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RESEARCH ARTICLE

Molecular characterization of *Staphylococcus aureus* nasal carriage among healthcare workers: Insights for infection control

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The purpose of the study was to identify the nasal carriage of *S. aureus* in healthcare workers of the clinical wards of the Târgu-Mureș Emergency County Hospital and to characterize the bacterial isolates phenotypically and genotypically. This study included 64 medical staff from the Târgu-Mureș Emergency County Hospital. Their data and nasal exudates were collected. The multiplex PCR method was used to identify *femA, PVL, mecA, eta, etb* and *tst* genes. ERIC-PCR was used to evaluate the genetic similarity of the bacterial isolates. A prevalence of 25% of nasal carriage of *S. aureus* was obtained. Of these 12% were methicillin-resistant and 47% showed clindamycin-inducible resistance phenotype. Almost half of the isolates (47%) were from ICU (Intensive Care Unit) personnel. PCR results confirmed the species and the presence of the *mecA* gene in MRSA (Methicillin-Resistant *Staphylococcus aureus*) isolates. Except for 4 strains that showed the gene for exfoliatin A, no other virulence factor genes were detected. ERIC-PCR identified the partially common origin of the *S. aureus* strains, all having a similarity of 55%, with some reaching up to 100% similarity. Although the strains did not spread clonally and did not carry important virulence factors, there were associations between the nasal carriage and respiratory infections, previous diagnosis with *S. aureus*, Intensive Care Units and Nephrology wards.

Keywords: *Staphylococcus aureus*, nasal carriage, molecular diagnosis, antibiotic resistance

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Introduction

Staphylococcus aureus is a human pathogen responsible for a variety of clinical manifestations with varying degrees of severity, ranging from local skin abscesses to systemic manifestations such as septic shock [1]. *S. aureus* colonizes the skin and mucous membranes. It is most commonly found nasally and under opportunistic conditions this colonization may represent an endogenous source of bacterial infection [2]. Approximately 30% of the general population has nasal carriage of *S. aureus* and they are considered to be at increased risk for infection [3,4]. *S. aureus* is one of the most important pathogens contributing to nosocomial infections. The main sources of staphylococcal transmission are colonized healthcare workers, other patients or contaminated surfaces [5].

Over the years, staphylococcal species have become resistant to antibiotics, which may be due to overuse of antimicrobial medication. As a result, there are therapeutic difficulties due to its ability to cause a wide range of infections and to adapt to different environmental conditions. One of the mechanisms leading to antibiotic resistance is related to the prolonged activation of genes encoding the staphylococcal efflux system, increasing the efflux capacity of drugs [6]. Acquired resistance can occur through mutations in ribosomal methylase RNA - clindamycin and erythromycin resistance. Bacterial biofilm, through the pseudocapsule formed, can provide the bacteria with conditions for adaptation [7]. Only a decade after the discovery of penicillin, penicillin-resistant *S. aureus* appeared in the clinic [8]. Later, after the development of a new penicillin: methicillin, the isolation of a methicillin-resistant *S. aureus* (MRSA) strain, resistance produced by the *mecA* gene, encoding PBP2a, was reported [9].

The purpose of this study was to identify and characterize strains of *Staphylococcus aureus* isolated from healthcare workers, based on the hypothesis that the prevalence of *S. aureus* is increased in the hospital environment and that it is one of the main pathogens involved in nosocomial infections. This study also aims to investigate the influence of the hospital wards on *S. aureus* nasal carriage, including key features in recent but undiagnosed staphylococcal infections. The study aims to characterize phenotypically and genotypically the bacterial isolates, including testing the similarity of the isolated strains and antimicrobial susceptibility.

Materials and methods

This study is prospective. Medical staff (doctors and nurses) from Târgu-Mureș Emergency County Hospital were included in this study. With the consent of the ethical board of the Târgu-Mureș Emergency County Hospital number 23195/9.10.2023 and of the persons involved in this study and the protection of personal data, a questionnaire was completed, nasal exudate samples were collected, and the

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data were analyzed and compared to prove the hypotheses previously established. Data were collected between September and December 2023.

