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Description and Interrelationship Analysis of the Phenotypic and Genotypic Characteristics of MSSA and MRSA Strains Isolated from Healthcare Workers in North-Eastern Brazil

Maraiza Alves De Oliveira[1], Yasmin Monara Ferreira De Sousa Andrade[2], Alisson Da Costa Souza[1], Icaro Bonyek Santos Da Silva[2], Guilherme Barreto Campos[3], Lucas Miranda Marques[3], Grace Anne Azevedo Doria[1], Rafael Ciro Marques Cavalcante[1]*

[1]Federal University of Sergipe, Campus Professor Antônio Garcia Filho. Lagarto, Sergipe, Brazil. Governador Marcelo Déda, 13, Centro, 49400-000. Lagarto, Sergipe, Brazil. Programa de Pós-graduação em Ciências aplicadas à Saúde

[2]Instituto Gonçalo Muniz, Fundação Oswaldo Cruz, Salvador, Brazil. Waldemar Falcao Street, 121, Candeal, 40296-710. Salvador, Bahia, Brazil

[3]Multidisciplinary Institute of Health, Federal University of Bahia (UFBA), Vitória da Conquista, Brazil

Research Article

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***For Correspondence:** Rafael Ciro Marques Cavalcante, Federal University of Sergipe, Campus Professor Antônio Garcia Filho. Lagarto, Sergipe, Brazil. Governador Marcelo Déda, 13, Centro, 49400-000. Lagarto, Sergipe, Brazil

E-mail: rafaelciro@academico.ufs.br

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ABSTRACT

Objective: To analyse the interrelationship between the phenotypic and genotypic characteristics of methicillin-susceptible and MRSA strains isolated from healthcare workers in north-eastern Brazil.

Methods: *Staphylococcus aureus* strains were isolated from nasal mucosa of nursing professionals. They are identified by biochemical analysis and the sensibility drug test were carried out by agar diffusion method. Biofilm formation was detected on the polystyrene plastic surface. Molecular characterization was conducted by Polymerase Chain Reaction (PCR). The Chi-squared or Fisher's exact tests were applied for the analysis of associations between phenotypic and genotypic characteristics.

Results: A total of 118 workers participated, 49.15% of whom presented *Staphylococcus aureus*. The inducible clindamycin resistance phenotype was detected in 48.84% of isolates. MRSA was found in 41.37% of isolates, while SCCmec type I was seen in 75%. In terms of virulence genes, *eta, tst, pvl, spa, clf*A, *ica*A, *ica*C and *ica*D genes were detected at least once. Biofilm formation was detected in 89.65% of the strains-18.97% of these were classified as strongly adherent. Multivariate analyses of clusters demonstrated variability between the strains, with the notable formation of three main clusters, according to phenotypic and genotypic characteristics.

Conclusion: Most isolates had multidrug resistance, significant virulence genes and the ability to form biofilms, increasing the severity of potential infections.

INTRODUCTION

Staphylococcus aureus is associated with endocarditis, bacteremia, osteomyelitis, and skin and soft tissue infections [1]. The severity of infections caused by this pathogen is intensified when Methicillin-Resistant *Staphylococcus aureus* (MRSA) is involved. Resistance occurs through the acquisition of a Staphylococcal Chromosomal Cassette mec (SCC*mec*), which carries the *mec*A or *mec*C gene, encoding an altered Penicillin-Binding Protein (PBP2a) which confers resistance to β-lactams [2]. Due to the growing spread of MRSA, there has been an increase in the use of antibiotics from the Macrolide-Lincosamide-Streptogramin B (MLSB) group, especially clindamycin. Resistance to this group can be expressed through various mechanisms, which may be constitutive ($cMLS_B$) or inducible ($iMLS_B$) [3].

Staphylococcus aureus ability to cause infections is related to the production and expression of virulence factors, that favour immune evasion and increase the severity of infections [4]. These include agglutination factors A and B, biofilm, Staphylococcal Enterotoxins (SE), Toxic Shock Syndrome Toxin 1 (TSST-1), exfoliative toxins, staphylococcal protein A (spa) and Panton-Valentine Leukocidin (pvl) [5,6].

