].

Molecular characterization

Detection of virulence genes

Conventional PCR was used to evaluate the presence of the enterotoxin genes *sea*, *seb*, *sec* [12] in addition to *spa* genes [13], *lukF-PV* and *lukS-PV* [14] and *clfA* [15].

Characterization of Staphylococcal cassette chromosome mec (SCCmec)

To evaluate the *SCCmec* types (I, II, III, IV, and V) carried by *mecA* positive isolates, a multiplex PCR was performed [16].

Pulsed-field gel electrophoresis (PFGE)

PFGE was performed according to Nunes *et al.*, [17] for all *S. aureus* isolates presenting the *mecA* gene described above.

Multilocus sequence typing (MLST)

Different PFGE pulsotypes were selected for further MLST analysis. PCR was performed for seven housekeeping genes [18] and, after amplification, the products were purified and then sequenced by Sanger (Applied Biosystems®). For allelic assignment, the sequences were analyzed using the electronic address: https://pubmlst.org/saureus/and the combination of the seven alleles indicated the sequence type (ST), and the clonal complex (CC) to which each of the strains belonged. The clonal complexes were generated using eBurst V3 and goeBURST 1.2.

Antigenicity characterization

The antigenicity assay was performed using a modified protocol described by Xia Zhang, 2010. Lu, Varley, 2013 [19,20]. The study was approved by the animal ethics committee (CEUA) of the Federal University of Bahia, Multidisciplinary Institute of Health, Campus Anísio Teixeira. To obtain murine macrophages, four male Balb/C mice aged six to eight weeks were used. After obtaining, the murine macrophages were incubated separately with the *Staphylococcus aureus* isolates (MOI 100: 1), and the assay was performed in triplicate. The standard inoculum of 0.135Abs (660 nm), equivalent to 10^8 CFU (colony forming units) was used for infection in peritoneal macrophages of Balb/C mice. As a positive control, Lipopolysaccharide-LPS (100 μg/mL) was used, and PBS (1X) was used as negative control. After the culture time, 6 hours, the cells were removed and resuspended with RNAlater™ and frozen at -70 °C for further extraction of the mRNA. Cell mRNA was extracted using TRIZOL® according to the manufacturer's instructions. Quantified in NanoDrop and immediately processed for cDNA synthesis by reverse transcriptase reaction using the SuperScript III Reverse Transcriptase kit (Thermo-Fisher Scientific, Waltham, MA, US). The cDNA was used to evaluate the gene expression of cytokines TNF-α, IL-1, IL-6, IL-17 and IL-10 by Real time PCR RT^2 qPCR, using gene specific primers. The reaction was performed using the StepOnePlus Real-Time PCR System (Applied Biosystems, Brazil) with SYBR Green (Qiagen-SABioscience, Brazil) and using the program recommended by the manufacturer. The Melting curve was evaluated at the end of the reaction to observe the specificity of the amplification. Data were analyzed by comparative method ($2^{\Delta\Delta Ct}$) and normalization was performed based on the expression of GAPDH.

Statistical analysis

SPSS 20.0® software (SPSS Inc., Chicago, USA) was used for phenotypic, genotypic, biofilm, and clinical-epidemiological production. Pearson's chi-square test was used to verify the association between variables. Odds Ratio (OR) was used to assess the risk factors associated with infections in a univariate analysis. Data from the quantification of gene expression were analyzed by GraphPad Prism. Non-parametric Kruskal-Wallis and Mann-Whitney tests were used, with $P < .05$ and 95% CI.

Results

Isolation and identification of microorganisms of interest

Of swabs collected from nasal (N), hand (H) and laboratory coats (LC) from 80 HCP, 240 bacterial samples were recovered. Among them, 178 (75%) were characterized as *S. aureus* according to positive Gram, catalase and coagulase tests. Sixty (34%) were isolated from N, 59 (33%) were obtained from H, and 59 (33%) were isolated from LC. Of the total number of professionals studied, 6 (7.5%) were positive for *S.aureus* in at least two sites of origin, at least one being an MRSA strain.

Antimicrobial resistance

Regarding antimicrobial resistance, it was observed that the isolates showed multiple resistance to antibiotics. Of the total of 178 isolates, 30 (16.9%) were resistant to oxacillin (Table I).

