



Nasal colonization with *Staphylococcus aureus* in nursing students: ground for monitoring

Colonização nasal por Staphylococcus aureus entre estudantes de Enfermagem: subsídios para monitorização
Colonización nasal por Staphylococcus aureus entre estudiantes de Enfermería: sugerencias para su monitoreo

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ABSTRACT

Objective: to monitor bacterial strains of *Staphylococcus aureus* that are resistant or not to oxacillin in nursing undergraduate students, with an emphasis on the process of colonization. **Method:** cross-sectional prevalence study carried out with 138 nursing students. The biological samples of the nasal cavity were collected in June 2015, by means of sterile swabs, which were subsequently submitted to confirmatory tests of catalase and coagulase. Isolated *Staphylococcus aureus* had their sensitivity profile determined by means of the Kirby Bauer method. Descriptive, univariate and bivariate analyses were performed. **Results:** the prevalence of *Staphylococcus aureus* was 21.7. Regarding the resistance profile, 24.1% of strains were resistant to oxacillin, with ampicillin being the antimicrobial with the greatest resistance (82.8%). **Conclusion:** the nasal cavity is an important bacterial flora of *S. aureus* in nursing students. The profile of isolated strains highlights the increase of *Staphylococcus aureus* resistance to antimicrobials such as oxacillin.

Descriptors *Staphylococcus Aureus*; Nursing; Infection; Monitoring; Nursing students.

RESUMO

Objetivo: monitorar as cepas de *Staphylococcus aureus* resistentes ou não à oxacilina, entre estudantes da graduação em enfermagem, com ênfase no processo de colonização. **Método:** estudo transversal de prevalência, realizado com 138 estudantes de Enfermagem. As amostras biológicas da cavidade nasal foram coletadas em junho de 2015, utilizando-se swabs estéreis, posteriormente submetidos a testes confirmatórios de catalase e coagulase. Os *Staphylococcus aureus* isolados tiveram seus perfis de sensibilidade determinados por meio da técnica de Kirby Bauer. Foram realizadas análises descritivas, univariadas e bivariadas. **Resultados:** a prevalência de *Staphylococcus aureus* foi de 21,7. Com relação ao perfil de resistência, 24,1% das cepas se mostraram resistentes à oxacilina, sendo a ampicilina o antimicrobiano com maior resistência (82,8%). **Conclusão:** a cavidade nasal é importante reservatório de *S. aureus* em estudantes de Enfermagem. O perfil das linhagens isoladas evidencia o aumento da resistência de *Staphylococcus aureus* a antimicrobianos, como a oxacilina.

Descritores: *Staphylococcus Aureus*; Enfermagem; Infecção; Monitorização; Estudantes de Enfermagem.

RESUMEN

Objetivo: monitorear las cepas de *Staphylococcus aureus* resistentes o no a oxacilina entre estudiantes de curso de graduación en enfermería, con énfasis en el proceso de colonización. **Método:** estudio transversal de prevalencia, realizado con 138 estudiantes de Enfermería. Muestras biológicas de la cavidad nasal recolectadas en junio de 2015, utilizándose swabs estériles, sometidos

posteriormente a tests confirmatorios de catalasa y coagulasa. Se determinaron perfiles de sensibilidad de los *Staphylococcus aureus* aislados mediante técnica de Kirby Bauer. Fueron realizados análisis descriptivos, univariados y bivariados. **Resultados:** la prevalencia de *Staphylococcus aureus* fue de 21,7. Respecto al perfil de resistencia, 24,1% de las cepas se mostraron resistentes a oxacilina, resultando la ampicilina el antimicrobiano con mayor resistencia (81,8%). **Conclusión:** la cavidad nasal constituye importante reservorio de *S. aureus* en estudiantes de Enfermería. El perfil de las cepas aisladas evidencia un aumento de resistencia del *Staphylococcus aureus* a antimicrobianos como oxacilina.

Descriptores: *Staphylococcus Aureus*; Enfermería; Infección; Monitoreo; Estudiantes de Enfermería.

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INTRODUCTION

Staphylococcus aureus is one of the most frequent causing factors of infections in healthcare environments. This gram-positive, spherical and pyogenic bacteria is characterized by its high capacity to quickly disseminate among people and in different environments, as well as by the capacity to infect healthy or immunocompromised individuals, exponentially increasing morbi-mortality rates, very often with a severe prognosis as a result of the change in the antimicrobial resistance profile⁽¹⁻²⁾.