The pathogen is endemic in a number of hospitals and presents a danger to patients, since they are vulnerable and frequently immunodepressed and thus more susceptible to colonization [7]. Within the hospital environment, healthcare workers may contribute to the nosocomial transmission of *Staphylococcus aureus* as reservoirs or vectors. Once colonization occurs, nurses become potential spreaders of microorganisms in health services and may cause outbreaks of infection and compromise patient health [8]. This study was undertaken to analyse the interrelationship of phenotypic and genotypic characteristics of methicillin-susceptible *Staphylococcus aureus* MSSA and MRSA strains isolated from healthcare workers in northeastern Brazil.

MATERIALS AND METHODS

An exploratory and cross-sectional study was undertaken with nursing staff from a university hospital in a rural area of Sergipe in north-eastern Brazil. Collections were performed in the hospital between april 2016 and june 2017. Analyses were conducted in the Research Laboratories of the Federal Universities of Sergipe and Bahia between April 2016 and December 2017. The Committee for Ethics in Research on Human Beings at the Federal University of Sergipe approved the research under opinion number: 2377921.

Sample collection and phenotype identification

All nursing staff, including technicians and nurses, were invited to participate in the study; a total of 118 accepted. After signing an informed consent form, a questionnaire was applied regarding sex, age, professional experience and occupation. In parallel, samples were collected with nasal swabs using a previously described method, incubated using medium Brain Heart Infusion (BHI, HiMedia, India) with 7.5% w/v NaCl, for 18 hours to 24 hours at 35°C under 200 rpm agitation [9]. Following growth, the samples were seeded on mannitol salt agar (HiMedia, India) and incubated for 18 hours to 24 hours at 35°C. Positive mannitol isolates were tested by Gram stain, catalase, coagulase and DNAse. Strains which show as gram-positive cocci, catalase, DNAse and coagulase positives were identified as *Staphylococcus aureus*.

Antimicrobial susceptibility

Antimicrobial susceptibility was detected through the disk diffusion method, following the recommendations of the Clinical and Laboratory Standards Institute (CLSI) [10,11]. Azithromycin 15 µg, ciprofloxacin 5 µg, erythromycin 15 µg, clindamycin 2 µg, linezolid 30 µg, penicillin 10 UI, rifampicin 5 µg and tetracycline 30 µg were tested. Strains resistant to at least three antimicrobial agents were classified as multi-resistant [12]. Cefoxitin disks (30 µg) were used to detect methicillin resistance. The D-test was performed to classify the MLSB phenotype in strains resistant to erythromycin. Isolate susceptibility to oxacillin and vancomycin was analysed using the agar dilution technique, according to CLSI recommendations [11]. *Staphylococcus aureus* ATCC 25923 was used as a control strain.

Biofilm formation

The ability to form biofilm on plates was assessed using an already described methodology [13]. Bacterial suspensions with turbidity equivalent to 0.5 on the Mcfarland scale were diluted in BHI at 1:100, in a flat-bottomed polystyrene plate with 96 wells (Kasvi, Brazil) to a final volume of 200 µL and incubated at 37°C for 24 hours. The plate was then inverted and rinsed three times with distilled water to remove non-adherent cells. Two hundred microliters of 99% methanol was then added and the plates were emptied and dried 15 minutes later. Two hundred microliters of 2% crystal violet (w/v) were added to each well, and the plates were again incubated for 5 minutes at 37°C. Following this, the plate was again inverted, rinsed and dried by inversion. The dye bound to the adhered cells was resolubilized with 150 µL of 33% acetic acid (v/v) and 125 µL were transferred from each well to a new plate. The biofilm formation was measured through an absorbance reading at 600 nm in a microtiter plate reader (Thermo Scientific Multiskan EX). The tests were performed in triplicate. *Staphylococcus aureus* ATCC 29213 and sterile BHI were used as positive and negative controls respectively. The strains were classified as Non-Adherent (NA), Weak Adherent (WA), Moderate Adherent (MA) and Strong Adherent (SA), according to previously established criteria [14].

