GMR
The Original

Virulent strains of *Staphylococcus aureus* isolated from healthcare personnel in a hospital center

PP Orellana[1,*], CF Andrade[1], MM Ginestre[2] and MR Orellana[3]

[1]Faculty of Dentistry, Laboratorio de Biología Molecular y Genética del Centro de Investigación, Innovación y Transferencia de Tecnología (CIITT) at the Universidad Católica de Cuenca. Cuenca, Ecuador.

[2]Chair of General Bacteriology. School of Bioanalysis, University of Zulia. Maracaibo-Venezuela.

[3]Regional Economics Research Group, The University of Cuenca and the Faculty of Economic and Administrative Sciences, Ecuador.

Corresponding Author: Paola Patricia Orellana Bravo
Email: porellana@ucacue.edu.ec

Genet. Mol. Res. 23 (3): gmr2346
Received June 20, 2024
Accepted September 24, 2024
Published September 27, 2024
DOI http://dx.doi.org/10.4238/gmr2346

ABSTRACT. *Staphylococcus aureus* (*S. aureus*) is a clinically significant pathogen with various virulence factors contributing to its pathogenicity. This study analyzed the frequency of *S. aureus* in healthcare personnel and evaluated the presence of virulence genes. Samples were collected from nasal secretions and the nail bed. Biochemical and molecular methods performed identification of *S. aureus*. PCR was used to detect virulence genes. The frequency of *S. aureus* in nasal secretion was 28% (14/50), and in the nail bed, 4% (2/50). The following virulence genes were detected in nasal secretion isolates: *hla/hld/hlg2*: 14/14(100%), *hlg*: 12/14(86%), *hlb*: 8/14(57%), *tst*: 7/14(50%). In the nail bed, isolates were detected: *hla/hld/hlg2*: 2/2(100%) and *hlg/hlb/tst*: 1/2(50%). No *lukS/lukF-PV* genes were detected. A significant association was found between nasal carriage of *S. aureus* and medical and nursing professions. A higher frequency of nasal carriage of *S. aureus* was detected, whereas it was deficient in the nail bed. The presence of virulence genes in *S. aureus* isolates demonstrates the importance of evaluating the virulence potential of strains circulating among carriers in hospital institutions.

Key words: *S. aureus*; Virulence genes; Carriers; Healthcare personnel

INTRODUCTION

S. aureus possesses an arsenal of elements such as surface components, proteins, toxins, and exotoxins that contribute to its pathogenicity by allowing it to adhere to tissue surfaces, evade the immune system, and induce toxic effects in the host. In addition, it has developed various resistance mechanisms to antimicrobials used to treat infections caused by this microorganism (Bien et al., 2011; Olarte et al., 2010).

Among the exoenzymes that facilitate its establishment and survival in the host are cytotoxins such as hemolysins (α, β, δ, γ) and leukocidins, especially Panton-Valentin leukocidin (PVL). Moreover, this microorganism produces three enzymes belonging to the group of pyrogenic superantigenic toxins (PTSAg), toxic shock syndrome toxin 1 (TSST-1), enterotoxins (SEs), and exfoliative toxins (ETs) A and B (Cervantes-García et al., 2014; Chamon et al., 2020).

Healthcare-associated infections are a global public health problem due to their frequency, severity, and high cost. The global prevalence is high, with an estimated 5-10% of hospitalized patients suffering from an infectious disease. Approximately 50% of these conditions are caused by *S. aureus*, a microorganism that inhabits the nasal passages, pharynx, and skin of asymptomatic carriers who can transmit the bacterium through respiratory secretions or contact with contaminated hands (Posada F, 2011).

Carrier status plays a vital role in the epidemiology and pathogenesis of staphylococcal infections. Several studies have shown that the patient's microflora usually causes nosocomial *S. aureus* infections. The initial reservoir of infection has not been clearly established; some patients are colonized with this bacterium at the time of hospitalization, and others are likely to acquire it during their hospital stay (Castellano González et al., 2005).

Healthcare personnel, especially physicians and nurses, play an essential role as reservoirs and carriers of *S. aureus* from person-to-person and patient-to-patient (Abraham et al., 2004). Direct contact with patients and poor hygiene practices are considered sources of infection and causes of the spread of *S. aureus* among hospitalized patients. According to published figures, the colonization of medical personnel with this microorganism ranges from 9.6 to 11.6% worldwide (Skov et al., 2012).

Identifying *S. aureus* reservoirs and preventive and sanitary control measures aimed at reducing colonization among healthcare personnel is of great value in preventing the spread of staphylococcal infections among hospitalized and ambulatory patients. Therefore, this research aims to analyze the frequency of colonization by *S. aureus* among members of the health personnel in a Cuenca-Ecuador hospital and to determine the virulence factors present in these strains that may contribute to developing infectious processes at the hospital level. In addition, to relate the presence of virulence genes in the isolates with characteristics of the health personnel, such as age, sex, profession, and service where they work.

MATERIALS AND METHODS

Population and sample

The population and sample comprised all health professionals working in a Cuenca-Ecuador hospital in 2021. All asymptomatic individuals who provided professional services during the study

period were included in the sample after signing the informed consent form and whose samples met the essential acceptance criteria for bacteriological culture.

Sample collection

A total of 50 individuals were studied. Nasal secretion and nail bed samples were collected from each participant. Nasal secretions were collected with a sterile swab moistened with 0.9% saline solution. The nail bed sample was obtained by rubbing the nail bed with a sterile swab moistened with 0.9% saline solution. Specimens were placed in Stuart's medium and transported to the laboratory for processing. Furthermore, each individual was administered a survey to obtain data related to the risk factors analyzed in this study.

Isolation and Identification of *S. aureus*

For isolation of *S. aureus*, the samples were inoculated on Salted Mannitol Agar (selective media for *Staphylococcus*) and Lamb Blood Agar (enriched media) and incubated under aerobic conditions at 35°C for 24-48 hours. At the end of the incubation period, macroscopic morphology was observed. Colonies with characteristics compatible with the genus *Staphylococcus* were streaked by Gram's staining. Phenotypic identification was performed by biochemical tests (coagulase, deoxyribonuclease, and mannitol fermentation).