Evaluation of biofilm production

Regarding biofilm production, 162 (91.0%), 8 (4.5%), 5 (2.8%) and 3 (1.7%) isolates were classified as strong producer, producer, moderate producer and weak producer, respectively.

Detection of virulence genes

Regarding MRSA isolates and the presence of virulence genes, it was possible to observe that in isolated laboratory coat samples, two (3.4%) isolates were positive for the *clfA* gene, one (1.7%) for the *spa* gene, two (3.4%) for the *sea* gene, and four (6.8%) for the *sec* gene. None of the laboratory coat isolates had the *seb* or *pvl* genes. Of the *S. aureus* strains obtained from hands, five (8.5 %), 18 (30.5%), five (8.5%), one

Table I
Profile of bacterial resistance to antibiotics according to the isolation site

Source (N)	Antibiotic resistance (N/%)					
	Oxacillin	Amoxicillin	Clindamycin	Erythromycin	Ciprofloxacin	Vancomycin
LC (59)	9 (15)	54 (92)	25 (42)	37 (63)	18 (30)	NI
Hands (59)	11 (19)	54 (92)	25 (42)	37 (63)	18 (30)	1 (2)
Nasal Cavity (60)	10 (17)	51 (85)	47 (78)	43 (72)	21 (35)	1 (3,3)

LC: lab coat.
N = total of samples; % percentage of resistant samples based on the total of each site; NI= Not identified.

Table II
Virulence genes found among MRSA isolates obtained from health professionals

Source	*clfA*	*spa*	*sea*	*seb*	*sec*	*pvl*
n (%)						
Lab coats	2 (3.4)	1 (1.7)	2 (3.4)	-	4 (6.8)	-
Hands	5 (8.5)	5 (8.5)	18 (30.5)	1 (1.7)	3 (5.1)	1 (1.7)
Nasal cavity	5 (8.3)	2 (3.3)	6 (10)	5 (8.3)	9 (15)	-
Nasal cavity	5 (8.3)	2 (3.3)	6 (10)	5 (8.3)	9 (15)	-

clfA (Clumping factor A), *spa* (*Staphylococcus aureus* Protein A), *sea* (Enterotoxin A), *seb* (Enterotoxin B), *sec* (Enterotoxin C) *and pvl* (Panton-Valentine Leukocidin)

(1.7%), three (5.1%) and one (1.7%) isolates presented *clfA, sea, spa, seb, sec* and *pvl* genes, respectively. And for nasal cavity isolates, five (8.3%), two (3.3%), six (10%), five (8.3%), nine (15%) isolates were positive for *clfA, spa, sea, seb* and *sec* genes, respectively. No isolates from this source presented the *pvl* gene (Table II).

Characterization of the mobile chromosome cassette - SCCmec

The *mec*A positive samples were classified as *SCCmec* I to V. Of the nine samples collected from laboratory coats, seven (77.7%) were Type I, one (11.1%) type III, and one (11.1%) of type V. Of the total samples obtained from the hands, six (54.5%) are type I, two (18.2%) type II, one (9.1%) type IV and two (18.2%) type V. And for samples isolated from the nasal cavity, four (40%) are type I, one (10%) type II, three (30%) are type IV and two (20%) samples were non-typeable (Table III).

PFGE

The analysis of the 30 isolates obtained by the PFGE technique presented 14 different pulsotypes (Figure 1). Pulsotypes A1, B and C grouped the largest number of isolates, presenting nine (30%); four (13.3%) and three (10%) MRSA isolates, respectively. In regard to SCC*mec* characterization, types IV and V were prevalent, corresponding to 56.7% and 30%, among pulsotypes, respectively.

MLST

MRSA isolates had their STs grouped into three CCs (Figure 2). Most of them belong to CC5, composed of STs 1, 5, 72 and 1635. CC398 presents ST1232. CC45 presents STs 45 and 1914. And five isolates were not typeable due to inherent problems with the samples.

Antigenicity characterization

14LB, 3H, 19H and 47N isolates induced high levels of TNF, IL-1, IL-6, while a larger number of strains led to increased expression of IL-10 and IL-17, especially the 19H isolate (Figure 3).

