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MICROBIOLOGICAL ANALYSIS OF SURFACES IN TWO SECTORS OF A HOSPITAL IN NORTHWESTERN PAULISTA WITH A FOCUS ON THE DETECTION OF *STAPHYLOCOCCUS AUREUS*•

ABSTRACT

Staphylococcus aureus is a common and virulent pathogen among healthcare-associated infections (HAIs), which can cause various diseases and includes antibiotic-resistant strains. The aim of this study was to analyze the presence of microbial contamination with a focus on S. aureus in the Intensive Care Unit (ICU) and Emergency Department (ED) sectors of a hospital in northwestern São Paulo. To do this, samples were taken from an isolation bed, a normal bed, the counter, a pen, and the door handles of the ED and ICU, before and immediately after cleaning the surfaces. The samples were transported in sterile BHI broth to the laboratory, where a 0.1 ml aliquot was inoculated into BHI agar and mannitol medium. After growth in an oven at 37 ◦C for 24-48 hours, the colonies indicative of S. aureus were subjected to the catalase, coagulase and Gram stain biochemical tests. The results indicated the presence of S. aureus in the ICU's isolation bed, in the ED's normal bed, and on the door handle and counter of the ICU before the rooms were cleaned. After they were cleaned by hospital staff, S. aureus colonies were found in the ICU's normal bed, isolation bed, and counter and in the ED's normal bed and counter. S. aureus was detected both before and after aseptic cleaning of surfaces, which demonstrates the need to implement more effective aseptic measures. The significant presence of S. aureus is a potential risk for both healthcare workers and patients, the latter being more susceptible to acquiring HAIs because they are immunocompromised.

Keywords: Staphylococcus aureus. Healthcare-Associated Infections. Intensive Care Unit. Emergency Department. Asepsis.

ANÁLISE MICROBIOLÓGICA DE SUPERFÍCIES EM DOIS SETORES DE UM HOSPITAL DO NOROESTE PAULISTA COM FOCO NA DETECÇÃO DE *STAPHYLOCOCCUS AUREUS*

RESUMO

Staphylococcus aureus é um patógeno comum e virulento em Infecções Relacionadas à Assistência à Saúde (IRAS), podendo ocasionar diversas enfermidades e apresentar cepas resistentes a antibióticos. O objetivo deste estudo foi analisar a presença de contaminação microbiana com foco no S. aureus em setores da Unidade de Terapia Intensiva (UTI) e Pronto Atendimento (PA) de um hospital do noroeste paulista. Para isso, amostras foram coletadas do leito de isolamento, leito comum, balcão, caneta e maçaneta do PA e UTI, antes e logo após a limpeza das superfícies. As amostras foram transportadas em caldo BHI estéril até o laboratório, no qual uma alíquota de 0,1 ml foi inoculada em meio BHI Agar e Manitol. Após crescimento em estufa a 37°C por 24-48 horas, as colônias indicativas para S. aureus foram submetidas a provas bioquímicas de catalase, coagulase e coloração de gram. Os resultados indicaram a presença de *S. aureus* no leito de isolamento da UTI, leito comum do PA, maçaneta e balcão da UTI antes da limpeza dos locais. Após a limpeza pela equipe hospitalar, foram encontradas colônias de S. aureus no leito comum, leito de isolamento e balcão da UTI, no leito comum e balcão do PA. Foi possível verificar a presença de S. aureus tanto antes como depois da assepsia das superfícies pela equipe de limpeza, evidenciando a necessidade de implementação de medidas assépticas mais eficazes. A notável presença de S. aureus pode ocasionar riscos tanto para os profissionais da saúde, quanto para os pacientes, já que estes estão em situação de imunocomprometimento e mais suscetíveis a adquirir IRAS.

Palavras-chave: *Staphylococcus aureus*. Infecções relacionadas à assistência à saúde. Unidade de Terapia intensiva. Pronto atendimento. Assepsia.

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1 INTRODUCTION

Healthcare-associated infections (HAIs) are defined as infections acquired by clients who are receiving treatment for other conditions in healthcare facilities. These infections were not present at the time of the client's admission, but were acquired during the course of care and stay in the health unit. According to the Centers for Disease Control and Prevention (CDC), in a single day, around 1 in 25 hospital clients acquires a HAI (TORTORA *et al.*, 2017). According to the National Health Surveillance Agency (ANVISA) (2017), intensive programs of HAI prevention and control in Brazil should consist of an efficient approach to controlling the spread of microbial resistance in the hospital environment.