Isolates phenotypically characterized as *S. aureus* were genotypically identified by polymerase chain reaction (PCR) using specific primers to amplify the *nucA* and *femB* genes. The primer sequence, amplicon size, and PCR conditions were taken from the techniques described by Hamdan et al.(Hamdan-Partida et al., 2015) and Ramesh et al.(Ramesh et al., 2002).

Detection of virulence genes

DNA extraction: The alkaline lysis (1% sodium dodecyl sulfate (SDS) solution in 0.25N NaOH) and boiling technique was used for DNA extraction from *S. aureus* strains, as briefly described: A suspension of colonies was prepared in 1 ml of sterile distilled water in Eppendorf tubes, centrifuged at 3000 rpm for 10 minutes, and the supernatant was discarded. Then 50 µl of lysis solution was added, vortexed, and placed in a thermoblock at 100°C for 15 minutes. Next, 450 µl of nuclease-free water was added and centrifuged at 3000 rpm for 20 seconds to obtain total DNA (Andrade T & Orellana B, 2019). The extracted DNA was stored at -20°C.

Polymerase Chain Reaction (PCR)

PCRs for the detection of the following virulence factors: *tst* gene encoding TSST-1, *lukS/lukF-PV* genes for Panton-Valentine Leukocidin production, *hla*, *hlb*, *hld*, *hlg* and *hlg-2* genes encoding alpha, beta, delta, gamma, and gamma variant hemolysins were performed according to the methodology as described by Jarraud et al.(Jarraud et al., 2002), Lozano et al.(Lozano et al., 2014); Lina et al.(Lina et al., 1999), genes, primers, and amplified fragments are listed in Table 1.

A final volume of 20 µl of reaction mixture was prepared containing: 10 µl Promega Green GoTaq 2X Mastermix, 2 µl DNA, 5 µl ultrapure water, and 1.5 µl of each primer. The amplification program consisted of 5 minutes at 94°C, 30 amplification cycles of 30 seconds at 94°C, 1 minute at 55°C and 1 minute at 72°C, and a final extension of 10 minutes at 72°C. The reactions were performed on an Agilent Sure Cycler 8800 Laica et al.(Laica et al., 2021).

Table 1. Primers used for the detection of virulence genes in *S. aureus.*

Genes	Primers (5′-3′)	Amplicon (pb)	Control strains (ATCC)
tst	F: TTCACTATTTGTAAAAGTGTCAGACCCACT	180	43300
	R: TACTAATGAATTTTTTTATCGTAAGCCCTT		
lukS/lukF-PV	F: ATCATTAGGTAAAATGTCTGGACATGATCCA	433	25923
	R: GCATCAASTGTATTGGATAGCAAAAGC		
hla	F: CTGATTACTATCCAAGAAATTCGATTG	209	25923
	R: CTTTCCAGCCTACTTTTTTATCAGT		
hlb	F: GTGCACTTACTGACAATAGTGC	309	25923
	R: GTTGATGAGTAGCTACCTTCAGT		
hld	F: AAGAATTTTTATCTTAATTAAGGAAGGAGTG	111	25923
	R: TTAGTGAATTTGTTCACTGTGTCGA		
hlg	F: GTCAYAGAGTCCATAATGCATTTAA	937	33592
	R: CACCAAATGTATAGCCTAAAGTG		
hlg-2	F: GACATAGAGTCCATAATGCATTYGT	535	25923
	R: ATAGTCATTAGGATTAGGTTTCACAAAG		

PCR products were separated by horizontal electrophoresis on 1.5% w/v agarose gel (50 ml gel with 2 µl SYBR Safe DNA Gel Stain10,000x from Invitrogen) immersed in 1X TAE buffer. The run was performed at 70 V, 70 A, and 50 W for 60 min according to the method described by Jarraud et al.(Jarraud et al., 2002) and Pacheco et al.(Pacheco et al., 2021). The 1kb Plus DNA Ladder molecular weight marker (Trackit from Invitrogen) was used. The size of the amplicons was calculated according to the migrations on agarose gels, compared to the migration of the standard DNA bands of the *100bp Plus DNA Ladder* molecular weight marker (Trackit from Invitrogen) and photographed on a UV transilluminator with a digital camera.

Statistical analysis

Continuous data are presented as mean ± standard deviation, and categorical data are presented as numbers and percentages. Pearson's chi-squared test, Fisher's test, and logistic regression were used to evaluate possible associations between the presence of virulence genes in *S. aureus* strains and the risk factors analyzed. For all tests, a *p-value* < 0.10 was considered statistically significant.

RESULTS

The incidence of *S. aureus* in nasal secretion samples from healthcare personnel was 28% (14/50), while only 4% (2/50) of nail beds were positive for this microorganism. Table 2 shows the results of virulence gene detection in *S. aureus* isolates. As can be seen, no genes for PVL production were detected. The presence of *hla*, *hld* and *hlg2* genes for hemolysin production was evident in all strains. In addition, the production of *tst*, *hlb* and *hlg* genes was detected in 50% or more of the isolates.

Table 3 shows the virulence gene profiles of the 16 *S. aureus* strains isolated from nasal secretions and nail beds. In 50% (8) of the isolates, the presence of genes for hemolysin production was detected, accompanied by the gene for TSST-1 expression.

Table 2. Presence of virulence genes in *S. aureus* isolates from healthcare personnel.

Virulence Genes	*S. aureus* strains	
	Nasal secretion (n=14)	**Nail bed (n=2)**
	N (%)	**N (%)**
hla	14 (100)	2 (100)
hld	14 (100)	2 (100)
hlg2	14 (100)	2 (100)
hlg	12 (86)	1 (50)
hlb	8 (57)	1 (50)
tst	7 (50)	1 (50)
lukS/lukF-PV	0 (0)	0 (0)

Table 3. Virulence gene profiles of *S. aureus* isolates from healthcare personnel.