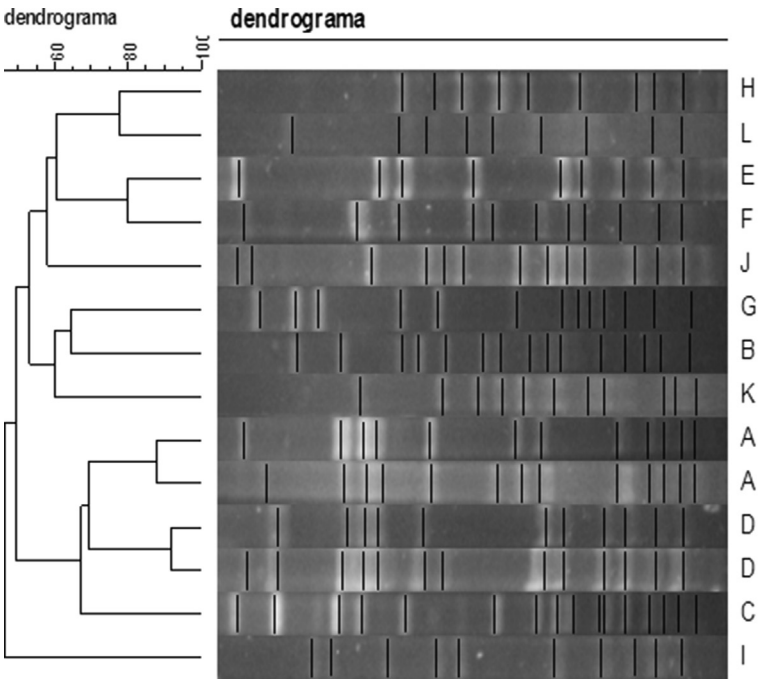
Professional habits

Eighty healthcare professionals working in different hospital sectors (emergency, medical clinics, paediatric surgery, administration, wound care, and assistants in general services) participated in this study. Of the total strains obtained from these professionals that were identified as *S. aureus*, 30 (16.9%) were MRSA isolated from different sites (hand, laboratory coat, nose). Using these confirmed MRSA strains, it was possible to associate them with the habits and practices of health professionals, such as handwashing (for example, washing hands upon arriving at the hospital, washing hands after every patient contact, handwashing before and after meals, and when leaving the hospital), having direct contact with patients, number of patient visits, handwashing technique, length of time working at the institution, and working in different hospital sectors. Thus, of the total MRSA strains, 16 (53.3%) were isolated from professionals who reported washing their hands before sample collection, while 14 (46.6%) were isolated from professionals who did not wash their hands before collection. As for the habit of hand washing, 25 (83.3%) samples were isolated from professionals who reported having this practice.

Tabela III
Number and percentage of SCCmec types found in lab coats, hands and nasal cavity

SCC*mec* type	Collection site							
	Lab coat		Hands		Nasal cavity		Total	
	N	%	N	%	N	%	N	%
I	7/9	77.7	6/11	54.4	4/10	40	17/30	56.7
II	0/9	0	2/11	18.2	1/10	10	3/30	10
III	1/9	11.1	0/11	0	0/10	0	1/30	3.3
IV	0/9	0	1/11	9.1	3/10	30	4/30	13.3
V	1/9	11.1	2/11	18.2	0/10	0	3/30	10
Non-typeable	0/9	0	0/11	0	2/10	20	2/30	6.7

N: total of samples.
%: percentage of SCC*mec* types based on the total of each site.



PFGE	Isolated (N)	Isolated (%)	Sccmec	LB/H/N	LB	H	N
H	1	3,33	IV	0/1/0	0	3 H	0
L	1	3,33	V	1/0/0	14 LB	0	0
E	2	6,66	IV	1/1/0	8LB	19 H	0
F	1	3,33	IV	0/0/1	0	0	47 N
J	1	3,33	NA	1/0/0	21 LB	0	0
G	1	3,33	IV	0/0/1	0	0	27N
B	4	13,33	V	3/0/1	11 LB; 22 LB; 77 LB	0	23N
K	1	3,33	II	0/0/1	0	0	63N
A1	9	30,0	IV	1/3/5	15 LB	6H; 13H; 15H	13 N; 15N; 17N; 21N; 54N
A2	2	6,66	II	1/1/0	3 LB	20 H	0
D1	2	6,66	V	0/2/0	0	54H; 68H	0
D2	1	3,33	V	0/1/0	0	61H	0
C	3	10,0	IV	1/1/1	7LB	47H	7N
I	1	3,33	V	0/1/0	0	12H	0