The main routes of transmission of HAIs are through direct contact between healthcare workers and patients or between patients and through indirect contact via fomites or ventilation systems. HAIs can also come from an endogenous source, i.e. from patients themselves when asepsis fails. Many diagnostic and therapeutic procedures in hospitals promote the transmission of infections by fomites such as urinary and intravenous catheters, respiratory devices, needles, and surgical bandages (TORTORA *et al.*, 2017).

Recognizing and controlling infectious microorganisms in healthcare facilities is extremely important for the entire team of professionals and for the patients who visit these facilities (ANVISA, 2017). HAIs are caused by various infectious agents that are often resistant to antibiotics, and lead to extended periods of hospitalization, increased costs for the health system, patients, and families, and sometimes death (ANVISA, 2017).

The genus *Staphylococcus*, belonging to the *Staphylococcaceae* family, stands out in HAIs due to its pathogenic potential and high mortality rate. *S. aureus* is the most common species of the genus to cause HAIs, and also the most virulent, being responsible for more than 30% of hospital-acquired infections (MARQUES *et al.*, 2017). According to Alvarez and Mimica (2012), *S. aureus* can cause simple ailments such as pimples, boils, and cellulitis to more severe infections such as pneumonia, meningitis, endocarditis, toxic shock syndrome, scalded skin syndrome, and septicemia.

It is of note that, due to its physiological and morphological characteristics, *S. aureus* is considered the most versatile of human pathogens (FARKAS *et al.*, 2019) because it has a high capacity for cell adhesion, for evading the immune response, for nutrient uptake, and for the production of toxins and enzymes that degrade antibiotics (SANTOS *et al.*, 2007).

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The first records of outbreaks caused by *S. aureus*, which had become resistant to penicillin in hospital settings, were reported in the 1950s. In the following decade, the first case of methicillin-resistant *S. aureus* (MRSA) appeared, leading to a pandemic at the end of the 1970s, with vancomycin being the antibiotic of choice for these strains. The first case of total *S. aureus* resistance to vancomycin was described in 2002 in the United States (GOMES; WARD; LAPLANTE, 2015). In this context, antibiotic-resistant bacteria have become an extensive problem for global health (TORTORA *et al.*, 2017).

Epidemiological data show a growing increase in cases of HAIs in the country and worldwide, whuch increase treatment costs and the constant need for surveillance teams and increased attention to asepsis (FOGLIA; FRASER; ELWARD, 2007; LIMA *et al*. 2015). Felix *et al.* (1995) conducted a nationwide study to determine the occurrence of HAIs in Brazilian hospitals and reported a 15% prevalence in 99 tertiary hospitals. In 2001, ANVISA began a diagnosis of HAI control in Brazil, which revealed structural problems for infection control because one third of Brazilian hospitals did not have a microbiology laboratory, 46% of which were in the Northeast and 24% in the Southeast (SANTOS *et al*. 2005).

In 2010, ANVISA set up a surveillance system for primary bloodstream infections associated with central venous catheters. Data obtained two years later showed an incidence of 5.7 per 1,000 central venous catheters per day in adult ICUs, according to laboratory criteria, and the main etiological agent was coagulase-negative *Staphylococcus* (ANVISA, 2011).

The potential of *S. aureus* to cause HAIs warrants obtaining scientific knowledge about the ecological, pathological, physiological, and epidemiological colonization of resistance of this species, as well as monitoring its presence in healthcare environments (NASCIMENTO, 2014).

The aim of this study was to analyze the presence of microbial contamination on surfaces, with a focus on *S. aureus*, in two hospital settings, the ICU and the ED, to evaluate the effectiveness of antisepsis protocols and assess the safety of patients, health professionals, and other staff.

2 METHOD

This was an experimental field study conducted in a hospital located in the interior of the state of São Paulo, where samples were taken from surfaces susceptible to contamination in two sectors, the ICU and ED, namely from the counter, a ballpoint pen of the ward, the door

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handle for accessing the rooms, a normal bed, and an isolation bed. The samples were collected before and immediately after cleaning in both sectors.

Three separate collections were performed in the ED and ICU of a hospital in the interior of São Paulo. The first collection was performed after cleaning and the second and third collections were performed before and just after cleaning, respectively. The collection sites were a normal bed in the ED, a common bed and an isolation bed in the ICU, the counters in the ED and ICU, pens of the ED and ICU nursing assistants, and door handles in the ED and ICU, totaling 10 samples in each collection.