Virulence genes	*S. aureus* strains	
	N	**%**
hla, hld, hlg2	3	18,75
hla, hld, hlg2, hlg	2	12,50
hla, hld, hlg2, hlg, hlb,	3	18,75
hla, hld, hlg2, hlg, tst,	2	12,50
hla, hld, hlg2, hlg, hlb, tst	6	37,50
Total	**16**	**100**

On the other hand, 8 (50%) *S. aureus* produced genetic profiles only for hemolysin expression, of which three showed the totality of hemolysin-producing genes. In contrast, in three other isolates, the *hla*, *hld*, *hlg2* profile was obtained, and in two strains, the latter profile was accompanied by the presence of the *hlg* gene (Table 3).

Figures 1-7 show the results of PCR used to detect the genes for hemolysins (*hla, hlb, hld, hlg, hlg2*), toxic shock syndrome (*tst*), and Panton Valentine leukocidin (*lukS/lukF-PV*) in *S. aureus* strains isolated from nasal secretion and nail bed samples.

A descriptive bivariate analysis of the risk factors considered in this study and the presence of virulence gene-producing *S. aureus* strains isolated from nasal secretions was performed. Qualitative variables were presented as frequencies and percentages. For each variable, the chi-squared test was applied and the probability (*p*), odds ratio and 95% confidence intervals (CI 95%) were calculated (Table 4).

The detection of *S. aureus* was 14% higher in the male sex. Concerning age, this bacterium's highest number of carriers occurred in individuals aged 30 years or younger. On the other hand, physicians were the professionals most likely to harbor this microorganism in their nostrils. The presence of *S. aureus* was 10% higher in individuals who worked more than 8 hours per day than those who worked 8 hours or less (Table 4).

Emergency, surgery, operating room, and intensive care units had the highest concentrations of *S. aureus* carriers. Those who reported an allergic condition had an 11% higher frequency of isolation compared with those who did not have an allergic condition. Pet ownership at home was a

variable for which there was a 15% difference between the professionals sampled, which was higher in those who reported living with pets (Table 4).

Table 5 shows the results of the logistic regression analysis. The independent variables from the bivariate analysis with a *p-value* < 0.1 were included. Odds ratios and their 95% confidence intervals are shown for each variable. These results confirm that *S. aureus* is mainly present in physicians and, to a lesser extent, in nurses; specifically, physicians were three times more likely to be carriers of this bacterium.

The bivariate analysis of the different virulence genes presents in the *S. aureus* strains isolated from nasal secretions and their association with risk factors is shown in Table 6.

In the 14 samples positive for this microorganism, a statistically significant association was found between the presence of hemolysin producing *S. aureus* and the variables physician and nurse. When analyzing the strains that tested positive for the *TSST-1* expression gene, no statistically significant evidence confirmed that the risk factors analyzed influenced the carriage of *S. aureus* producing the *tst* gene (Table 6).

Table 4. Bivariate analysis of variables associated with nasal carriage of *S. aureus* in healthcare personnel.

Variable	**Category**	**Total**	***S. aureus***					**Odd Ratio**		
			Negative (72%)		**Positive (28%)**		**p***	**OR**	**CI (95%)**	
		N	**N**	**%**	**N**	**%**			**CIi**	**CIs**
Sex	Woman	34	26	76.5	8	23.5				
	Man	16	10	62.5	6	37.5	0.305	1.95	0.4	8.4
Age	≤ 30 years	29	20	69.0	9	31.0				
	> 30 years	21	16	76.2	5	23.8	0.574	0.7	0.2	2.9
Occupation	Physician (yes/no)	19	11	57.9	8	42.1	**0.082**	3.0	0.7	13.2
	Nurse (yes/no)	22	19	86.4	3	13.6	**0.045**	0.2	0.0	1.2
	Other (yes/no)	9	6	66.7	3	33.3	0.697	1.4	0.2	7.8
Workday	≤ 8 hours	28	21	75.0	7	25.0				
	> 8 hours	22	15	68.2	7	31.8	0.594	1.4	0.3	5.8
Years of service	≤ 1 year	26	18	69.2	8	30.8				
	> 1 year	24	18	75.0	6	25.0	0.650	0.8	0.2	3.1
Service Work	Outpatient/Clinical visit (yes/no)	15	12	80.0	3	20.0	0.507	0.5	0.1	2.7
	Emergency/OR/Sx/ ICU (yes/no) **	18	12	66.7	6	33.3	0.529	1.5	0.3	6.3
	Other(yes/no)	17	12	70.6	5	29.4	1.000	1.1	0.2	4.7
Allergic	No	39	29	74.4	10	25.6				
	Yes	11	7	63.6	4	36.4	0.476	1.7	0.3	8.3
Pet ownership	No	12	10	83.3	2	16.7				
	Yes	38	26	68.4	12	31.6	0.468	2.3	0.4	24.5
ATB*** last 3 months	No	44	31	70.5	13	29.5				
	Yes	6	5	83.3	1	16.7	0.663	0.5	0.0	5.0

*p<0.1; **OR: operating room, Sx: surgery; ICU: Intensive Care Unit; ***ATB: antibiotics

Table 5. Logistic model for the variables Physician and Nurse.

Variables	Coefficients β		Odd Ratio	
	Model 1	**Model 2**	**exp(β)**	**[95% CI]**
Profession (0 = other)				
Physician	1.11*		3.03	[-0.21; 6.27]
Nurse		-1.41*	0.24	[-0.05; 0.54]
Constant	-1.43***	-0.44		

*: $p < 0.1$; ***: $p < 0.01$

Table 6. Bivariate logistic regression analysis of variables associated with *S. aureus* carrying virulence genes.