Figure 1. Dendrogram of Pulsed Field Gel Electrophoresis (PFGE) patterns and characteristics related to SCCmec of *Staphylococcus aureus* isolates obtained from the General Hospital of Vitória da Conquista-Bahia. Isolates with a similarity coefficient $\geq$ 85% were considered genetically related; LB = lab coats; H = Hands; N = Nasal Cavity.

Twenty-three (76.6%) MRSA samples were associated with professionals who had direct contact with patients, 21 (70%) were isolated from professionals who perform up to 20 daily consultations and 16 (53.3%) were from professionals who performed the handwashing technique correctly. When considering a stratified analysis, according to the collection site, the variable of time working at the institution presented *P*-significant value related to isolated samples of hands and laboratory coats ($P = 0.005$). The association between strains isolated from laboratory coats of professionals working in different sectors of the hospital and those in the habit of washing their hands was statistically significant ($P = 0.027$ and $P = 0.005$, respectively) and as for the isolates of the nasal cavity, the only variable that showed statistical significance was handwashing before collection ($P = 0.029$). (Table IV).

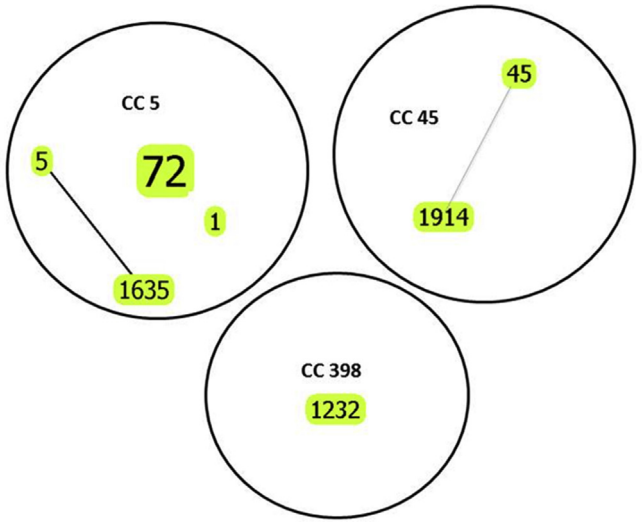


Figure 2. Clonal complex groups of MRSA isolates collected from health professionals. Seven sequence types (STs) were identified and classified into three groups. The size of the nodes (highlighted in green) is proportional to the number of isolates in each ST of the MLST database. Clonal complexes were generated using eBurst V3 and Figure 1 goeBURST 1.2.

Discussion

In the present study, the prevalence of colonization by *S. aureus* in HCP (74.16%) was higher than that found in other studies reporting 43.8% [21], and 22.22% [22]. The prevalence of MRSA found in healthcare professionals (16.8%) is similar to that of the USA (18%) [23]. Studies in Saudi Arabia, Jordan, Taiwan and China showed different colonization rates for MRSA, 73%, 10.1%, 6.8% and 1%, respectively [24—27], suggesting that these professionals are more exposed to *S. aureus* colonization compared to the general population and that the hospital environment exerts selective pressures that increase the incidence of resistant strains [28].

Regarding enterotoxin genes (*sea, seb,* and *sec*) and other proteins (PVL, spa and *clfA*), 14.6% of the isolates of *S. aureus*, have the gene *sea*; 3.4% have *seb* and 9% have *sec*. *PLV* was identified in 0.6% of the samples, in contrast to 2.3% of *S. aureus* isolated from patients [29]. We identified that 6.7% of samples presented *clfA*, while 9.1% of *S. aureus* presented this gene [30]. The *spa* gene was detected in 4.5% of the strains, a value close to a study that isolated *S. aureus* from insects in a hospital setting (6.3%) [31].