Molecular characterization

The total DNA was extracted by boiling, using an adapted protocol [15]. Bacterial suspensions were centrifuged at 14,000 rpm for 15 minutes to obtain a pellet. The pellet was then rinsed twice and re-suspended in 100 µL of Phosphate-Buffered Saline 1X (PBS). The bacterial suspension was exposed to a heat shock of 100°C for 10 minutes, then 0°C for 5 minutes. DNA concentration and purity were measured using a Nanodrop UV-vis spectrophotometer (Thermo Fisher Scientific, USA). Conventional Polymerase Chain Reaction was performed to detect *mecA*, *pvl*, *eta*, *tst*, *spa*, *clfA*, *icaA*, *icaC* and *icaD* genes with already described primers [16]. Positive *mec*A strains were subject to a multiplex PCR reaction to amplify the genes from chromosomal cassettes I to V, using previously described primers and methods [17]. The reactions were prepared in 25 µL, containing: 12.5 µL Master Mix (Promega, USA), 1 µL of each primer, 2.5 µL of DNA template and enough ultrapure water to a total of 25 µL. The amplifications were performed according to conditions established by the authors of each primer. These were performed in a thermal cycler (Bio-Rad®, model T100TM) and the products were visualized by electrophoresis in 2% agarose gel in a 1X Tris-Acetate-EDTA (TAE) buffer. Finally, the gels were stained with ethidium bromide, visualized and photo-documented (UVP-Multidoc-It digital imaging system, USA).

Multivariate analyses for grouping data

Data regarding strain clusters, according to genotypic and phenotypic characteristics, were represented through heat maps and Principal Components Analysis (PCA). Isolates were analysed using http://www.heatmapper.ca/expression/, where unsupervised hierarchical grouping was performed using average distance and euclidean distance as metrics, and https://cytoscape.org/ (version 3.8.2) to assess the interrelationships between components.

Statistical analysis

Statistical analyses of the results were performed using SPSS 16.0 software (SPSS Inc., Chicago, IL, USA). The Chi-squared or Fisher's exact tests were applied for the analysis of association. The latter was only applied when the expected frequencies were below five. Differences between means presenting $p<0.05$ were considered significant.

RESULTS

In total, 118 samples were collected and 58 isolates were identified as *Staphylococcus aureus* (49.15%). Most isolates were from female nursing technicians (79.3%), under 40 years old (69.0%), with five or more years of professional experience (Table 1). The univariate analysis demonstrated no significant association between carrier status and demographic characteristics.

Table 1. Demographic characteristics of volunteers and distribution of carriers and non-carriers.

Variables		Non-carrier N=60%	Carrier N=58%
Sex	Female	53 (88.3)	46 (79.3)
	Male	7 (11.7)	12 (20.7)
Age	< 40 years	39 (65.0)	40 (69.0)
	≥ 40 years	21 (35.0)	18 (31.0)
Professional experience	< 5 years	18 (30.0)	17 (29.3)
	≥ 5 years	42 (70.0)	41 (70.7)
Occupation	Nurse	26 (43.3)	23 (39.7)
	Nursing technician	34 (56.7)	35 (60.3)
Note: No differences were observed in the frequency of carriers and non-carriers (p value 0.14–0.93).			

Antimicrobial susceptibility

The isolates were more frequently resistant to penicillin (86.21%), erythromycin (74.14%), azithromycin (67.24%) and ciprofloxacin (53.45%) and less frequently to tetracycline (36.21%), clindamycin (19.87%), rifampicin (12.07%), and linezolid (1.72%). Multidrug resistance was present in 42 (72.41%) isolates. The MLS_B phenotype was observed in 43 isolates. Of these, 11 (25.58%) were resistant to clindamycin, as detected by disk diffusion, and were therefore classified as $cMLS_B$, while 21 (48.84%) were $iMLS_B$ positive. Resistance to methicillin was found *via* disk diffusion in

25.58% of isolates, while through the agar dilution test was 34.38%. One strain with intermediate resistance to vancomycin was also found.

Methicillin resistance

The *mecA* gene was detected in 41.37% of the isolates (24/58). Detections *via* disk diffusion and agar dilution have shown 62.50% and 83.33% of sensibility and 79.07% and 89.47% of Negative Predictive Values, respectively. Specificity and Positive Predictive Values obtained 100%. The resistance index for the azithromycin, ciprofloxacin, erythromycin and tetracycline antimicrobials was significantly higher among the MRSA strains (Figure 1). In the SCCmec typing, the highest frequency was SCCmec type I, found in 18 of the 24 MRSA isolates (75.00%), and followed by SCCmec type III (12.50%), SCCmec type V (8.33%) and SCCmec type IV (4.17%). SCCmec type II was not found. Of the 24 MRSA strains, 23 had different genotypic and phenotypic profiles (Table 2).

Figure 1. Antibiotic resistance profiles of methicillin-susceptible and methicillin-resistant *Staphylococcus aureus* isolated from nursing staff. **Note**: MRSA n=24; MSSA n=34.