Nasal exudates were harvested in the morning at the start of the working day. After that, nasal swabs were placed in TSB (Tryptone Soy Broth, Oxoid™) medium with 7% NaCl and kept in a thermostat at 37 ºC for 24 h for bacterial growth to occur. They were subsequently cultured on blood-agar and Chapman's medium. Identification of suspect colonies was based on biochemical properties and coagulase tests (Oxoid Staphytect Plus kit). Antibiotic susceptibility testing was performed on Mueller Hinton medium by Kirby-Bauer disk-diffusion method, following the EUCAST standard.

S. aureus isolates were stored for further testing at -70°C in TSB medium (Tryptone Soya Broth, Oxoid™) with glycerol (cryoprotectant role).

Bacterial DNA was purified using silica columns (IndieSpin Pathogen kit, Indical Bioscience, Germany).

The first triplex PCR (Polymerase Chain Reaction) method was used for species identification *(femA)*, identification of methicillin resistance gene (*mecA*) and detection of PVL (Panton Valentine Leukocidin) virulence gene. The reaction mix consisted of 1 µl bacterial DNA, 12.5 µl DreamTaq Green PCR Master Mix 2X (Thermo Fisher Scientific, Waltham, MA, USA), 0.5 µl femF, 0.5 µl femR, 0.5 µl pvlF, 0.5 µl pvlR, 1 µl mecAF, 1 µl mecAR and 7.5 µl H2O (Table 1), with a total volume of 25 µl. The MiniAmp Thermal Cycler analyzer was used to perform the PCR cycles: initial denaturation: 5 min at 94 ºC; followed by 35 cycles of denaturation 94 ºC/30 sec, primer annealing 55 ºC/30 sec, elongation 72 ºC/ 40 sec; followed by final elongation 72 ºC for 2 min.

The electrophoresis gel was prepared by mixing 50 ml Tris-borate-EDTA (TBE) Buffer solution with a 0.5g TopVision agarose tablet (Thermo Scientific, UK). For staining, 1.5 µl GelRed (Biotium, UK) was added. 10 µl of each amplification product was loaded into the electrophoresis gel and the results were visualized using UV radiation with DNR Bio-imaging Systems.

The second triplex PCR was performed to identify *eta* (Exfoliatin A), *etb* (Exfoliatin B) and *tst* (Toxic shock toxin) genes. A volume of 25 µl was used, consisting of 1 µl bacterial DNA, 12.5 µl DreamTaq Green PCR Master Mix 2X (Thermo Fisher Scientific, Waltham, MA, USA), 1 µl etaF, 1 µl etaR, 0.5 µl etbF, 0.5 µl etbR, 0.5 µl tstF, 0.5 µl tstR and 7.5 µl H2O (Table 1). Initial denaturation: 5min at 94 ºC, followed by 35 cycles of denaturation 94 ºC/30 sec, primer annealing 56 ºC/30 sec, elongation 72 ºC/ 40 sec, followed by final elongation 72 ºC for 2 min. Electrophoresis and data analysis was performed similar to the previous PCR.

For ERIC-PCR, the reaction mix contained 12.5 µl DreamTaq Green PCR Master Mix 2X (Thermo Fisher Scientific, Waltham, MA, USA), 0.4 µl of ERIC primer-Forward 5′ ATGTAAGAGCTCCTGGGGATTCAC 3′, 0.4 µl of ERIC primer-Reverse 3′ AAGTAGTAGTGAGAGTG5 ′ (Thermo Fisher Scientific, Waltham, MA, USA), one micro liter of bacterial DNA and pure water (DNAse Free Water) to the final volume of 25 µl. The protocol followed was: initial denaturation: 5 min at 95 ºC, followed by 30 cycles of denaturation 94 ºC/30 sec, primer annealing 52 ºC/ 1 min, extension 72 ºC for 2 min, followed by final extension 72 ºC for 8 min. Electrophoresis and data reading was performed, similar to the previous PCR assay [11].

For statistical data, the results were analyzed using Microsoft Excel and GraphPad Prism 10.

Results

This study involved 64 healthcare workers of the Târgu Mureș Emergency County Hospital, from several clinical wards. The study participants ranged in age from 20 to 60 years, the majority being women (84.38%; n=54).