Variable	**Category**	**Total**	**Hemolysins**			**TSST-1**		
			Positive		**p***	**Positive**		**p***
			N	**%**		**N**	**%**	
Sex	Woman	34	8	23.5		4	11.8	
	Man	16	6	37.5	0.305	3	18.8	0.507
Age	≤ 30 years	29	9	31.0		4	13.8	
	> 30 years	21	5	23.8	0.574	3	14.3	0.960
Occupation	Physician (yes/no)	19	8	42.1	**0.082**	4	21.1	0.261
	Nurse (yes/no)	22	3	13.6	**0.045**	2	9.1	0.375
	Others (yes/no)	9	3	33.3	0.697	1	11.1	0.783
Workday	≤ 8 hours	28	7	25.0		2	2.0	
	> 8 hours	22	7	31.8	0.594	5	5.0	0.115
Years of service	≤ 1 year	26	8	30.8		4	15.4	
	> 1 year	24	6	25.0	0.650	3	12.5	0.769
Service Work	Outpatient/Clinical visit(yes/no)	15	3	20.0	0.409	2	13.3	0.929
	Emergency/OR/Sx/ICU (yes/no) **	18	6	33.3	0.529	3	16.7	0.684
	Others(yes/no)	17	5	29.4	0.873	2	11.8	0.744
Allergic	No	39	10	25.6		6	15.4	
	Yes	11	4	36.4	0.484	1	9.1	0.595
Pet ownership	No	12	2	16.7		2	16.7	
	Yes	38	12	31.6	0.316	5	13.2	0.760
***ATB last 3 months	No	44	13	29.5		7	15.9	
	Yes	6	1	16.7	0.510	0	0.0	0.292
*p<0,1; **OR: operating room, Sx: surgery; ICU: Intensive Care Unit; ***ATB: antibiotics								

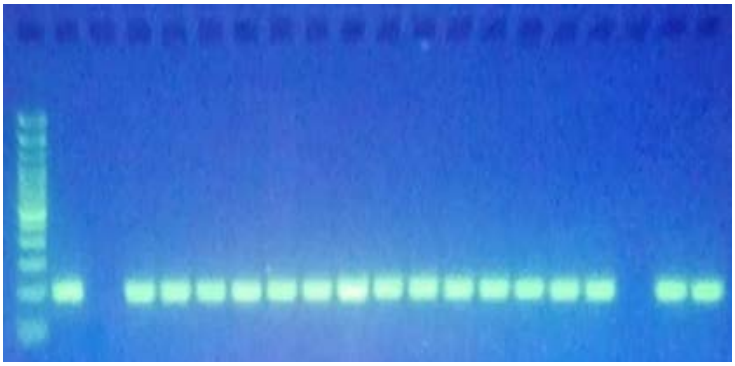

Figure 1. PCR product for *hla* (209 pb) gene in *S. aureus***:** lane 1 ladder, lane 2 positive control *S. aureus* ATCC 25923, lane 3 negative control *S. pyogenes* ATCC 12344, lane 4-17 strains positive for nasal secretion, lane 19-20 strains positive for nail bed.

Figure 2. PCR product for *hld* (111 pb) gene in *S. aureus***:** lane 1 ladder, lane 2 positive control *S. aureus* ATCC 25923, lane 3 negative control *S. pyogenes* ATCC 12344, lane 4-17 strains positive for nasal secretion, lane 19-20 strains positive for nail bed.

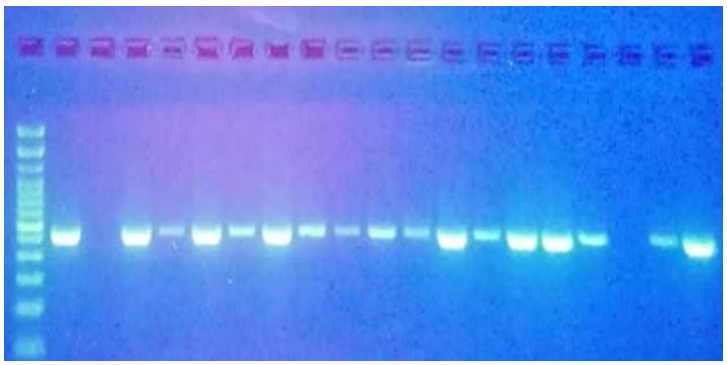

Figure 3. PCR product for *hlg2* (535 pb) gene in *S. aureus*: lane 1 ladder, lane 2 positive control *S. aureus* ATCC 25923, lane 3 negative control *S. pyogenes* ATCC 12344, lane 4-17 strains positive for nasal secretion, lane 19-20 strains positive for nail bed.

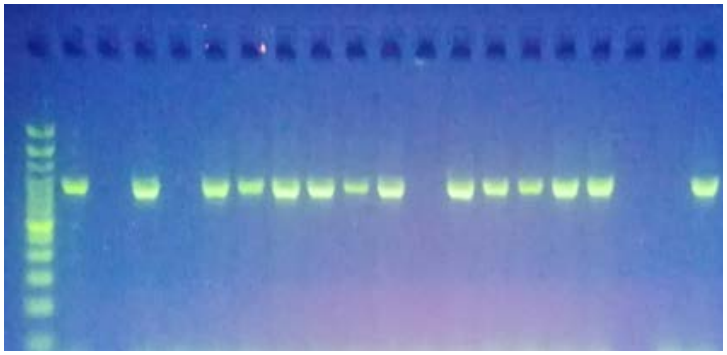

Figure 4. PCR product for *hlg* (937 pb) gene in *S. aureus*: lane 1 ladder, lane 2 positive control *S. aureus* ATCC 33592, lane 3 negative control *S. pyogenes* ATCC 12344, lane 4, 6-11, 13-17, strains positive for nasal secretion, lane 20 strains positive for nail bed.