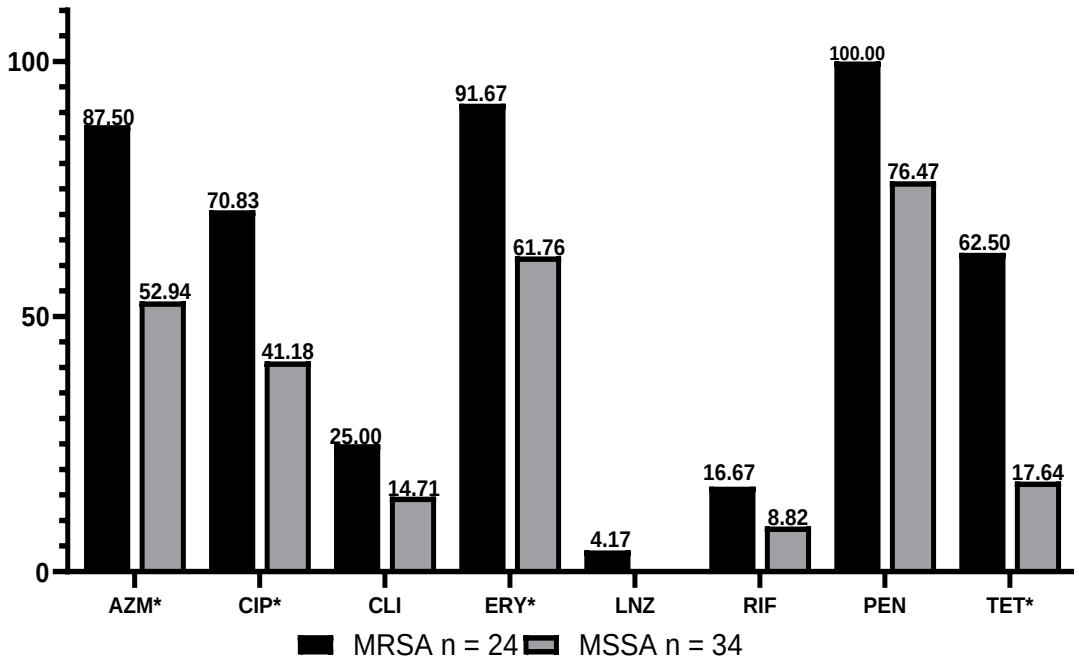


Table 2. Phenotypic and genotypic profile of MRSA strains isolated from nursing staff, Brazil.

Isolate n=24	Phenotypic profile			Genotypic Profile	
	Antimicrobial resistance	MLSB Phenotype	Biofilm formation	Virulence	SCCmec type
C44	PEN	*	WA	*icaAC*	I
C46	PEN	*	WA	*spa*, *clfA*, *icaA*	I
C27	AZM CIP ERY PEN	MS	NA	*eta*, *icaC*	IV
C11	AZM ERY PEN	$iMLS_B$	SA	*icaADC*	I
C35	AZM ERY PEN	$iMLS_B$	SA	*eta*, *icaADC*	I
C32	AZM ERY PEN TET	$iMLS_B$	WA	*clfA*, *icaC*	I
C34	AZM CIP ERY PEN	MS	MA	*spa*, *icaD*	III

C52	AZM CIP ERY PEN	MS	MA	*clf*A, *ica*ADC	I
C54	AZM CIP ERY PEN	iMLS$_B$	MA	-	I
C4	AZM CIP ERY PEN TET	iMLS$_B$	MA	*spa*	I
C15/C58	AZM CIP ERY PEN TET	iMLS$_B$	MA	-	I
C28	AZM CIP ERY PEN TET	iMLS$_B$	SA	*spa*, *clf*A, *ica*C	I
C39	AZM CIP ERY PEN TET	iMLS$_B$	NA	*spa*, *clf*A, *ica*D	I
C17	AZM CLI ERY PEN TET	cMLS$_B$	MA	*ica*ADC	III
C18	AZM CLI ERY PEN TET	cMLS$_B$	NA	*ica*C	I
C19	CIP CLI ERY PEN TET	cMLS$_B$	WA	*spa*	I
C50	AZM CIP ERY LZD PEN RIF	MS	WA	*ica*AC	V
C51	AZM CIP ERY PEN RIF TET	MS	SA	*clf*A, *ica*AC	I
C56	AZM CIP ERY PEN RIF TET	MS	MA	*clf*A, *ica*ADC	I
C40	AZM CIP CLI ERY PEN TET	cMLS$_B$	NA	*clf*A, *ica*A	III
C53	AZM CIP CLI ERY PEN TET	cMLS$_B$	SA	*ica*AD	I
C55	AZM CIP CLI ERY PEN TET	cMLS$_B$	MA	*spa*, *ica*AD	V
C45	AZM CIP ERY PEN RIF TET	MS	WA	*spa*, *ica*DC	I

