ORIGINAL ARTICLE

Evaluation of nasal colonization by *Staphylococcus aureus* in university students in the healthcare field: analysis of the relationship with anthropometric indices

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ABSTRACT

Introduction: *Staphylococcus aureus* is an opportunistic pathogen present in the normal microbiota of the skin and mucous membranes of healthy people. Some studies reported that obesity may be a factor that favors this bacterium's colonization of the nasal site. **Objective:** The present study evaluated the frequency of nasal colonization by *S. aureus* in health students in São Luís (MA), analyzing the possible relationship with anthropometric factors (body mass index – BMI; waist-hip ratio – WHR). **Methodology**: Eighty-three students (51 female and 32 male) enrolled in courses in the health area were recruited to obtain demographic, behavioral, and anthropometric data (BMI, WHR). The samples obtained from the nasal cavities were inoculated in Mannitol Salt Agar medium with subsequent identification of the isolates. Finally, biofilm formation was analyzed. **Results**: In the population studied, the prevalence rates of obesity and overweight/obesity were 26.51% and 55.42%, respectively. In addition, altered WHR was found in 37.35% of the students. Most of the evaluated samples (91.57%) were positive for *Staphylococcus* sp. ($p<0.0001$), with the prevalence of *S. aureus* (61.45%) statistically higher than that of negative catalase *Staphylococcus* (30.12%; $p=0.0028$). *S. aureus* isolates were predominantly classified as poor biofilm formers. No significant associations were observed between nasal colonization by *S. aureus* and obesity in the studied population. **Conclusion**: The data obtained in this work demonstrate a high frequency of healthy students with *S. aureus* in their nostrils. In addition, most students were classified as overweight/obesity. However, no significant relationships were found between the prevalence of *S. aureus* and the anthropometric indices evaluated. The high rates of nasal colonization by *S. aureus* in health students demonstrate the importance of control measures to prevent the dissemination of this microorganism.

Descriptors: *Staphylococcus aureus* infections, Body composition, Asymptomatic colonizationc Biofilmsc Overweight, Obesity.

INTRODUCTION

Staphylococcus spp. is a Gram-positive bacterium present in the normal microbiota of the skin and mucous membranes of healthy people [1,2]. This bacterium is considered one of the main pathogens due to its high capacity to acquire genes related to antimicrobial resistance and pathogenicity determinants [3–5]. One-third of the world population is estimated to have *S. aureus* as a normal transient skin microbiota, and around 50% of adults may carry *S. aureus* in the upper respiratory tract at some point in their lives [6,7].

However, despite being considered a human commensal, the carriage of *S.*

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https://doi.org/10.11606/issn.2176-7262.rmrp.2024.208876

aureus increases the risk of several types of invasive infections [8–10]. For example, nasal carriers can spread the microorganism to healthy individuals through respiratory droplets, oropharyngeal secretions, and their hands [11–13]. Nasal colonization is considered a risk factor for the emergence of healthcare-associated infections (HAIs) in healthcare professionals [4,14,15].

Practical classes in clinical and hospital settings, an essential part of the learning process can expose healthcare students to occupational risks, becoming microbial carriers, with *S. aureus* being one of the main concerns [12,14]. On the other hand, these students can disseminate pathogenic bacteria to patients, being a potential source of nosocomial infections [14,15].

Several aspects of the host have been associated with the presence of *S. aureus* in the respiratory tract of humans [8,16]. Among them, obesity has been related as a determining factor for nasal colonization among hospitalized patients and non-hospitalized adults [17–19]. Supposedly, this occurs due to high serum glucose concentrations and type 2 diabetes, which may be associated with altered immune response [18]. Obese patients and those with diabetes are classified as having an increase of up to five times related to the risk of infections, and several studies show that defective immune responses contribute to increased infection rates in these pathologies [20–22]. In this context, this work aimed to evaluate the rate of nasal colonization by *S. aureus* in health students, analyzing the relationship with their anthropometric indices. The ability of *S. aureus* isolates to form biofilm was also evaluated.

MATERIAL AND METHODS

Type of study, recruitment of individuals and sample characterization

This quantitative experimental research was carried out on health students at a private University in São Luís, Maranhão. The project was approved by the CEUMA University Research Ethics Committee (number 3,240,896). The students were recruited in the second semester of 2019. The sample consisted of eighty-three students (51 female and 32 male).