As for biofilm formation, 91% of the *S. aureus* samples from this study were strongly producing, being consistent with other studies reporting 72.83% [32] and 96.8% [29]. These data

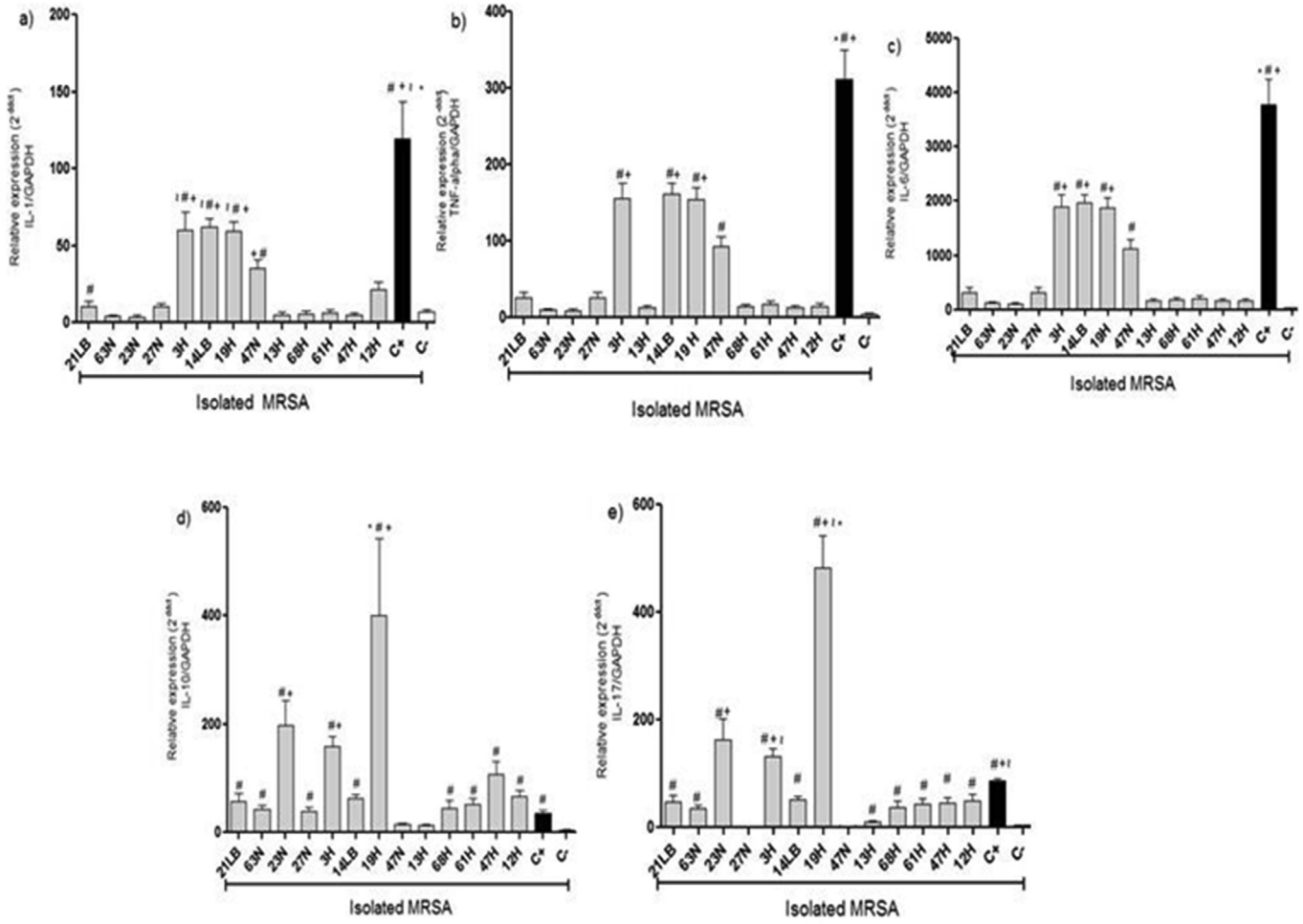


Figure 3. Cytokine gene expression in murine macrophages inoculated with *Staphylococcus aureus* after 6 h of infection (a) IL-1 gene expression (b) TNF-α gene expression of (c) IL-6 gene expression. (d) IL-10 gene expression. (e) IL-17 gene expression. Statistical significance ($P < .05$). The Mann-Whitney test was used with GraphPad Prism® software version 5.0.

suggest the robust ability of *S. aureus* to produce biofilm. This structure contributes to pathogenicity, forming a barrier against the action of drugs and the immune system [32].