Note: PEN=Penicillin; AZM=Azithromycin; ERY=Erythromycin; CIP=Ciprofloxacin; RIF=Rifampin; TET=Tetracycline; LZD=Linezolid; cMLS$_B$=Constitutive resistant phenotype; iMLS$_B$=Inducible resistant phenotype; MS=Resistant phenotype; NA=Non-Adherent; WA=Weak Adherent; MA=Moderate Adherent; SA=Strong Adherent; *clf*A=Gene encoding clumping factor A; *eta*=Gene encoding exfoliative toxin A; *ica*ADC=Gene encoding ica operon and polysaccharide intercellular adhesin; pvl=Gene encoding Panton–Valentine Leucocidin toxin; *tst*=Gene encoding toxic shock syndrome toxin; *spa*=Gene encoding staphylococcal protein A; SCC*mec*=Staphylococcal chromosomal cassette *mec*; *=Not determined;-=Absent.

Biofilm formation

Formation of biofilm on plates was confirmed in 89.65% (52) of the strains. Of these, 18.97% (11/58) were classified as SA, 32.76% (19/58) MA, 37.93% (22/58) WA, while 10.34% (6/58) were classified as NA. The distribution of resistance and biofilm formation phenotypes among the isolates can be found in Table 3. The univariate analysis demonstrated that iMLS$_B$ are strong biofilm-forming strains (p=0.035). Similarly, isolates that did not have an association with *ica*A, *ica*C or *ica*D genes did not form biofilms (p=0.044).

Table 3: Phenotypes of resistance and biofilm formation in *Staphylococcus aureus* isolated from nursing staff, Brazil.

Isolate	AZM	CIP	CLI	ERY	FOX	LZD	PEN	RIF	TET	iMLS$_B$	cMLS$_B$	MS	NA	WA	MA	SA
C1	■			■			■			■					■	
C2		■													■	
C3																■
C4	■	■		■			■		■	■					■	
C5	■														■	
C6	■						■							■		
C7		■					■								■	
C8		■					■		■							■
C9	■	■		■			■			■					■	
C10		■		■			■			■				■		

Note: Coloured boxes indicate a positive phenotype; AZM=Azithromycin; CIP=Ciprofloxacin; ERY=Erythromycin; FOX=Cefoxitin; LZD=Linezolid; PEN=Penicillin; RIF=Rifampicin; TET=Tetracycline; $cMLS_B$=Constitutive resistant phenotype; $iMLS_B$=Inducible resistant phenotype; MS=Resistant phenotype; NA=Non-Adherent; WA=Weak Adherent; MA=Moderate Adherent; SA=Strong Adherent.

Virulence genotype

The most frequently found virulence gene was *ica*A, which was detected in 62.07% (36/58) of the isolates, while the least common virulence gene was *pvl*, which was present in only 1 (1.72%). There was a difference in the distribution of genes between the MRSA and MSSA isolates and the distribution of *ica*D was significantly lower among the methicillin-resistant (Table 4). Forty-two isolates had more than one virulence gene, while three isolates harboured six (Table 5).

Table 4. Distribution of virulence genes in methicillin-susceptible and methicillin-resistant *Staphylococcus aureus*, isolated from nursing staff.

Virulence gene	MRSA% n=24	MSSA% n=34	Total% n=58	p value*
*clf*A	33.33 (8)	38.23 (13)	36.21 (21)	0.7020 †
eta	8.33 (2)	0	3.45 (2)	0.1670 ϕ
ica	50.00 (12)	70.58 (24)	62.07 (36)	0.1115 †
*ica*C	54.26 (13)	64.70 (22)	60.34 (35)	0.4190 †
*ica*D	41.66 (10)	73.53 (25)	60.34 (35)	**0.0146** †
spa	16.66 (4)	35.29 (12)	27.59 (16)	0.1180 †
pvl	4.16 (1)	0	1.72 (1)	0.4138 ϕ
tst	0	5.88 (2)	3.45 (2)	0.5064 ϕ

Note: MRSA=methicillin-resistant *Staphylococcus aureus*; MSSA=methicillin-susceptible *Staphylococcus aureus*; *clf*A=gene encoding clumping factor A; *eta*=gene encoding exfoliative toxin A; *ica*ADC=gene encoding ica operon and polysaccharide intercellular adhesin; *pvl*=gene encoding panton-valentine leucocidin toxin; *tst*=gene encoding toxic shock syndrome toxin; *spa*=gene encoding staphylococcal protein A; p value* in bold=statistical significance ($p<0.05$); †=Chi-squared test; ϕ=Fisher's exact test.