According to the questionnaire data, 20.31% (n=13) of the subjects had administered antibiotics such as amoxicillin/clavulanic acid, ampicillin/sulbactam, cefuroxime, azithromycin, doxycycline, doxycycline, ofloxacin, norfloxacin, or sulfamethoxazole/ trimethoprim in the last 3 months. No statistical associations were found between antibiotic usage in the last 3 months and *S. aureus* nasal carriage (p=0.28, OR=2.273 with 95% CI 0.618 - 8.354). Also, no statistical associations were found between previous pathologies in the last 6 months and the presence of *S. aureus* carriage: 3.12% of participants (n=2) had skin infections (p=1.000, OR=0.564 with 95% CI 0.025 - 12.370); 7.81% (n=5) had food poisoning (p=0.319, OR=0.239 with 95% CI 0.012 - 4.582); 4.68% (n=3) had surgery

Tabel 1. Primers and nucleotide sequences used for PCR [10]

	Gene	Primer	Sequence (5`-3`)	PCR amplicon size (bp)
Triplex 1	*femA*	femAF	AAAAAAGCACATAACAAGCG	132
		femAR	GATAAAGAAGAAACCAGCAG	
	mecA	mecAF	TGCTATCCACCCTCAAACAGG	286
		mecAR	AAGTTGTAACCACCCCAAGA	
	pvl	pvlF	ATCATTAGGTAAAATGTCTGGACATGATCCA	441
		pvlR	GCATCAACTGTATTGGATAGCAAAAGC	
Triplex 2	*eta*	etaF	GCAGGTGTTGATTTAGCATT	93
		etaR	AGATGTCCCTATTTTTGCTG	
	etb	etbF	ACAAGCAAAAGAATACAGCG	226
		etbR	GTTTTTGGCTGCTTCTCTTG	
	tst	tstF	ACCCCTGTTCCCTTATCATC	326
		tstR	TTTTCAGTATTTGTAACGCC	

(p=0.1, OR=1.533 with 95% CI 0.1296 - 18.142); No subject had osteomyelitis; 28.12% (n=18) of the study participants had contact within the last 6 months with other people diagnosed with *S. aureus* (p=0.522, OR=0.5 with 95% CI 0.125 = 2.051).

Of all subjects, 40.62% (n=26) had recent respiratory infections (in the last 6 months – pharyngeal hyperemia, fever, dyspnea, productive cough or purulent nasal secretions) , and of these, only 3 were found *S. aureus* carriers. Nevertheless, most *S. aureus* carriers (81.25%; n=13) did not confirm respiratory tract infection in the last 6 months. Thus, the medical personnel without respiratory infections in the past 6 months had a significantly higher likelihood of being diagnosed with *S. aureus* carriage compared to those with recent respiratory infections (p = 0.042, OR = 3.98, 95% with 95% CI 1.005 - 15.808).

On blood agar medium, some samples showed S-type colonies, large, 1-3 mm, round, smooth, bulging, with regular margins, yellowish character and presence of β-hemolysis. On Chapman medium they decomposed mannitol (Figure 1). The coagulase test was performed for these samples using the Oxoid Staphytect Plus Kit, where latex-agglutination was observed. For sample no. 1, two colonies with macroscopically different appearances were observed, so each was reseeded and named 1-1 and 1-2.

25% of the study participants had nasal carriage of *S. aureus*. As presented in Table 2, most positive samples originated from the Intensive Care Unit (ICU) (p=0.229, OR=2.094 with 95% CI 0.646 - 6.783) and the Nephrology Unit (p=0.03, OR=7.667 with 95% CI 1.251 to 46.980).

According to the antibiograms, the majority of the samples showed susceptibility to antibiotics. Significant resistance was observed to penicillin (76%) and to erythromycin (65%). Two of the 17 *S. aureus* isolated strains (11.76%) showed resistance to cefoxitin, and 47% showed inducible resistance to clindamycin (Figure 2). Therefore, of the positive samples 11.76% were MRSA, MLSBi; 35.29% were MSSA, MLSBi, and 52.94% were MSSA.

The first Triplex PCR test was for *femA, mecA* and *pvl* genes. All samples showed the *femA* gene, specific for *S. aureus*. Only two samples showed the *mecA* gene, specific for methicillin-resistance, and no samples showed PVL. The next Triplex PCR was performed for exfoliatin A, exfoliatin B and toxic shock toxin TST. None of the samples showed *tst* and *etb*. 23.52% (n=4) showed the presence of exfoliatin A gene.

Electrophoresis of the ERIC-PCR showed partially common origin of the *S. aureus* strains, all having 55% similarity. Strains are further divided, reaching similarities up to 100% (Figure 3).