The samples were collected using sterile swabs. These were placed in the solution for twenty seconds, which was then transferred to test tubes containing 1 mL of brain heart infusion (BHI) agar for transportation in a cooled container. In a laminar flow hood, an aliquot of 0.1 mL from each tube was inoculated onto mannitol salt agar (medium selective and differential for *S. aureus*) and BHI-Agar (for aerobic and anaerobic bacteria and fungi). The plates were incubated at 37 °C for 24 to 48 hours and then analyzed for colony shape and color and number of colony-forming units (CFUs).

Colonies indicative of *S. aureus* (change in color of the mannitol medium) were subjected to catalase and coagulase biochemical tests and Gram staining to confirm the species, which are referred to as qualitative analyses. The catalase test was performed using 10-volume hydrogen peroxide on samples of the colony on a histological slide. The presence of air bubbles was indicative of positivity. The coagulase test was performed by mixing 0.2 mL of the broth with suspected colony growth with 0.5 mL of coagulase (Laborclin®) and incubating at 37 °C to visualize clot formation.

For the quantitative assessment of the microbial population, the number of CFUs was counted using a mechanical counter. All data were analyzed descriptively, allowing an understanding of the characteristics of the collection sites before and after cleaning. In this study, the data was organized, summarized, and interpreted using count metrics, namely simple and relative frequency and visual representations.

3 RESULTS AND DISCUSSION

Table 1 shows the number of CFUs obtained in the mannitol salt agar and BHI-agar media in the first collection performed before the rooms were cleaned. The presence of *S. aureus*, confirmed by Gram staining and biochemical tests, was detected in the isolation beds and normal beds in the ED and ICU. *S. aureus* was also found on the counter in the ED. With regard to contamination, the highest CFU numbers were obtained in the normal beds of the two

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environments, on the counter of the ED and on the pen of the ICU, a finding that may be related to the flow of use and handling.

Table 1 - Colony-forming units obtained from hospital surfaces in two different culture media and presence of *S. aureus* confirmed by biochemical tests after differentiation in mannitol medium.

Surfaces	CFU/ mannitol medium	CFU/BHI medium	Presence of S. aureus / Mannitol medium
Isolation bed ED	1	2	+
Isolation bed ICU	22	16	+
Normal bed ED	47	40	+
Normal bed ICU	18	54	+
Counter ED	47	14	+
Counter ICU	3	5	-
Pen ED	2	4	-
Pen ICU	24	50	-
Door handle ED	2	5	-
Door handle ICU	1	11	-
TOTAL	167	201	

ED: emergency department. ICU: intensive care unit.
Source: By the authors.

Tables 2 and 3 show the number of CFUs on the surfaces before and after cleaning in two subsequent samples taken in the ED. The samples showed the presence of microorganisms in all the analyzed environments.

Table 2 - Colony-forming units obtained from hospital surfaces in two different culture media in the emergency department (ED) before and after cleaning by hospital staff.

		Mannitol		**BHI**	
	Period	Counting	%	Counting	%
Isolation bed ED	Before	5	42	3	33
	After	10	38	9	9
Normal bed ED	Before	3	25	4	44
	After	11	42	0	0
Counter ED	Before	4	33	0	0
	After	4	15	35	35
Pen ED	Before	0	0	0	0
	After	1	4	32	32
Door handle ED	Before	0	0	2	22
	After	0	0	24	24
Total		38		109	

Source: By the authors.

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Table 3 - Frequency of CFU in the intensive care unit (ICU) before and after cleaning by hospital staff.

		Mannitol		BHI	
	Period	Count	%	Count	%
Isolation bed ICU	Before	15	11	8	5
	After	5	11	47	30
Normal bed ICU	Before	40	29	55	35
	After	20	45	15	9
Counter ICU	Before	80	58	80	52
	After	17	39	55	35
Pen ICU	Before	1	1	0	0
	After	0	0	1	1
Door handle ICU	Before	3	2	12	8
	After	2	5	41	26
Total		183		314	

Source: By the authors.

BHI medium is non-selective, i.e. it allows both gram-positive and gram-negative bacteria to grow, while mannitol medium is selective for salt-tolerant gram-positive cocci (mainly *Staphylococcus)*.

Although in the ICU there was less movement of people, there was also significant contamination of surfaces.