Table 5. Co-existence of *Staphylococcus aureus* virulence genes isolated from nursing staff in Lagarto, Brazil.

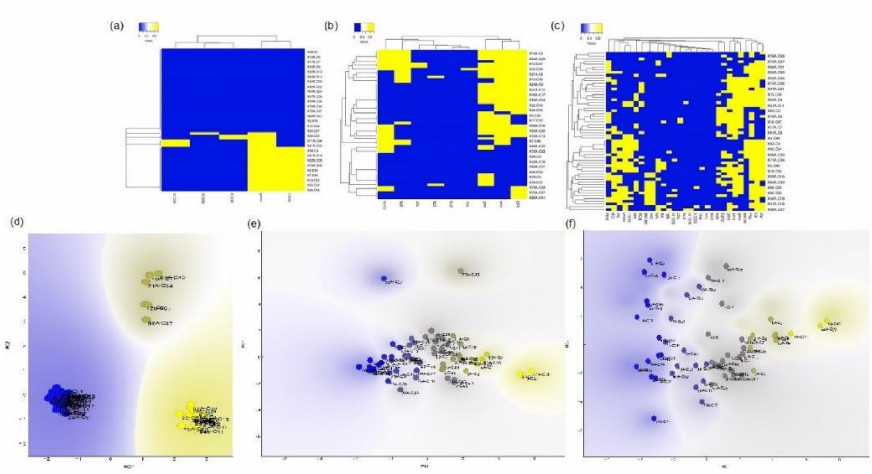
Genes	n%
*clf*a+*ica*C	2 (3.45)
*clf*a+*ica*D	2 (3.45)
eta+*ica*C	1 (1.72)
*ica*A+*ica*C	3 (5.17)
*ica*A+*ica*D	3 (5.17)
*ica*C+*ica*D	1 (1.72)
*ica*A+*ica*C+*ica*D	8 (13.79)
*clf*a+*icaa*A+*ica*C	1 (1.72)
*clf*a+*icaa*A+*ica*D	1 (1.72)
*clf*a+*spa*+*ica*A	2 (3.45)
spa+*ica*A+*ica*C	1 (1.72)
spa+*ica*A+*ica*C	1 (1.72)

spa+*ica*A+*ica*D	2 (3.45)
*clf*a+*ica*A+*ica*C+*ica*D	4 (6.90)
*clf*a+*spa*+*ica*A+*ica*D	1 (1.72)
spa+*ica*A+*ica*C+*ica*D	2 (3.45)
*clf*a+*spa*+*ica*A+*ica*C+*ica*D	4 (6.90)
eta+*spa*+*ica*A+*ica*C+*ica*D	1 (1.72)
*clf*a+*pvl*+*spa*+*ica*A+*ica*C+*ica*D	1 (1.72)
*clf*a+*spa*+*tst*+*ica*A+*ica*C+*ica*D	2 (3.45)
Note: *clf*A=gene encoding clumping factor A; *eta*=gene encoding exfoliative toxin A; *ica*ADC=gene encoding ica operon and polysaccharide intercellular adhesin; *pvl*=gene encoding panton-valentine leucocidin toxin; *tst*=gene encoding toxic shock syndrome toxin; *spa*=gene encoding staphylococcal protein A.	

Staphylococcus aureus strain clustering

Based on our data, the interrelationship and clustering of *Staphylococcus aureus* strains can be found in Figure 2. The heat maps show that different clusters (groups) were formed for strains with similar characteristics in terms of resistance (a), virulence (b) and the whole dataset (phenotypic and genotypic). The PCA analysis reinforces the heat map results, demonstrating the formation of different groups according to the above-mentioned characteristics: resistance (d), virulence (e) and for the whole dataset (phenotypic and genotypic). This data therefore demonstrates the diversity of *S. aureus* strains isolated from healthcare workers, as highlighted by the formation of 3 main groups.