The inclusion criteria used were those over 18 years old, university students in the health field, and the exclusion criteria were university students from other educational institutions, pregnant women, students who did not approve the Free and Informed Consent Form. Participation in the project was entirely voluntary, and after thoroughly explaining the procedures and objectives of the entire work, the students signed the Free and Informed Consent Form (FICF). The variables were obtained through a questionnaire to characterize the sample (age, course, use of antibiotics, adherence to antibiotic treatment, previous hospital/health institution contact) and through anthropometric assessment (weight, height, mass index (BMI), and waist and hip circumferences).

Obtaining anthropometric indices

An assessment of body height in centimeters (cm) and weight in kilograms (kg) was carried out, with subsequent calculation of the body mass index (BMI) – by the ratio between weight and height squared, to evaluate the state nutritional status of the studied population. The measurement of anthropometric measurements was carried out at the institution itself, in a closed location, by previously trained examiners. A calibrated digital scale was used to measure weight, with a maximum capacity of 150 kg. The student was weighed barefoot in the center of the scale, with as little clothing as possible. Height was measured using a portable stadiometer, measuring 2 meters. The student was standing barefoot, weight equally distributed between the feet, with the body raised to maximum extension.

The World Health Organization (WHO) parameters were taken into account: individuals with a BMI <18.5 kg/m² as underweight; eutrophy, BMI between 18.5 kg/m² and 24.9 kg/m²; overweight, BMI between 25 kg/m² and 29.9 kg/m²; grade I obesity, BMI between 30 kg/m² and 34.9 kg/m²; grade II obesity, BMI between 35 kg/m² and 39.9 kg/m²; and grade III obesity, BMI > 40 kg/m².

The waist-hip ratio was measured with an inelastic measuring tape, measuring the waist circumference (WC) (from the midpoint of the last rib to the iliac crest) and the hip circumference (CD) (at the level of the greater trochanter of the femur), to assess the risk for developing cardiovascular disease. The waist-hip ratio (WHR) is calculated by dividing the WC measurement by the HC measurement. The *cutoff* index for WHR is less than 0.85 for women and 0.89 for men.

Isolation and identification of *S. aureus* from the nasal cavity

For collection, sterile *swabs* moistened with previously sterilized saline were used. The *swab* was introduced into the nasal cavity, carefully making circular movements three times. Then, the collected materials were placed inside dry sterile tubes and immediately sent for seeding in the Mannitol Salt Agar medium. Then, the plates were incubated for 48 hours at 37ºC.

Soon after confirming the characteristics of the colonies, the strains were subjected to catalase and coagulase tests. During the catalase test, a drop of 3% hydrogen peroxide was added to a colony of the strain to be tested. The presence of bubbles or effervescence classified the samples as catalase-positive. In the coagulase test, two drops of distilled water or sterile saline solution were inserted onto a slide. With the help of a bacteriological thread, the colony under study was added, gently homogenizing it. A drop of plasma was inserted into one of the circles on the slide. Another drop of distilled water was added as a control in the other circle. The blade was gently tilted back and forth, and agglutination was observed. The presence of a clot represents a positive result, and its absence means a negative result.

Biofilm Formation

The biofilm formation test was carried out on polystyrene plates, inserting 200 µL of bacterial suspensions in quadruplicate; for the negative control, BHI broth without bacterial inoculum was used, and for the positive control, the *S. aureus* ATCC 6538 strain (strong producer of biofilm). The plates were incubated at 37ºC for 24 hours. After this period, the bacte-

rial suspensions were removed, and each well was washed three times with 200 µL of PBS (Phosphate Buffer Saline). Subsequently, procedures were carried out to evaluate the biofilm and absorb absorption using a microplate reader, at a wavelength of 570 nm [23]. To this end, the isolates are classified, considering the Optical Density cutoff point (DOc), at three standard deviations above the average of the control ODs, into the categories: DO≤DOc= non-forming; DOc<DO≤2x DOc= weakly forming; 2x DOc<DO≤4x DOc= Moderate Forming and 4x DOc< DO= strong forming [24].

Data Analytics

The data obtained were analyzed using the *GraphPad Prism* software, version 8.4.3. The differences between categorical variables were assessed using Fisher's Exact Test or Binominal Test. *P* values less than *0.05* were considered statistically significant.