Strains 32 and 33 had 100% similarity, and according to the questionnaire data, both samples come from the ICU ward, but no other correlation was found in the questionnaire between the two samples.

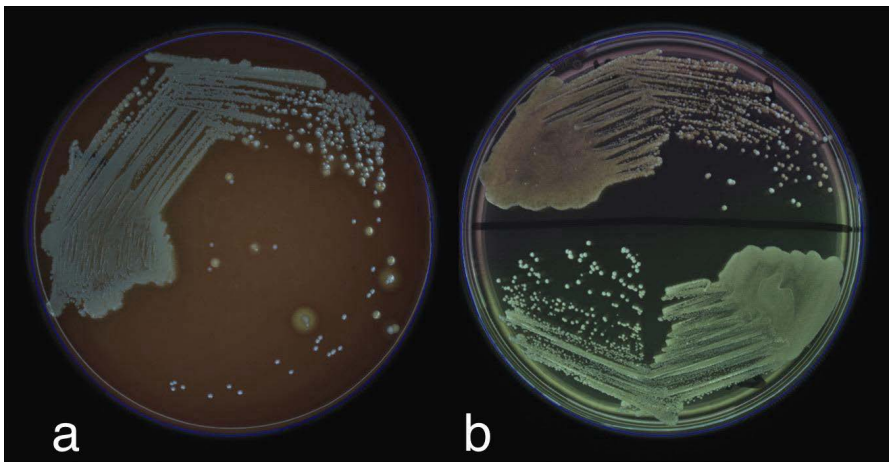

Fig. 1. Isolates of *Staphylococcus aureus* on Blood Agar (a) and Chapman Medium (b)

Tabel 2. Prevalence of *Staphylococcus aureus* nasal carriage in clinical wards

Ward	Tested healthcare staff	*S. aureus* carriers	*S. aureus* carriage (%)
Nephrology	6	4	66.7%
Pediatrics	2	1 (2 strains)	50.0%
Intensive care unit	20	7	35.0%
Diabetes	9	2	22.2%
Pediatric Intensive Care Unit	5	1	20.0%
Rheumatology	5	1	20.0%
Orthopedics	8	0	0.0%
Obstetrics	5	0	0.0%
Surgery	3	0	0.0%
Cardiology	1	0	0.0%
TOTAL	64	16 (17 isolates)	25.0%