Considering the samples from the ED after the rooms were cleaned, the highest CFU counts were obtained in the isolation bed (19 CFUs), normal bed (11 CFUs), counter (39 CFUs), pen (33 CFUs), and door handle (34 CFUs) (Figure 1). It is of note that the collections were performed on different days, so there may have been greater movement of people at any of the collections.

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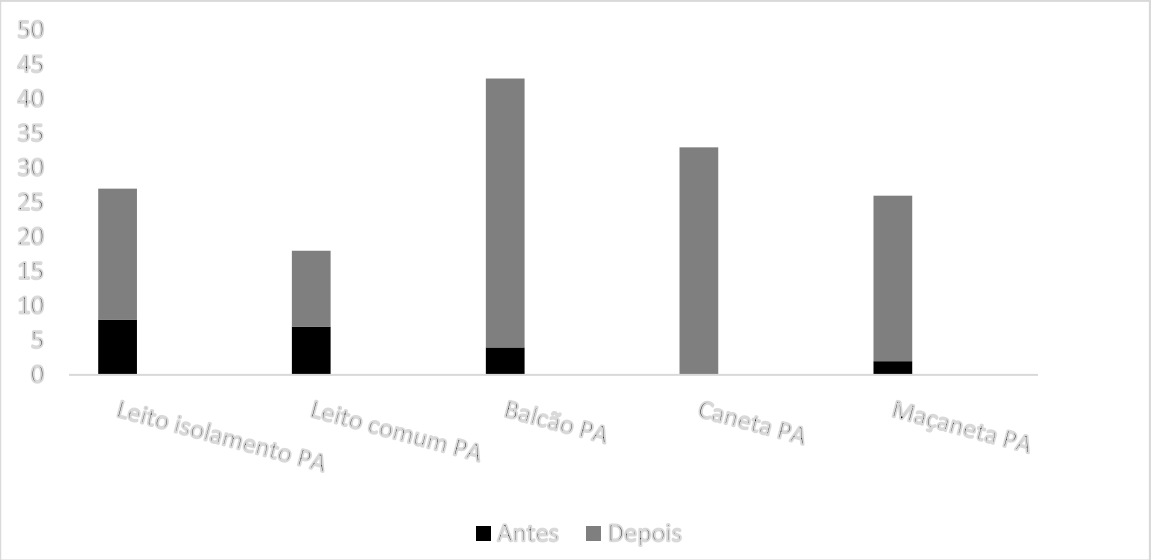
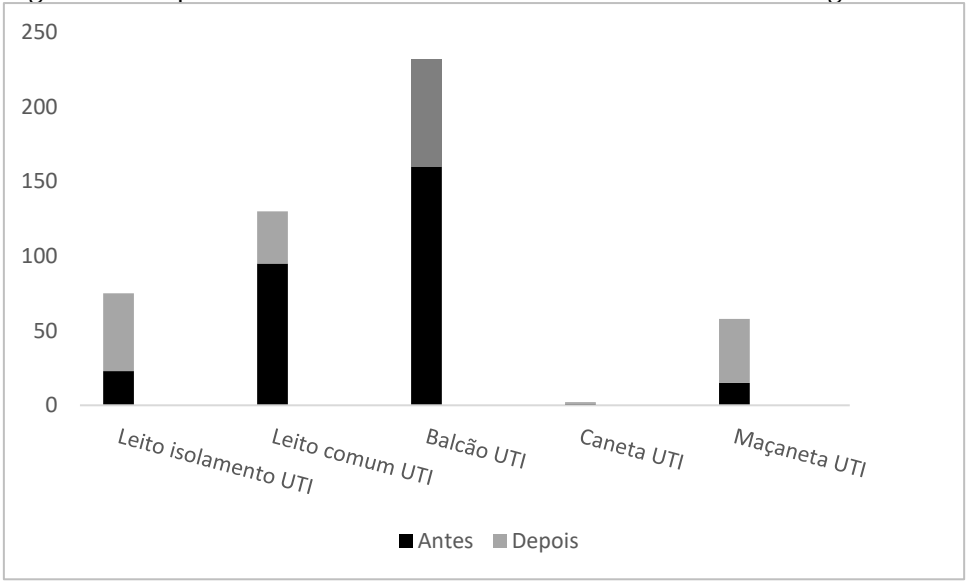
Figure 1- Comparison of the number of CFUs before and after the surfaces in the emergency department (ED) were cleaned



Source: By the authors.