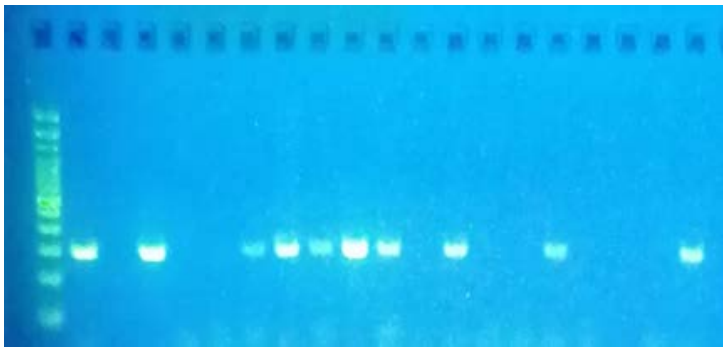

Figure 5. PCR product for *hlb* gene (309 pb) in *S. aureus*: lane 1 ladder, lane 2 positive control *S. aureus* ATCC 25923, lane 3 negative control *S. pyogenes* ATCC 12344, lane 4, 7-11, 13,16 strains positive for nasal secretion, lane 20 strains positive for nail bed.

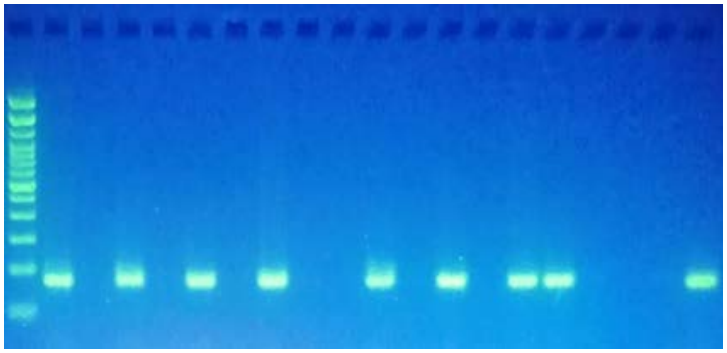

Figure 6. PCR product for *tst* gene (180 pb) in *S. aureus*: lane 1 ladder, lane 2 positive control *S. aureus* ATCC 43300, lane 3 negative control *S. pyogenes* ATCC 12344, lane 4, 6, 8, 11, 13, 15 ,16 strains positive for nasal secretion, lane 20 strains positive for nail bed.

Figure 7. PCR product for *lukS-PV/lukF-PV* gene (433 pb) in S. *aureus*: lane 1 ladder, lane 2 positive control *S. aureus* ATCC 25923, lane 3 negative control *S. pyogenes* ATCC 12344, lane 4-17 strains negative for nasal secretion, lane 19-20 strains negative for nail bed.

DISCUSSION

Colonization of the nasal passages and skin favors the persistence of *S. aureus* in the organism and is considered a reservoir for staphylococcal infections. In the present study, the frequency of *S. aureus* isolation from the nasal passages of healthcare personnel was 28%, which is significant for transmitting this bacterium to patients, particularly those with an immunodeficient due to severe diseases.

This study's results agree with data from other investigators (Boncompain et al., 2017; Laceb et al., 2022; Odu NN, 2012; Walana et al., 2020), who reported 25.5%-32% nasal carriage of *S. aureus* in students and healthcare personnel. Several authors have reported relatively lower isolation rates of 19-22% (Chen et al., 2015; Conceição et al., 2014; González et al., 2016; Hefner & Linde, 2018; Karimi et al., 2017; Rongpharpi et al., 2013; Sedaghat et al., 2018; Xie et al., 2018) among workers in various hospital services. On the other hand, other studies (Cimera-Proaño & Pérez-Pazmiño, 2010; Erdenizmenli et al., 2004; Khanal et al., 2021; Rongpharpi et al., 2013) reported a frequency of *S. aureus* carriers in professionals of hospital institutions ranging from 8.8 to 15.7%.

Results inconsistent with those obtained in this study were reported by Capozzi et al. (Capozzi et al., 2015), who evaluated the presence of *S. aureus* in the nursing staff of a hospital in Venezuela and found a nasal carrier rate of 50%; this corresponds to that reported in a hospital in Brazil by Prates et al. (Prates et al., 2010), where they notified a frequency of nasal isolation of 41%. Similarly, other studies (Boisset et al., 2019; Danelli et al., 2020; Sarkar et al., 2016) have reported a 39-43% prevalence of asymptomatic carriers in healthcare workers and students.

Studies of hand carriers are limited compared to those of nasal ones. In this study, all carriers of *S. aureus* strains in the nail bed also carried them in the nostrils. The frequency of this microorganism in this anatomical site was similar to that reported by Rai et al.(Rai et al., 2022) in hospital workers in Nepal.

Strict compliance with prevention measures due to the pandemic, such as hand washing, using disinfectant solutions, gloves, and masks, may have influenced the low isolation of *S. aureus* in the fingernail beds of the healthcare personnel participating in this investigation.

All the *S. aureus* strains isolated in this study showed virulence determinants; the detection of the *hla*, *hld* and *hlg2* genes, which encode for the production of hemolysins in all isolates, stands out.

On the other hand, half of the isolates presented the *tst* gene. No genes for PVL production (*lukS/lukF-PV*) were obtained.

Similar results for hemolysin gene production were reported by Xie et al. (Xie et al., 2018), who studied the production of exotoxins in strains from the clinical laboratory staff of a hospital and reported the detection of *hla*, *hld* and *hlg* genes in 98% or more of the isolates. However, they differed about the *tst* gene, found in only 23%, and the presence of *lukS/lukF-PV* genes, found in 15% of *S. aureus*.

Data from a study by Lozano et al. (Lozano et al., 2014), which evaluated the presence of exoenzymes in *S. aureus* obtained from asymptomatic carriers, are consistent with this research's results concerning the production of *hla*, *hld* and *hlb* genes and the absence of *lukS/lukF-PV* genes. However, they differ in the presence of the *tst* gene in 28% of the strains and a lower proportion of the *hlg2* gene (50%).