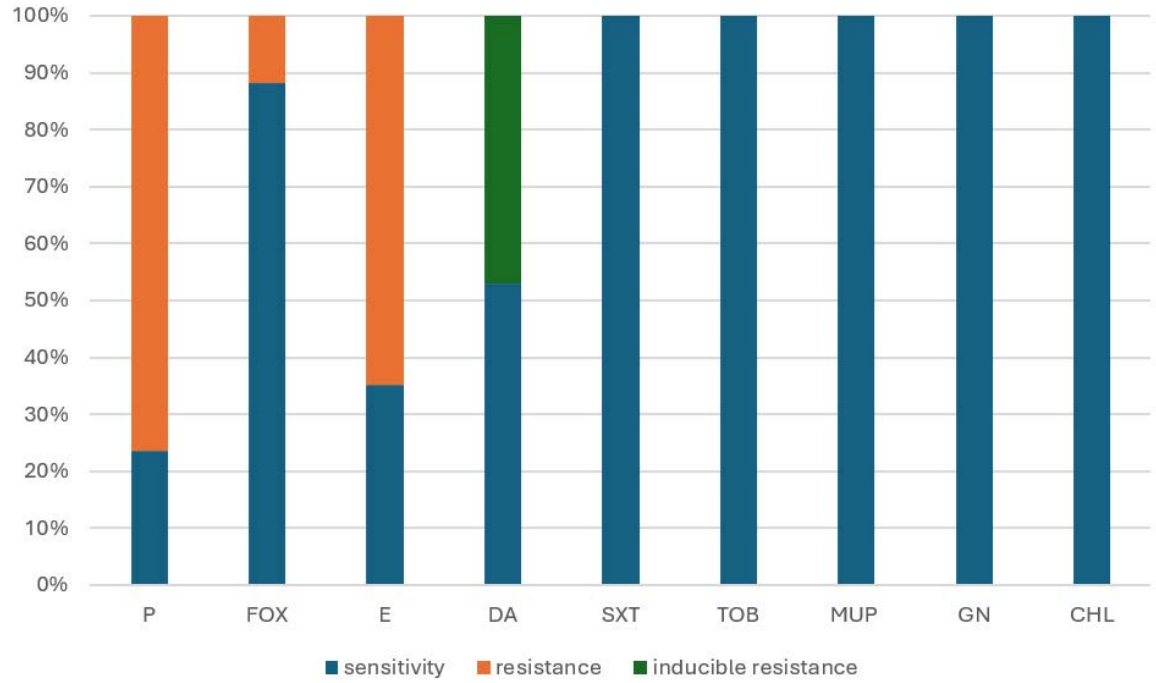

Fig. 2. *S aureus* antibiotic resistance and sensitivity (P – Penicillin, FOX- Cefoxitin, E – Erythromycin, DA – Clindamycin, SXT - Trimethoprim/Sulfamethoxazole, TOB – Tobramycin, MUP - Mupirocin, GN – Gentamycin, CHL – Chloramphenicol)

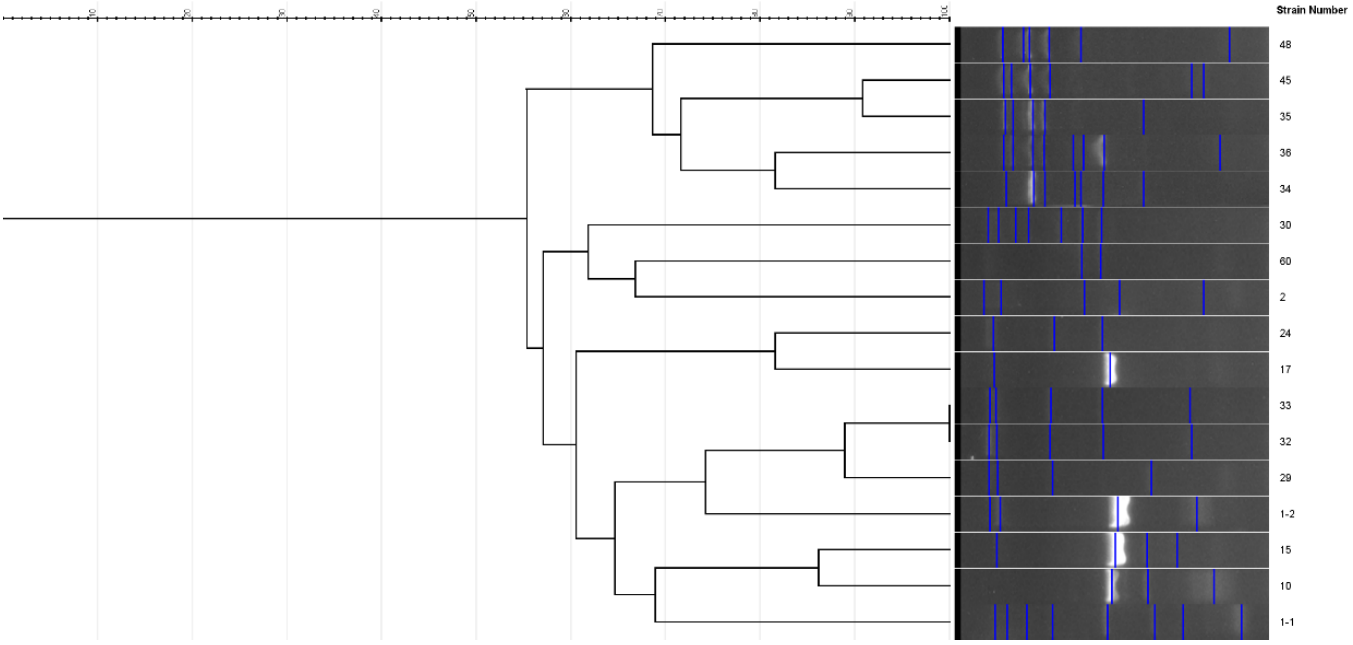

Fig. 3. Similarity of isolated strains (ERIC-PCR) - sample identification number on right axis; percent similarity on top axis

Samples 45 and 35 had 91% similarity, and according to the questionnaire data, sample 35 originated from the Nephrology ward, the subject having respiratory infections in the last 3 months, and was diagnosed with *S. aureus* in May 2023. Sample 45 originated from the Diabetes, Nutrition and Metabolic Diseases ward and had surgery in the last 6 months. No correlation was found from the questionnaire between the two samples.