In regard to *SCCmec* I—V typing, 56% of our isolates are type I, 9% type II, 3% type III. The same percentage of type IV and V strains (13%) were detected. Two isolates (6%) were non-typeable. We found a higher incidence of hospital *SCCmec* (I, II, III), consistent with other studies [8,27,33—37]. However, types IV and V (CA-MRSA) are often in hospitals [38,39]. Type IV is smaller in size than types I, II, and III, increasing its capacity to transfer [40,41] and reach hospitals through health professionals [42]. The low prevalence of non-typeable isolates is also described, indicating that these isolates may be of other *SCCmec* types, or that a mec-complex recombination has occurred [8,34—37].

The dendrogram in Figure 1 shows the variability in PFGE profiles, suggesting changes in the genome [43]. Similarities were identified between profiles A1, A2; D1 and D2; E and F. We also observed the genetic profile of isolated strains for the same professionals, identifying similarities (lines 7J and 7N, 15J, 15M, 15N, 13M, 13N) and divergences (3J and 3M, 21J and 21N, 54M and 54N; 47M and 47N), indicating the occurrence of cross-transmission among professionals, which contributes to increasing the genetic diversity of hospital isolates.

The MLST allele profile analysis, in this study, grouped the isolates into three clonal complexes: CC5, CC398, and CC45, with the majority of MSRA isolates belonging to CC5. A study conducted in São Paulo with samples from patients infected with MRSA strains showed that CC5 was the only clone identified [44]. We did not identify isolates of the Brazilian epidemic clone (CEB), similar to the data observed in isolates obtained from human milk samples [45]. The authors observed that within CC5, two samples showed ST1635 and ST5 that may be related to the paediatric clone USA800. CC45, which presented ST1914 and ST45, may be related to the Berlin USA600 clone. A study carried out in hospitals in Recife, Brazil also found a prevalence of the CC45 clone [43]. In this study, one sample presented ST 1232, which belongs to CC 398, being one of the most important clones of *S. aureus* associated with cattle, and contact with these animals represents a risk factor for human colonization [46]. However, a study carried out in Rio de Janeiro with 1,852 children identified 6 CC398 bacterial isolates, and the individuals did not have previous contact with animals, suggesting that the transmission of this clone can occur routinely in urban areas regardless of previous exposure [47]. The distribution of complexes between different environments highlights the importance of developing preventive measures in the control of bacterial transmission [48].

Table IV
Population profile of the HCP with MRSA on hands, laboratory coats, and nasal cavity