The findings of this study are not in agreement with those reported by Schaumburg et al. (Schaumburg et al., 2011), who analyzed *S. aureus* strains obtained from asymptomatic carriers and found a low frequency of *tst* (10%) and a high percentage of *lukS/lukF-PV* genes (40.5%).

The results published by Conceição et al. (Conceição et al., 2014) in isolates obtained from patients and health personnel carriers are in agreement with this investigation regarding the high number of strains carrying hemolysin genes. However, they reported the presence of PVL genes in 36% of the isolates. This was not the case in this study, where no PVL genes were detected.

S. aureus strains isolated by Li et al.(Li et al., 2019) in China from clinical samples of patients with various infectious pathologies were analyzed for various virulence determinants. As in the present study, most isolates carried genes expressing some type of hemolysin. However, they differed regarding PVL gene production since the strains carried *lukS/lukF-PV* genes (48%), which were not found in this investigation.

In agreement with this study, Sedaghat et al.(Sedaghat et al., 2018) detected the *hla* gene in almost all *S. aureus* strains from healthcare personnel and patients in an Iranian hospital. In contrast, they reported the genes for PVL in 22% of isolates and a lower frequency for *tst* (17%).

All *S. aureus* isolates obtained in this study had three or more genes for hemolysin production; similarly, other investigators report the presence of at least one hemolysin gene in isolates of clinical and carrier origin. The constant production of these exotoxins may be related to the chromosomal location of the genes for their expression.

An essential finding of this research is detecting of the *tst* gene in half of the *S. aureus* strains. The product of this gene, TSST-1, is a potent exotoxin belonging to the superantigen family that activates T lymphocytes, resulting in a systemic inflammatory response due to cytokine overproduction and toxic shock. Although the *tst* gene is located on a pathogenicity island, a bacteriophage can transfer it between isolates(Gillet et al., 2019).

The chromosomal and extrachromosomal localization of virulence genes, and the constant exchange of genetic material between strains of *S. aureus* and even between different species of Gram-positive bacteria have contributed to this microorganism's genetic evolution with a wide variety of virulence markers that enhance its pathogenicity.

On the other hand, the expression of virulence genes in *S. aureus* is regulated by a quorum-sensing mechanism that controls the processes of colonization and survival of the microorganism

in the host cells. Thus, in the initial growth phase, the bacterium mainly produces adhesion factors, whereas genes for the production of exoenzymes are mainly expressed in the exponential development phase(Garza-Velasco et al., 2013).

The presence of virulence genes in *S. aureus* strains does not necessarily imply their expression, as genes may not be expressed due to mutations in the coding or regulatory region. However, nasal colonization by *S. aureus* carrying exotoxin genes in hospital workers and healthcare students represents a potential risk for the emergence of highly pathogenic *S. aureus* strains adapted to the hospital environment and capable of spreading these virulence markers, with the consequent production of severe and difficult-to-treat infections in hospitalized patients. In addition, continuous exposure makes carriers a vehicle for the cross-transmission of toxigenic pathogens between the hospital and the community (Danelli et al., 2020).

Risk factors such as age, sex, cohabitation with healthcare personnel, occupation, geographic location, smoking habit, antibiotic use, underlying diseases, hospitalization, nasal cleaning habits, contact with pets, working hours, hospital service where the patient works, ethnicity, among others, have been associated with nasal colonization by *S. aureus* in asymptomatic carriers.

In this study, nine descriptive characteristics of the participants (age, sex, occupation, working day, working hours, service works, allergic, pet ownership, and antimicrobial use) and their potential risk for nasal carriage of *S. aureus* were analyzed.

The applied bivariate logistic regression analysis results indicate a dependency relationship between nursing and medical professions and nasal carriage of *S. aureus*. In other words, nursing and medical professionals have a higher risk of being nasal carriers of *S. aureus* than the other professionals considered.

The Odd Ratio (OR) was used to measure the strength of the relationship between the variables. The calculated OR value indicates that physicians have a three times higher risk of being nasal carriers of *S. aureus* than the rest of the professionals.

Several authors have evaluated the association between population characteristics and nasal carriage of *S. aureus* in healthcare personnel. Statistically significant associations have been reported for the variables male sex (Halablab et al., 2010; Sedaghat et al., 2018), occupation (Danelli et al., 2020; Laceb et al., 2022; Walana et al., 2020), age (Halablab et al., 2010; Yan et al., 2015), ethnicity (Yan et al., 2015), geographic región (Yan et al., 2015), diabetes mellitus (Sedaghat et al., 2018), health worker relative (Halablab et al., 2010; Sedaghat et al., 2018; Walana et al., 2020), asthma (Halablab et al., 2010), antibiotic use (Halablab et al., 2010).

Among the health personnel studied, nasal carriage of *S. aureus* is independent of the other variables considered in this study; therefore, the carrier status of this bacterium is not associated with sex, age, working hours, years of service, service in which work, pet ownership, and antimicrobial use.

CONCLUSIONS

This investigation described the presence of *S. aureus* in health personnel carriers in a Cuenca-Ecuador hospital. The results indicate a higher frequency of carriers in the nasal cavities compared to the nail bed, where the recovery of this microorganism was much lower.

The presence of genes for the expression of virulence factors in the *S. aureus* isolates obtained in this study demonstrates the importance of evaluating the virulence potential of strains circulating among carriers of healthcare personnel in hospital facilities since it may reflect the direct and constant exposure to intrahospital pathogens and their toxins.

The medical and nursing professions were the risk factors for which a statistically significant association with nasal carriage of *S. aureus* was found. Although it is true that carrier status per se does not necessarily imply suffering from an infectious process, this condition facilitates colonization and transmission of staphylococcal infections among hospitalized patients in direct contact with healthcare personnel, especially those with poorly competent immune systems as a result of invasive processes or chronic diseases.