RESULTS

Profile of evaluated individuals

Eighty-three university students participated in the study, with the number of women (51) being statistically higher than the number of men (32) (p>0.05). The predominant age group was 21-24 (59.04%). According to the classification proposed by the WHO, the individuals were classified as (i) malnourished = 2 (2.41%; 1 female and 1 male); (ii) eutrophic = 35 (42.17%; 24 females and 11 males); (iii) Overweight = 24 (28.92%; 15 females and 9 males); (iv) Obesity grade I = 13 (15.66%; 5 females and 8 males); (v) Obesity grade II = 4 (4.82%; 1 female and 4 male); (vi) Obesity grade III = 5 (6.02%; 5 female). Therefore, the proportion of obese students (26.51%) was lower than that of non-obese students (69.86%; p<0.05). The prevalence of overweight/obesity was 55.42% (n= 46).

Similarly, most students (62.65%) had normal WHR (p<0.05). WHR is the calculation based on waist and hip measurements to check an individual's risk of cardiovascular disease. Individuals with cardiovascular risk were considered to be those whose WHR result exceeded 0.80 cm for women and 0.95 cm for men. No significant associations were observed between the different BMI or WHR classification ranges and the students' gender (Table 1; p>0.05).

The use of antibiotics and the occurrence of hospitalizations in the last 12 months were also investigated in the population participating in this study. Eighteen students reported hospitalizations, with a higher number among female students but without a statistical difference (p=0.0533). The use of antibiotics was reported by 33 (39.76%) students, with 9 (10.84%) using them before the time indicated in the prescription.

Table 1. Characterization of the population of university students in the healthcare field recruited.

Parameter	TOTAL		FEMININE (N =51)		MASCULINE (N=32)		p[9]
	N	%	N	%	N	%	
18-20 years old	27	32.53	17	62.96	10	37.04	-
21-24 years old	49	59.04	32	65.31	17	34.69	-
> 24 years old	7	8.43	2	28.57	5	71.43	-
Underweight[1]	2	2.41	1	50.00	1	50.00	>0.9999
Eutrophi[c]2	35	42.17	24	68.57	11	31.43	0.3612
Overweight[3]	24	28.92	15	62.50	9	37.50	>0.9999
Obesity grade 1[4]	13	15.66	5	38.46	8	61.54	0.1180
Obesity grade 2[5]	4	4.82	1	25.00	3	75.00	0.2932
Obesity grade 3[6]	5	6.02	5	100.00	0	0.00	0.1510
RCQ normal[7]	52	62.65	31	59.62	21	40.38	0.8161
RCQ change[d]8	31	37.35	20	64.52	11	35.48	0.8161
Hospitalization in the last 12 months	18	21.69	15	83.33	3	16.67	0.0533
Use of ATB in the last 12 months	33	39.76	21	63.64	12	36.36	0.8197
Discontinuation of ATB use	9	10.84	7	77.78	2	22.22	0.4293

Source: Prepared by the authors.

N (number). BMI (Body Mass Index). WHR (waist-to-hip ratio). ATB (Antibiotic).[1] BMI < 18.5 kg/cm2; [2] 18.5 kg/cm2 ≤ BMI < 25.0 kg/cm2;[3] 25 kg/cm2 ≤ BMI < 30 kg/cm2; [4]30 kg/cm2 ≤ BMI < 35 kg/cm2; [5] 35 kg/cm2 ≤ BMI < 40 kg/cm2; [6]BMI ≥40.0 kg/cm2; [7]Female ≤ 0.84 and male ≤ 0.89; [8]Female ≥ 0.85 and male ≥ 0.90. [9] P value obtained using Fisher's exact test.

Frequency of *Staphylococcus* in the nasal cavities of university students

Assessment of the frequency of *Staphylococcus sp.* in the nasal cavities of university students is represented in Table 2. The majority of samples evaluated (76; 91.57%) were positive for *Staphylococcus sp.* (p<0.0001), with the prevalence of *S. aureus* (61.45%) statistically higher than that of catalase-negative *Staphylococcus* (30.12%; p=0.0028). The prevalence of *S. aureus* was statistically higher than that of catalase-negative *Staphylococcus* in men (p=0.0125). No significant differences were observed in comparing the prevalence of bacteria between the sexes of individuals (p>0.05).

Table 2. Relationship between the frequency of bacteria of the genus *Staphylococcus* in the nasal cavities of university students in the health field and biological sex.

Culture result	TOTAL		FEMININE		MASCULINE		
	N	%	n	%	N	%	*p*[1]
Negative culture	7	8.43	3	42.86	4	57.14	0,4208
Catalase negative Staphylococcus	25	30.12	18	72.00	7	28.00	0,2269
Staphylococcus aureus	51	61.45	30	58.82	21	41.18	0,6448

Source: Prepared by the authors.