Figure 2. The Heatmapper platform demonstrated the different groups formed from the *S. aureus* strains, according to resistance factors (a), virulence (b) and these factors together (c). In these heat maps, yellow indicates presence, while blue indicates absence of the respective factors. The PCA also demonstrated the diversity of the strains through the formation of groups according to resistance (d), virulence (e) and these factors together (f), as evidenced in the graph's colours.



DISCUSSION

As far as we are aware, this is the first report that demonstrates the phenotypic and genotypic profiles of *Staphylococcus aureus* resistance and virulence isolated from nursing staff and undertaken in Sergipe, Brazil.

Knowledge about colonization in healthcare workers supports the development of infection control strategies. The general prevalence of *Staphylococcus aureus* found in this study was 49.15%. Collecting samples from healthcare workers in a university hospital, obtained a prevalence of 25.7% [18]. Furthermore, in a study of nursing staff working in specialized HIV units, obtained a similar prevalence (22.9%) [19]. However, a study of working with healthcare workers in an urban university hospital, recorded a prevalence of 43.8%, similar to that found here [20]. These variations may be related to the patient population, natural microbiota on skin or collection procedures.

Multidrug resistant bacteria are difficult to treat and treatment results are less effective than for susceptible bacteria. Multidrug resistance was found in 72.41% of isolates in this study, principally to penicillin, erythromycin and azithromycin. In the same way, in a study of healthcare workers in a children's hospital in Iran, found high levels of resistance to these drugs [21]. However, when analysing isolates from general clinical patients in Austria, researchers found low resistance to azithromycin and erythromycin [22]. This may at least partially be explained by the duration of drug use, in addition to drug availability in pharmacies and potential irrational use. It is worth noting that knowledge about the profile of *Staphylococcus aureus* susceptibility to antimicrobials supports the choice and use of antimicrobial agents [23].

On the other hand, when we used a conventional antibiogram, we observed low levels of resistance to clindamycin (19.87%). In a similar way, a study, found that 17.2% of strains were resistant to clindamycin in healthcare workers in two hospitals in Ethiopia [24]. In our study, the $iMLS_B$ phenotype was found in 48.84% of isolates. However, in a study of healthcare workers in four hospitals in Tanzania, found 32.5% positive $iMLS_B$ strains, lower than the level detected here [25]. We therefore note that this level varies and depends on the population studied. This finding is important, since it emphasizes that when only using conventional antibiograms, many strains are falsely identified as sensitive to clindamycin, leading to the ineffective treatment of infections [26].

In our study, 41.37% of *Staphylococcus aureus* isolates were characterized as MRSA strains. Similarly, collecting samples from healthcare workers in a university hospital in Nepal, researchers obtained an MRSA prevalence of 41.3% [27]. However, in a study of healthcare workers in a public hospital in north-eastern Brazil, an index of 16.9% was obtained, lower than that detected here [18]. We therefore note the variability in levels between these populations. The high level found here is important, given that methicillin-resistant *Staphylococcus aureus* infections are hard to resolve, due to a lack of therapeutic options [1]. In a meta-analysis of 127 studies, was reported that transmission of MRSA to patients was probable in 93% of studies [28]. Here, 75% of isolates were SCCmec type I, while 12.5% were type III. In the same way, researchers in north-eastern Brazil found that SCCmec I was the most prevalent (40%) [18]. However the most frequently isolated MRSA clone in Brazil is Brazilian Endemic Clone (BEC) which is SCCmec-III (ST239) [29]. This suggests the circulation of different ST239 clones, which may indicate an, at least partial, replacement of this strain in Brazil. One strain found among the isolates was VISA (1.72%). This percentage agrees with the meta-analysis, which reported a prevalence of 1.0% among *Staphylococcus aureus* isolates in the United States [30].

Staphylococcus aureus is frequently linked to infections associated with biomaterials, due to the production of biofilm. Here, 89.65% of isolates were biofilm producers. Another study obtained a slightly lower level (72.83%) when analysing clinical isolates from three hospitals in Thailand [31]. In our study, there was no significant association between harbouring ica operon genes and intensity of biofilm formation. Other researchers did not observe any such relationship when analysing isolates from bovine mastitic milk [32]. In the same way, observed biofilm formation in

the absence of these genes [33]. It has therefore been suggested that other genes, in addition to the components of the ica operon, are involved in biofilm formation in these strains [34]. The isolates high biofilm-forming ability is worth noting, since it promotes adhesion to surfaces, clogs medical devices and causes infections tolerant to multiple drugs [35].