Strains 29, 32 and 33 had a similarity of 89%, and according to the questionnaire data, all 3 samples come from the ICU ward. Strains 10 and 15 had a similarity of 86%: sample 10 originated from the Pediatric ICU ward, had recent contact with persons diagnosed with *S. aureus* and was diagnosed in 1968 with this pathogen. Sample 15 originated from the ICU ward, finding no correlation resulting from the questionnaire between the two samples.

Discussion

The study showed a prevalence of 25% of nasal carriage of *S. aureus* among the medical staff of the Târgu Mureș Emergency County Hospital. Of these, 12% were methicillin-resistant strains (MRSA). In another longitudinal

cohort study conducted in 2012 in a hospital from England, which included both medical staff and patients, it was found that 37% of healthcare workers were *S. aureus* nasal carriage, 4% of which were MRSA carriers [12]. In another study targeting medical staff in a university hospital in 2008, nasal carriage was demonstrated in 43.8% of the medical staff, of which 15.2% with MRSA strains [13]. In another clinical hospital, *S. aureus* was isolated in 22.22% of the medical staff, and methicillin resistance was found by disk-diffusion method in 11.43% of the isolates [14]. It can be seen that the percentage of medical staff with nasal carriage differs, depending on the hospital, the sanitary standards of each hospital or the country.

In the present study, the prevalence of nasal carriage of *S. aureus* was higher in women (69%) than in men (31%), similar to other studies in the literature, but this may be due to the predominance of female participants in the studies, and also to the fact that approximately 70% of the medical personnel in Romania are women, according to the National Institute of Statistics [15].

There was an association between nasal carriage and respiratory infections. 19% of the cases had a history of respiratory infections in the last 6 months. The samples were collected in the fall/winter when respiratory infections are on the rise, most of them are caused by viruses. Respiratory tract viral infections are also associated with staphylococcal infections, in some cases remaining the nasal carriage [16].

27% of healthcare workers who had been diagnosed with *S. aureus* during their lifetime had nasal carriage at this moment. In another study, screening tests were performed on hospitalized patients with the aim of controlling nosocomial infections. That article showed that the previous positive screening greatly increased the probability of a new positive result [17].

Intensive Care Unit and Nephrology wards are associated with increased rates of *S. aureus* nasal carriage. Patients who are repeatedly exposed to skin lesions - patients on renal replacement therapy - have higher rates of nasal carriage, in addition to transmission by touching different objects or not washing hands properly [18]. Of MRSA strains, 6% originated from ICU wards in agreement with other studies, where 5.1% of MRSA strains originated from ICU wards [13].

Genotyping for *S. aureus* is important because it is the way to know the bacterial characteristics and to formulate a prognosis [19]. The ERIC-PCR is also important, providing information on the existence of community or intrahospital outbreaks, based on which the necessary measures can be taken to stop them.

We have to acknowledge the study limitations. The small sample size, especially as the participation was voluntarily, can induce a bias and influence the power of data analysis. Also, the study is transversal, implemented in a single center, in short period, thus the results may not be suggestive for the entire region. The bacterial similarity was assessed by ERIC-PCR, a method that does not provide the same resolution as other fingerprinting methods such as pulse-field gel electrophoresis, but is still usable for *S. aureus* genetic similarity evaluation [20], with cheaper costs and faster processing.

Conclusions

Staphylococcus aureus colonizes the nasal mucosa of healthy people, highlighting the existence of healthy carriers among healthcare workers, in a significant proportion. The antimicrobial susceptibility of the identified strains demonstrates the effect of widespread antibiotic administration and the ability of the pathogen to adapt to environmental conditions. Both ICU and nephrology wards are risk factors for *S. aureus* nasal carriage, accounting for the majority of positive samples. Also, previous diagnosis with *S. aureus* or recent respiratory infections are associated with increased prevalence of nasal carriage. No important virulence factors were identified and MRSA prevalence was low. All this demonstrates that *S. aureus* can be considered an occupational risk in the hospital environment, posing a threat to both healthcare workers and patients.

Authors' contribution

EPV - Investigation; Data curation; Methodology; Visualization; Writing – original draft

AB - Investigation; Data curation; Methodology; Visualization; Writing – original draft

AM - Conceptualization; Formal analysis; Methodology; Project administration; Resources; Validation; Writing – review & editing

Conflict of interest

None to declare.

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