Considering the ICU samples, contamination was detected in the isolation bed (52 CFUs), normal bed (35 CFUs), counter (72 CFUs), pen (1 CFUs) and door handle (43 CFUs). However, after cleaning the environment, the highest CFU count was obtained only in the normal bed and on the door handle.

Figure 2- Comparison of the number of CFUs before and after cleaning



Source: By the authors.

Table 4 shows the contamination of the beds in qualitative terms, i.e. the presence of *S. aureus* was assessed in the evaluated periods, namely before and after disinfection, and

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contamination was found in the normal bed of the ED before and after cleaning. In the ICU, the isolation bed was contaminated by *S. aureus* before and after cleaning, the common bed was contaminated after cleaning, and contamination of the door handle was reduced after cleaning.

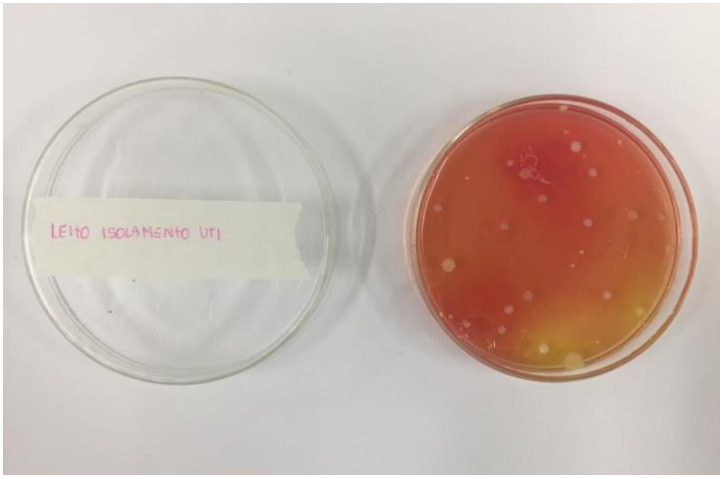
Table 4- *Staphylococcus aureus* contamination in the emergency department (ED) and intensive care unit (ICU) before and after cleaning the analyzed surfaces.

Analyzed surfaces	**ED**		**ICU**	
	Before	After	Before	After
Isolation bed	-	-	+	+
Normal bed	+	+	-	+
Counter	-	-	-	-
Pen	-	-	-	-
Door handle	-	-	+	-
Total Positives	1 (20%)	1 (20%)	2 (40%)	2 (40%)

Source: By the authors.

The presence of *S. aureus* on the surfaces was confirmed both by the differential staining pattern in mannitol salt agar (Figure 3) and by biochemical tests for catalase and coagulase (Figure 4). Mannitol medium is differential for *S. aureus* because the bacterium causes the color of the medium to change by altering in pH, and the presence of *S. aureus* has been shown in recent cultures (LEVINSON, 2016).

Figure 3- Appearance of the *S. aureus* colony in mannitol salt agar. A change in the color of the medium is observed.



Source: By the authors.

The catalase test allows differentiating the *Staphylococcus* genus from the *Streptococcus* genus, because all *Staphylococcus* are catalase positive while *Streptococcus* do not have the catalase enzyme and are therefore catalase negative. The presence of catalase is an

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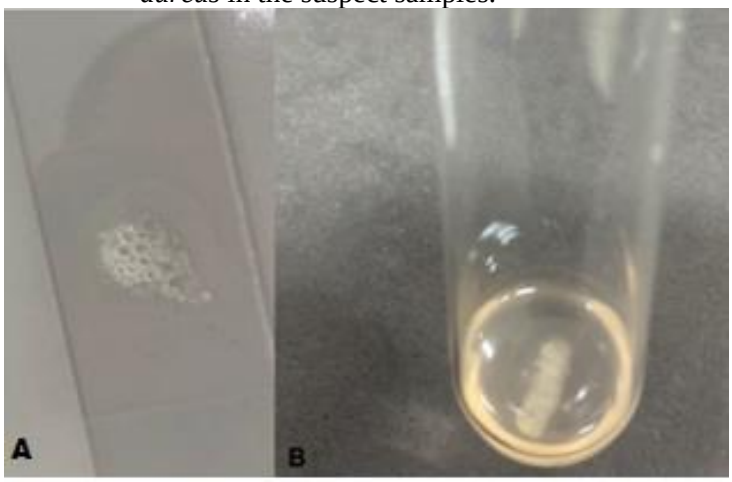
adaptive advantage of the group as it allows it to survive the oxidative reactions caused by phagocytes as a defense strategy (LEVISON, 2010).