[1]p value obtained using Fisher›s exact test.

Relationship between anthropometric indices and the prevalence of *Staphylococcus* in the nasal cavities of university students

Next, possible relationships between the prevalence of *Staphylococcus* in the nasal cavities of university students and anthropometric indices (BMI and WHR) were analyzed. The frequency values of total colonization by *Staphylococcus sp.* in the nasal cavity did not show significant associations (p>0.05) with the different BMI or WHR classification ranges. On the other hand, comparing colonization profiles, eutrophic individuals and those with grade 1 obesity were more colonized by *S. aureus* than by coagulase-negative *Staphylococcus* (p=0.0351 and p=0.0386, respectively). The prevalence rates of microorganisms were similar for the other classifications (Table 3).

Table 3. Colonization profile by *Staphylococcus sp.* in the nasal cavity in different anthropometric classifications.

Classification		***Staphylococcus***		**Catalase negative *Staphylococcus***		***S. aureus***		*p*[1]
	N	**(n)**	**%**	**(n)**	**%**	**(n)**	**%**	
Underweight	2	2	100	0	0.00	2	100.00	0.5000
Eutrophic	35	32	91.43	10	28.57	22	62.86	**0.035**1
Overweight	24	21	87.50	7	29.17	14	58.33	0.1892
Obese individuals	22	21	95.45	8	36.36	13	59.09	0.3833
Obesity grade 1	13	12	92.31	2	15.38	10	76.92	**0.0386**
Obesity grade 2	4	4	100	3	75.00	1	25.00	0.6250
Obesity grade 3	5	5	100	3	60.00	2	40.00	>0,9999
No cardiovascular risk	52	50	96.15	26	50.00	24	46.15	0.8877
With cardiovascular risk	31	26	83.87	9	29.03	17	54.84	0.1221

Source: Prepared by the authors.

[1]P value obtained through binomial test. N= total number of individuals; n= number of positive samples.

The colonization rates of bacteria of the genus *Staphylococcus* in the nasal cavities of university students in the health-care field were compared in different anthropometric classification ranges (Table 4). No significant associations were found comparing the colonization rates of obese and normal-weight individuals or comparing students with and without increased cardiovascular risk (p>0.05) (Table 4).

Table 4. Comparison between the frequency of bacteria of the genus *Staphylococcus* in the nasal cavities of university students in the health field in different anthropometric classification ranges.

Classification	*p*[1]
Eutrophic x Obese	0.7874
Eutrophic x Obese I	>0.9999
Eutrophic x Obese II and III	0.0829
Eutrophic x Overweight/Obese	0.8194
Without cardiovascular risk x With cardiovascular risk	0.5006

Source: Prepared by the authors.

[1]P value obtained using Fisher's exact test.

Assessment of biofilm formation by *S. aureus* isolates obtained from the nasal cavities of university students

S. aureus isolates were evaluated for their ability to adhere to a polystyrene surface (Table 5). The majority of isolates (88.24%) showed the ability to form biofilms (p<0.05). Three isolates were classified as strong biofilm formers, obtained from samples from the nasal cavity of men (2 eutrophic individuals and 1 with grade 1 obesity). None of these students used antibiotics or were hospitalized during the last 12 months. Nine isolates formed a moderate biofilm, all obtained from female samples (1 malnourished, 5 eutrophic, 2 overweight, 1 with grade 3 obesity). Five of these students (50%) used antibiotics in the last 12 months (1 stopped treatment prematurely), and only 1 remained hospitalized last year.

Of the 33 isolates with poor adherence (18 from female samples), one was obtained from a student with mild malnutrition, twelve from eutrophic students, eleven from overweight, and ten from obese individuals (grade 1= 8; grade 2= 1; grade 3= 1). Six of these students were hospitalized, and thirteen used antibiotics in the last 12 months. Finally, only 6 isolates did not form a biofilm. No significant differences were detected in the prevalence rates of biofilm-forming isolates between obese and non-obese individuals (p>0.05).

Table 5. Biofilm formation by *S. aureus* isolates obtained from healthcare students in São Luís, MA.

Classification		NA		WbF		MF		SF	
	N	(n)	%	(n)	%	(n)	%	(n)	%
Under weight	2	1	50,00	0	0.00	1	50,00	0	0.00
Eutrophic	22	3	13,64	12	54.55	5	22,73	2	9.09
Overweight	14	1	7.14	11	78.57	2	14,29	0	0.00
Obese individuals	10	1	10,00	8	80.00	0	0.00	1	10,00
Obesity grade 1	1	0	0.00	1	100,00	0	0.00	0	0.00
Obesity grade 2	2	0	0.00	1	50.00	1	50,00	0	0.00

Source: Prepared by the authors.