The isolation of *S. aureus* strains carrying the *tst* gene for the expression of *TSST-1* and genes for the production of hemolysins (*hla*, *hlb*, *hld*, *hlg* and *hlg2*), cytolytic exotoxins for blood and endothelial cells, is an important finding that merits the implementation of control measures aimed at preventing the spread of these strains among healthcare personnel and patients.

ACKNOWLEDGMENTS

This article is part of the project: "Detection of resistance and virulence genes of *Staphylococcus aureus* isolated from cellular screening of health personnel" of the Research Group in Genetics and Molecular Biology of Microorganisms of the Universidad Católica de Cuenca.

The authors thanks Universidad Católica de Cuenca (Cuenca-Ecuador) and their authorities for all support and allowing the use of the Laboratorio de Biología Molecular y Genética del Centro de Investigación, Innovación y Transferencia de Tecnología (CIITT), as well as access to clinics, materials, chemicals, human resources for the development of this research. Finally, this study was carried out with resources from the CIITT of the Universidad Católica de Cuenca, and self-managed resources.

Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

Abraham J, Mansour C, Veledar E, Khan B, et al. (2004). *Staphylococcus aureus* bacteremia and endocarditis: The Grady Memorial Hospital experience with methicillin-sensitive *S aureus* and methicillin- resistant *S aureus* bacteremia. *Am Heart J.* 147: 536–9.

Andrade C, Orellana P. (2019). Frecuencia y susceptibilidad a penicilina y meticilina de aislamientos ambientales de *Staphylococcus aureus* en un hospital de Cuenca. *Kasmera*. 47: 123–30.

Bien J, Sokolova O, Bozko P. (2011). Characterization of Virulence Factors of *Staphylococcus aureus*: Novel Function of Known Virulence Factors That Are Implicated in Activation of Airway Epithelial Proinflammatory Response. *J Pathog.* 2011:1–13.

Boisset S, Saadatian-Elahi M, Landelle C, Bes M, et al. (2019). Unexpected categories at risk of *S. aureus* nasal carriage among hospital workers. *Int J Hyg Environ Health.* 222: 1093–7.

Boncompain CA, Suárez CA, Morbidoni HR. (2017). *Staphylococcus aureus* nasal carriage in health care workers: First report from a major public hospital in Argentina. *Rev Argent Microbiol.* 49: 125–31.

Capozzi E, Molbili D, Martinez I. (2015). Portadores nasales de *S. aureus* en el personal de enfermería de un centro de salud del Estado Carabobo, Venezuela. *Kasmera.* 43: 139–47.

Castellano González M, Bermúdez Navarro E, Armindo Perozo Mena L, Camacho Molina B, et al. (2005). *Staphylococcus aureus*: estado de portador en personal de enfermería y patrones de susceptibilidad antimicrobiana. *Rev Soc Venez Microbiol.* 25: 72–8.

Cervantes-García E, García-González R, Salazar-Schettino PM. (2014). Características generales del *Staphylococcus aureus*. *Rev Latinoam Patol Clin Med Lab.* 61: 28–40.

Chamon RC, de Oliveira TLR, Ferreira RBR, Moreira LO, et al. (2020). Panton-Valentine leukocidin (PVL) isoforms among *Staphylococcus aureus* lineages isolated from hospitals in Rio de Janeiro, Brazil. *Braz J Microbiol.* 51: 1509–13.

Chen B, Dai X, He B, Pan K, et al. (2015). Differences in *Staphylococcus aureus* nasal carriage and molecular characteristics among community residents and healthcare workers at Sun Yat-Sen University, Guangzhou, Southern China. *BMC Infect Dis.* 15: 1–12.

Cimera-Proaño D, Pérez-Pazmiño F. (2010). Prevalencia de portadores nasales asintomáticos de *Staphylococcus aureus* meticilino-resistente y su relación con factores de riesgo y protectores en el personal de salud del Hospital General de las Fuerzas Armadas. *Rev Latinoam Patol Clínica Med Lab.* 57: 196–204.

Conceição T, Santos Silva I, De Lencastre H, Aires-De-Sousa M. (2014). *Staphylococcus aureus* nasal carriage among patients and health care workers in são tomé and príncipe. *Microb Drug Resist.* 20: 57–66.

Danelli T, Duarte FC, de Oliveira TA, da Silva RS, et al. (2020). Nasal carriage by *Staphylococcus aureus* among healthcare workers and students attending a university hospital in Southern Brazil: Prevalence, phenotypic, and molecular characteristics. *Interdiscip Perspect Infect Dis.* 2020.

Erdenizmenli M, Yapar N, Senger SS, Özdemir S, et al. (2004). Investigation of colonization with methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* in an outpatient population in Turkey. *Jpn J Infect Dis*. 57: 172–5.

Garza-Velasco R, Zúñiga-Rangel O, Perea-Mejía LM. (2013). Current perspective on clinical significance of *Staphylococcus aureus* in the hospital environment. *Educ Quimica.* 24: 8–13.

Gillet Y, Henry T, Vandenesch F. (2019). Fulminant staphylococcal infections. *Gram-Posit Pathog.* 712–22.

González M, Hernández N, Apaulaza K, Díaz M, et al. (2016). Portadores asintomáticos nasal y faríngeo de *Staphylococcus aureus* en trabajadores de un hospital pediátrico Nasal and pharyngeal asymptomatic carriers of *Staphylococcus aureus* in pediatric-hospital workers. *Cienc Médicas Pinar Río.* 20: 298–305.

Halablab MA, Hijazi SM, Fawzi MA, Araj GF. (2010). *Staphylococcus aureus* nasal carriage rate and associated risk factors in individuals in the community. *Epidemiol Infect.* 138: 702–6.

Hamdan-Partida A, González García S, Bustos-Martínez J. (2015). Identificación de *Staphylococcus aureus* utilizando como marcadores los genes *nucA* y *femB*. *Cienc Clínicas.* 16: 37–41.