Variables	Hands					Laboratory coats					Nasal cavity				
	Presence of MRSA n (%)	Absence of MRSA n (%)	Total (N=80) n (%)	Odds ratio	P*	Presence of MRSA n (%)	Absence of MRSA n (%)	Total (N=80) n (%)	Odds ratio (IC 95%)	P*	Presence of MRSA n (%)	Absence of MRSA n (%)	Total (N=80) n (%)	Odds ratio	P*
Alteration of the work sector in the hospital, n(%)															
Yes	9 (81.8)	45 (62.2)	54 (67.5)	2.400 (0.480; 12.010)	0.456	9 (100.0)	45 (63.4)	54 (67.5)	0.833 (0.740; 0.939)	**0.027**	9 (90.0)	45 (64.3)	54 (67.5)	5.000 (0.598; 41.783)	0.154
No	2 (18.2)	24 (34.8)	26 (32.5)			0 (0.0)	26 (36.6)	26 (32.5)			1 (10.0	25 (35.7)	26 (32.5)		
Washing hands before collection, n (%)															
Yes	5 (45.5)	18 (26.1)	23 (28.8)	2.361 (0.642; 8.688)	0.187	5 (55.6)	18 (25.4)	23 (28.8)	3.681 (0.890; 15.217)	0.111	6 (60.0)	17 (24.3)	23 (28.8)	4.676 (1.179; 18.553)	**0.029**
No	6 (54.5)	51 (73.9)	57 (71.3)			4 (44.4)	53 (74.6)	57 (71.3)			4 (40.0)	53 (75.7)	57 (71.3)		
Handwashing habit, n (%)															
Yes	8 (72.7)	39 (56.5)	47 (58.8)	2.051 (0.501; 8.399)	0.494	9 (100.0)	38 (53.5)	47 (58.8)	0.809 (0.703; 0.929)	**0.009**	8 (80.0)	39 (55.7)	47 (58.8)	3.179 (0.629; 16.061)	0.184
No	3 (27.3)	30 (43.5)	33 (41.3)			0 (0.0)	33 (46.5)	33 (41.3)			2 (20.0)	31 (44.3)	33 (41.3)		
Changing the type of contact with the patient, n (%)															
Direct	8 (72.7)	45 (65.2)	53 (66.3)	1.422 (0.345; 5.863)	0.742	8 (88.9)	45 (63.4)	53 (66.3)	4.622 (0.547; 39.059)	0.260	7 (70.0)	46 (65.7)	53 (66.3)	1.217 (0.289; 5.137)	1.000
Indirect	3 (27.3)	24 (34.8)	27 (33.8)			1 (11.1)	26 (36.6)	27 (33.8)			3 (30.0)	24 (34.3)	27 (33.8)		
Number of visits per day, n (%)															
Under 20	7 (63.6)	41 (59.4)	48 (60.0)	1.195 (0.320; 4.470)	1.000	8 (88.9)	40 (56.3)	48 (60.0)	6.200 (0.736; 52.230)	0.078	6 (60.0)	26 (37.1)	32 (40.0)	2.538 (0.655; 9.840)	0.187
Above 20	4 (36.4)	28 (40.6)	32 (40.0)			1 (11.1)	31 (43.7)	32 (40.0)			4 (40.0)	44 (62.9)	48 (60.0)		
Correct hand washing technique, n (%)															
Yes	4 (36.4)	28 (40.6)	32 (4.0)	3.080 (0,820; 11,563)	0.083	5 (55.6)	27 (38.0)	32 (40.0)	2.037 (0.503; 8.255)	0.472	3 (30.0) 7 (70.0)	26 (37.1) 44 (62.9)	29 (36.3)	0.725 (0.172; 3.051)	0.740
No	7 (63.6)	25 (36.2)	32 (40.0)			4 (44.4)	44 (62.0)	48 (60.0)			3 (30.0) 7 (70.0)	26 (37.1) 44 (62.9)	29 (36.3) 51 (63.75)		
Works in another institution, n (%)															
Yes	3 (27.3)	26 (37.7)	29 (36.3)	0.620 (0.151; 2.549)	0.380	2 (22.2)	27 (38.0)	29 (36.3)	0.466 (0.090; 2.407)	0.476	10 (100.0)	66 (94.3)	76 (95.0)	0.868 (0.796; 0.948)	1.000
No	8 (72.7)	43 (62.3)	51 (63.8)			7 (77.8)	44 (62.0)	51 (63.8)			0 (0.0)	4 (5.7)	4 (5.0)		
Scale of hours worked, n (%)															
6-12 hours	11 (100.0)	65 (94.2)	76 (95.0)	0.855 (0.780; 0.938)	1.000	8 (88.9)	68 (95.8)	76 (95.0)	0.353 (0.033; 3.809)	0.386	2 (20.0)	11 (15.7)	13 (16.3)	1.341 (0.250; 7.179)	0.662
2-3 hours	0 (0.0)	4 (5.8)	4 (5.0)			1 (11.1)	3 (4.2)	4 (5.0)			8 (80.0)	59 (84.3)	67 (83.8)		
Length of time working at institution:															
Less than 5 years	5 (45.5)	8 (11.6)	13 (16.3)	6.354 (1.572; 25.691)	**0.005**	4 (44.4)	9 (12.7)	13 (16.3)	5.511 (1.243; 24.432)	**0.034**	2 (20.0)	11 (15.7)	13 (16.3)	1.341 (0.250; 7.179)	0.662
Over 5 years	6 (54.5)	61 (88.4)	67 (83.80			5 (55.6)	62 (87.3)	67 (83.8)			8 (80.0)	59 (84.3)	67 (83.8)		