NA (non-adherent). WbF (weak biofilm forming). MF (moderate biofilm forming). SF (strong biofilm former).

DISCUSSIONS

The present study evaluated the frequency of colonization by *Staphylococcus* in health students in São Luís and its possible association with anthropometric factors. BMI and WHR are anthropometric measurements used to classify obesity, being predictors strongly correlated with the emergence of chronic non-communicable diseases, such as diabetes mellitus 2 and cardiovascular risk, respectively [25]. The prevalence of obesity and overweight/obesity in healthcare students recruited in this study was 26.51% and 55.42%, respectively. In addition, altered WHR was found in 37.35% of students. These data are superior to those found in other research with university students [26–28].

In the studied population, the colonization rate by *Staphylococcus sp.* was 91.57%, with the prevalence of *S. aureus* being 61.45%. These results corroborate previous research with university students and health professionals who reported colonization rates by *S. aureus* in the nasal cavity between 20% and 70% [14,15,27]. For example, the prevalence of nasal colonization by *S. aureus* was 28% in a study of medical students in Colombia [26]. In another study carried out in Londrina (Brazil) with students from different health courses, the rate of nasal colonization by *S. aureus* was 45.31%, higher than that detected by health professionals [14]. Higher rates were found in surveys of medical students in Nepal (54.50%) and Nigeria (66.6%) [12,29].

Obesity is a serious public health problem due to its multifactorial and progressive nature, resulting in high health system costs [29–31]. Excess fat can cause complications in various functions of the body, representing a risk factor for several pathologies such as type 2 diabetes mellitus, fatty liver disease, cardiovascular diseases, and stroke, among other pathologies [32]. Furthermore, adipocytes exhibit plasticity and can assume different phenotypic profiles, influencing the immune system [33,34]. This means that obesity can trigger chronic inflammation, directly affecting the immune response [20,21], making it deficient, and favoring the colonization and installation of infection by *S. aureus* [18,19].

Next, the relationship between fat deposition in the abdominal region and

the colonization rate of *Staphylococcus sp*. was evaluated in students' nostrils. In the present study, it was impossible to observe a significant association between obesity and WHR and colonization by *S. aureus*. WHR is a specific indicator to determine central adiposity and is not always associated with BMI [25,35]. The relationship between hip circumference and *S. aureus* colonization rate was positive for men in a study conducted in Norway [18].

Finally, the ability of *S. aureus* isolates to form a biofilm on a polystyrene surface was analyzed. Biofilms are a set of microorganisms covered by an extracellular polymer or exopolysaccharide matrix, allowing them to adhere to different types of biological or non-biological surfaces, allowing them to survive in different environments [36,37]. The formation of biofilms is characterized as an essential virulence factor of *S. aureus*, is also associated with therapeutic failure [38,39].

The prevalence of biofilm-forming S. aureus isolates was 88.24%, most of which were weak biofilm-formers. In a study with individuals treated in an Otorhinolaryngology service at a University Hospital in Turkey, there were 87 *S. aureus* isolates from a total of 658 nasal samples (13.2%), with 86 (98.85%) isolates having the ability to form biofilm [40]. Similarly, research using nasal samples (colonization and invasive infections) from patients at a University Hospital in Brazil isolated *S. aureus* from 94 samples (63.95%) [41].

Some limitations must be considered in the present study. For example, the use of samples from a single educational institution. Therefore, a study with a greater number of samples from different educational institutions would provide a more comprehensive overview of the prevalence of *S. aureus*. Another limitation is the absence of a group of students from other areas to analyze whether the prevalence of *S. aureus* would be higher in Health students. It will also be essential to include molecular techniques for identifying isolates and evaluating profiles in a future study of antibiotic resistance.

CONCLUSIONS

The results obtained in this work point to a high colonization by *S. aureus* in the nostrils of the population of academics in the health area of São Luís. The *S. aureus* isolates were predominantly classified as weak biofilm-formers. No significant associations were observed between S. aureus colonization and obesity in the studied population. The high frequency of students carrying *S. aureus* denotes the importance of control measures to prevent the spread of this microorganism.

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Received: mar 01, 2023
Aprovado: aug 04, 2023
Editor: Profa. Dra. Ada Clarice Gastaldi