In relation to the virulence genes in the isolates, clfA was detected in 36.21% of *Staphylococcus aureus*, more than that found by similar study (8.3%) [18]. The spa gene was detected in 25.86% of the *Staphylococcus aureus* in this study. When analysing isolates from doctors, nurses and laboratory scientists in a reference hospital in Zambia, researchers obtained an almost equal amount (25.8%) [36]. Nevertheless, a previous study cited, obtained a lower level among nasal isolates (3.3%) [18]. One isolate (1.72%) harboured PVL. Other researchers did not find PVL positive strains in isolates from healthcare workers in a hospital centre in Portugal [37]. The tst gene was detected in 3.45% of isolates. Similarly, in the Czech Republic, found tst in 1.7% of isolates from patients in a university hospital [38]. The presence of multiple virulence genes found here is significant, since the more virulence factors the pathogen expresses, the greater its capacity to cause infection; It is worth noting that one of the spa positive isolates also harboured PVL, which, in association with spa, may cause fatal pneumonia [39].

Based on the similarity between genotypic and phenotypic factors, strains of *Staphylococcus aureus* were grouped and different clusters formed, demonstrating variation in the isolates we assessed. Studies which have made more in-depth analyses of the molecular aspects of MSSA and MRSA isolates from hospitals have also demonstrated diversity among these strains, alerting researchers to the role of healthcare workers as sources for the spread of more virulent and/or resistant strains within the hospital environment, and consequently to the risk of spread to the community [18,40]. The emergence of variable, more virulent or resistant strains hinders the treatment of infections and demonstrates the evolution and adaptability of these bacteria as a result of the exertion of selective pressure, principally in hospitals [18]. We therefore note the need for studies such as ours, which demonstrate the importance of understanding these microorganisms, in order to prevent spread and infection.

CONCLUSION

We observed high prevalence, multidrug resistance and the presence of MRSA strains. We also noticed that phenotypic tests to detect methicillin resistance may generate false negatives and should therefore be linked to molecular analysis. Additionally, we found numerous strains containing the iMLS$_B$ phenotype, emphasizing the importance of performing the D-test before use of clindamycin. The majority of the isolates carried important virulence genes and had the ability to produce biofilm, increasing the severity of infections they could cause. This study therefore supplements knowledge about strains that circulate in hospitals, highlighting the need to monitor the strains circulating within this hospital and to intensify precautionary measures in order to prevent the spread of these strains.

ABBREVIATIONS

AZM-Azithromycin; BHI- Brain Heart Infusion; BEC-Brazilian Endemic Clone; CIP-Ciprofloxacin; CLSI-Clinical and Laboratory Standards Institute; PCR-Conventional Polymerase Chain Reaction; ERY-Erythromycin; LZD-Linezolid; MLS$_B$-Macrolide-Lincosamide-Streptogramin B; cMLS$_B$-Macrolide-Lincosamide-Streptogramin B constitutive; iMLS$_B$-Macrolide-Lincosamide-Streptogramin B inducible; MRSA-methicillin-resistant *Staphylococcus aureus*;MA- Moderate

Adherent; NA-Non-Adherent; PVL-Panton-Valentine Leukocidin; PEN-Penicillin; PBP2a-penicillin-binding protein; PCA-principal components analysis; RIF-Rifampin; SCCmec-staphylococcal chromosomal cassette mec; SE-staphylococcal enterotoxins; SPA-staphylococcal protein A; SA-Strong Adherent; TET-Tetracycline; TSST-1-toxic shock syndrome toxin 1; WA-Weak Adherent.

AUTHORS CONTRIBUTIONS

Cavalcante RCM, Doria GAA and Marques LM coordinate the research. Oliveira MA, Andrade YMFS, Sousa AC and Campos GB collected the samples, performed the experiments, analyzed the data and wrote the main manuscript text. Silva IBS prepared figures 1-2. All authors critically reviewed the manuscript.

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DECLARATIONS

Ethical approval and consent to participate

The Committee for Ethics in Research on Human Beings at the Federal University of Sergipe approved the research under protocol number: 2377921. The participating professionals, aware of the procedures performed and the research objectives, signed the Informed Consent Term (FICF) and only then were admitted.

Consent for publication

Not applicable.

Availability of data and material

Data analyzed during this study are included in this article. Sensitive data of participant are stored confidentially.

Competing interests

The authors declare that there is no conflict of interest.

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