Figure 4- Results of the catalase (A) and coagulase (B) biochemical tests confirming the presence of *S. aureus* in the suspect samples.



Source: By the authors.

The coagulase test distinguishes between species of the *Staphylococcus* genus, because *S. aureus* is the only species of the genus that induces clot formation when incubated with blood or serum. The formation of clots around its colonies during infection hinders the action of the host's immune system, being an important virulence factor of the bacterium (FARKAS *et al.*, 2019). The catalase and coagulase biochemical tests, associated with the differential appearance in mannitol medium, are confirmatory laboratory diagnosis for *S. aureus* (ZURITA; MEJIA; GUZMAN-BLANCO, 2010).

Gram staining confirmed the identification, showing gram-positive cocci in the shape of a cluster, making it another presumptive method of diagnosis.

The results show that *S. aureus* was detected on the ICU access door handle before cleaning and was eliminated from the door after cleaning. However, after cleaning, *S. aureus* remained present in the ICU's isolation bed, in the ED's normal bed, and in one sample from the ICU's normal bed (Table 3).

Antisepsis in healthcare environments aims to reduce and/or eliminate microorganisms from the environment to promote the health of patients and professionals working there, as well as prevent HAI-s (GARCIA *et al.*, 2019).

In Brazil, the regulation of biocides is the responsibility of the Ministry of Health, through the Household Sanitizers Division (DISAD), which deals with sanitizers and

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disinfectants, and the Medicines Division (DIMED), which regulates antiseptics. According to Ordinance no. 2.616/98 of ANVISA, which determines the guidelines and standards for the prevention and control of hospital-acquired infections (BRASIL, 1998), the use of antiseptics, disinfectants, and sterilizers in health services should be guided by the provisions of Ordinance No. 15, of August 23, 1988, of the Health Surveillance Secretariat (SVS) of the Ministry of Health (MS) and by the publication "Processing of Objects and Surfaces in Health Facilities" ("*Processamento de Artigos e Superfícies em Estabelecimentos de Saúde"*) of the MS of 1994, or others that complement or replace them (REIS *et al.*, 2011).

An enquiry about the hospital's cleanliness to the Hospital Infection Control Commission (CCIH) of the institution showed that it follows the standard operating procedures (POP) based on ANVISA Ordinance No. 2.616-98, which is updated every three years (BRAZIL, 1998).

S. aureus is one of the microorganisms most associated with HAIs, with high levels of morbidity and mortality (SOUSA *et al.*, 2016; VALADÃO; MIKALOUSKI, 2018) because it produces toxins that facilitate the rupture of epidermal barriers, it has mechanisms to neutralize phagocytosis and the cellular immune response, and some strains are resistant to multiple antibiotics (ALBUQUERQUE *et al.*, 2013; SOUZA; FEILSTRECKER; HUBNER, 2015). Its presence in the hospital unit is therefore worrying, especially in the ICU environment, which is specialized in the care of patients with severe clinical conditions and often immunocompromised (SANTOS, 2007; SANTOS; RIBEIRO, 2016). The presence of the pathogen combined with antibiotic therapy and immunocompromisation pose the risk of serious infection (MUNDIM *et al.*, 2008).

The presence of *S. aureus* on the surfaces analyzed is probably associated with their contact with the skin of patients, staff, and visitors. Several studies have demonstrated the presence of the pathogen on the hands of these individuals (MOREIRA; SANTOS; BEDENDO, 2013) and found a percentage of occurrence of the pathogen of 67.39% on the hands of patients and of 61.54% on the hands of hospital staff. Moura *et al*. (2011) found a prevalence of *S. aureus* in 17.7% of nursing professionals, with 2.5% of the strains being resistant to methicillin.

It is therefore essential to control this microorganism in the hospital setting, through preventive measures and appropriate practices guided by ANVISA, to avoid the occurrence of HAIs and guarantee the safety of the patients and all healthcare staff.

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4 CONCLUSION

It can be concluded that the detection of *S. aureus* in both the ED and the ICU poses risks for both healthcare professionals and patients, because the latter are immunocompromised and thus at greater risk of acquiring HAIs. Therefore, based on the presented data, we emphasize the importance of testing for *Staphylococcus aureus* on hospital surfaces and the need to review the cleaning protocols and the practices to achieve asepsis in the institution.

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