Hefner JT, Linde KC. (2018). Chapter 20 - Analytical Methods. In: Hefner JT, Linde KC, editors. Atlas of Human Cranial Macromorphoscopic Traits. *Academic Press*; 287–92.

Jarraud S, Mougel C, Thioulouse J, Lina G, et al. (2002). Relationships between *Staphylococcus aureus* genetic background, virulence factors, *agr* groups (alleles), and human disease *Relation. Infect Immun.* 70: 631–41.

Karimi M, Esfahani BN, Halaji M, Shahin M, et al. (2017). Molecular characteristics and antibiotic resistance pattern of *Staphylococcus aureus* nasal carriage in tertiary care hospitals of Isfahan, Iran. *Infez Med.* 25: 234–40.

Khanal A, G.C. S, Gaire A, Khanal A, Estrada R, et al. (2021). Methicillin-resistant *Staphylococcus aureus* in Nepal: A systematic review and meta-analysis. *Int J Infect Dis.* 103: 48–55.

Laceb ZM, Diene SM, Lalaoui R, Kihal M, et al. (2022). Genetic Diversity and Virulence Profile of Methicillin and Inducible Clindamycin-Resistant *Staphylococcus aureus* Isolates in Western Algeria. *Antibiotics*. 11.

Laica SP, Andrade CF, Orellana PP, Ramos RR. (2021). Resistance to beta-lactams in *Staphylococcus aureus* isolated from cell phone screens of dentistry students based on an antibiogram and detection of *blaZ* and *mecA* genes. *Genet Mol Res.* 20.

Li X, Huang T, Xu K, Li C, et al. (2019). Molecular characteristics and virulence gene profiles of *Staphylococcus aureus* isolates in Hainan, China. *BMC Infect Dis.* 19: 1–12.

Lina G, Piémont Y, Godail-Gamot F, Bes M, et al. (1999). Involvement of Panton-Valentine Leukocidin—Producing *Staphylococcus aureus* in Primary Skin Infections and Pneumonia. *Clin Infect Dis.* 29: 1128–32.

Lozano C, Marí A, Aspiroz C, Gómez-Sanz E, et al. (2014). Nasal carriage of coagulase positive staphylococci in patients of a Primary-Healthcare-Center: Genetic lineages and resistance and virulence genes. *Enferm Infecc Microbiol Clin.* 33: 391–6.

Odu NN OI. New York Science Journal 2012;5(7) http://www.sciencepub.net/newyork Nasal carriage and antibiotics susceptibility of. *N Y Sci J.* 5:56–63.

Olarte NM, Valderrama IA, Reyes KR, Garzón MI, et al. (2010). Colonización por *Staphylococcus aureus* resistente a la meticilina en una unidad de cuidados intensivos de adultos de un hospital colombiano: caracterización fenotípica y molecular con detección de un clon de circulación en la comunidad. *Biomédica.* 30: 353.

Pacheco MA, Orellana PP, Andrade CF, Torracchi JE. (2021). Virulence genes in *Staphylococcus aureus* isolated from cell phone screens of dentistry students in Cuenca-Ecuador. *Genet Mol Res.* 20.

Posada F, Posada P, Retureta M, Ferrer Martín Y. (2011). Riesgo de infecciones respiratorias agudas en la población infantil del municipio Ciego de Ávila. *MEDICIEGO.* 17.

Prates KA, Torres AM, Garcia LB, Yamada Ogatta SF, et al. (2010). Nasal carriage of methicillin-resistant *Staphylococcus aureus* in university students. *Braz J Infect Dis.* 14: 316–8.

Rai JR, Amatya R, Rai SK. (2022). Hand and nasal carriage of *Staphylococcus aureus* and its rate of recolonization among healthcare workers of a tertiary care hospital in Nepal. *JAC-Antimicrob Resist.* 4: 1–5.

Ramesh A, Padmapriya BP, Chrashekar A, Varadaraj MC. (2002). Application of a convenient DNA extraction method and multiplex PCR for the direct detection of *Staphylococcus aureus* and Yersinia enterocolitica in milk samples. *Mol Cell Probes.* 16: 307–14.

Rongpharpi SR, Hazarika NK, Kalita H. (2013). The prevalence of nasal carriage of *Staphylococcus aureus* among healthcare workers at a tertiary care hospital in Assam with special reference to MRSA. *J Clin Diagn Res.* 7: 257–60.

Sarkar A, Raji A, Garaween G, Soge O, et al. (2016). Antimicrobial resistance and virulence markers in methicillin sensitive *Staphylococcus aureus* isolates associated with nasal colonization. *Microb Pathog.* 93: 8–12.

Schaumburg F, Ngoa UA, Kösters K, Köck R, et al. (2011). Virulence factors and genotypes of *Staphylococcus aureus* from infection and carriage in Gabon. *Clin Microbiol Infect.* 17: 1507–13.

Sedaghat H, Esfahani BN, Halaji M, Jazi AS. (2018). *Genetic Iran.* 10: 82–9.

Skov R, Christiansen K, Dancer SJ, Daum RS, et al. (2012). Update on the prevention and control of community-acquired meticillin-resistant *Staphylococcus aureus* (CA-MRSA). *Int J Antimicrob Agents.* 39: 193–200.

Walana W, Bobzah BP, Kuugbee ED, Acquah S, et al. (2020). *Staphylococcus aureus* nasal carriage among healthcare workers, inpatients and caretakers in the Tamale Teaching Hospital, Ghana. *Sci Afr.* 8: e00325.

Xie X, Dai X, Ni L, Chen B, et al. (2018). Molecular epidemiology and virulence characteristics of *Staphylococcus aureus* nasal colonization in medical laboratory staff: Comparison between microbiological and non-microbiological laboratories. *BMC Infect Dis.* 18: 1–10.

Yan X, Song Y, Yu X, Tao X, et al. (2015). Factors associated with *Staphylococcus aureus* nasal carriage among healthy people in Northern China. *Clin Microbiol Infect.* 21: 157–62.