The infectious power of *S. aureus* is not only limited to its dissemination and multiplication, but is also indicated in the way in which this microorganism activates the host immune response [49]. These bacteria can bind to the histocompatibility complex (MHC) in the antigen-presenting cell and T cell receptor (TCR) and trigger T lymphocyte activation and release of cytokines by macrophages [50]. Thus, we evaluated the gene expression of TNF-α, IL-1 and IL-6, IL-10 and IL-17 cytokines. Nandi *et al*. evaluated the expression of TNF-α, IL-6, and IL-10 in culture of peritoneal macrophages and observed an increase of TNF-α and IL-6 compared to IL-10 [51], which negatively regulates procytokine inflammatory response [52]. In this study we observed that IL-10 expression was induced by most of the MRSA isolates (84.61%). Wang *et al*. observed that peptidoglycan and *S. aureus* lipoteichoic acid induced IL-10 expression in monocytes [53]. This cytokine acts in *S. aureus* infections suppressing activities of monocytic cells that would aid in the pathogenicity of the microorganism [52]. For the IL-17 cytokine, induction was observed in 84.61% of the inoculated MRSA samples. IL-17 is secreted mainly by Th17 cells; however it can be secreted by other cells such as macrophages [54,55]. Mandel *et al*. evaluated human blood cells and perceived a significant increase of IL-17 after exogenous infection with *S. aureus* [56]. In addition to its role in stimulating proinflammatory cytokines, IL-17 also plays a protective role in host defense and is important for the clearance of *S. aureus* [54]. Probably the action of IL-17 against *S. aureus* together with IL-10 could explain the lower production of proinflammatory cytokines observed in macrophages after infection.

With the establishment of the epidemiological profile of MRSA strains associated with the habits of health professionals, it was possible to observe that there was higher MRSA colonization in professionals who moved between different sectors of the hospital and had direct contact with patients. Good hygiene and handwashing habits and appropriate techniques should be practiced by these professionals, reducing the risk of contamination [57]. Although long-term workers are expected to be more adept at hygiene standards [58], our data suggests that senior workers working at an institution for many years have lost the habit of constant hygiene. In addition, their coats are often colonized and act as transmission vehicles for microorganisms in hospitals and in the community [59]. In this way, our data can contribute to better strategies, practices and awareness by health professionals to reduce the spread of resistant strains.

Conclusion

Understanding the epidemiological profile of isolates from HCP is critical in controlling microbial infections in health centers. This study provided a better understanding of the evolution of MRSA strains, and contributes to literature on epidemiological monitoring, which contributes to elaborating measures to prevent and control infections caused by strains of *S. aureus*.

Credit author statement

Lucas S. C. da Silva: Conceptualization, Methodology, Formal analysis, Investigation, Data Curation, Writing - Original Draft and Writing - Review & Editing. **Yasmin M. F. S. Andrade**: Conceptualization, Methodology, Formal analysis, Investigation, Data Curation, Writing - Original Draft and Writing - Review & Editing. **Arianne C. Oliveira**: Methodology and Formal analysis. **Beatriz C. Cunha**: Methodology and Formal analysis. **Eliana G. Oliveira:** Methodology and Formal analysis. **Tamara S. Cunha**: Methodology and Formal analysis. **Suzelle S. Mafra**: Methodology and Formal analysis. **Jéssica B. Almeida**: Methodology, Supervision and Formal analysis. **Suzi P. Carvalho**: Methodology, Supervision and Formal analysis. **Flávia S. Nascimento**: Methodology and Formal analysis. **Manoel Neres Santos Junior**: Methodology and Formal analysis. **Raiane C. Chamon**: Methodology, Supervision and Formal analysis. **Kátia R. N. Santos**: Methodology, Supervision and Formal analysis. **Guilherme B. Campos**: Conceptualization, Formal analysis, Investigation, Data Curation and Writing - Original Draft. **Lucas M. Marques**: Conceptualization, Formal analysis, Investigation, Data Curation, Writing - Original Draft, Writing - Review & Editing, Project administration and Funding acquisition.

Conflict of interest statement

The present study does not present any conflict of interests.

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