The *S. aureus* transmission occurs by direct (especially hands) or indirect contact (contaminated surfaces or fomites), especially the colonization in which the individual becomes the carrier of the microorganism, without necessarily showing characteristic signs and symptoms of infection (asymptomatic carrier)⁽³⁻⁵⁾.

Colonization is pointed as a risk factor for the development of infections related to health care, especially when it concerns *S. aureus*. The nasopharyngeal microbiota represents the primary site of colonization for this pathogenic agent. It is estimated that this bacteria is part of the skin transient microbiota of one third of the general population, and this prevalence may vary greatly, especially in Brazil, where there are no systematic data on colonization rates among the general population⁽⁶⁻⁷⁾.

When it comes to health professionals and students, colonization rates range from 20 to 40%, with high percentages of multi-resistant strains, especially among health professionals who work in hospitals and are a great source of infection, more particularly to patients treated by them every day^(3-5,8).

Clinical practice among students of life sciences is part of the teaching-learning process and exposure to occupational risks is inherent to it, since there are different types of care provided to patients. As nursing undergraduate students move further along the school program and go deeper into the complexity of care practices, whether they are in hospitals or other healthcare settings, they become microbial carriers, and *Staphylococcus aureus* is a major concern⁽⁸⁻¹⁰⁾.

This issue has been widely discussed; nevertheless, the dissemination of methicillin-resistant *Staphylococcus aureus* (MRSA) among students has been growing gradually, despite the epidemiological monitoring efforts made to determine the rates and control its dissemination. Therefore, other studies aimed at the isolation of the microorganism and the identification of the species are necessary, since there is no consensus on the positivity rates of strains and on the susceptibility profile of isolated *Staphylococcus aureus* to antibiotics in specific regions and population groups⁽⁷⁻¹¹⁾.

This information is essential so preventive and therapeutic measures can be successfully directed, regardless of signs and clinical symptoms of infection. In view of the above, the objective is to monitor bacterial strains of *Staphylococcus aureus* that are resistant to oxacillin or not in nursing undergraduate students, with an emphasis on the process of colonization and thus relate the characterization of these students in terms of age, gender, comorbidities, term, among other variables.

METHOD

This is a cross-sectional study of the prevalence of *Staphylococcus aureus* in the nasal cavity of nursing students.

Study population

The study included students from the Nursing Program of the Federal University of Piauí. The aforementioned course started in 1973 and offers 40 seats per semester. The study sample totaled 138 students, with an average of 15 students per term. The choice of this course was due to the fact that nursing students develop part of their academic training in hospital, outpatient or laboratory settings.

Students who agreed to participate in the study were informed about the objectives of the study and, after signing of a free and informed consent form, a questionnaire was applied in which the following variables were assessed: gender, age, marital status, academic term, internship (curricular or extracurricular), means of transportation used and the presence of comorbidities. Students who had been under antimicrobials for the last 30 days before the collection of biological samples were excluded.

Collection, isolation and microbiological procedures

The biological samples taken from the nasal cavity of students were collected in June 2015, with the help of sterile wooden swabs (Labor import), by means of friction in circular movements in both nasal cavities. After collection, the material was transported to the microbiology laboratory in a box, at room temperature, for a maximum period of one hour, in tubes (5 mL - 10.50x90 mm) containing Luria Bertani Miller (LB Miller) (KASVI, Curitiba, Mato Grosso, Brazil). After that, the samples were incubated at 36° C for 24 hours.

After this period, the samples were cultured in flat petri dishes (disposable polystyrene plates, 90 x 15 mm) containing salted mannitol agar (HiMedia Laboratories Pvt Ltd, Mumbai, India). After the incubation period (24 hours at 36° C in the oven), the samples were analyzed. Those which had a

Staphylococcus aureus growth in gold color were transferred to a Brain-heart infusion medium (BHI) (HiMedia Laboratories Pvt Ltd, Mumbai, India). The colonies that were positive for mannitol agar and which were subsequently cultured in BHI were examined for their colony morphology by means of Gram staining, and the samples which had a morphology that corresponded to *S. aureus* were submitted to confirmatory tests of catalase (hydrogen peroxide), Gram staining and coagulase test (Laborclin, Vargem Grande, Pinhais, Paraná).

The isolated *Staphylococcus aureus* had their sensitivity profile determined by means of the Kirby Bauer method, following the Clinical and Laboratory Standards Institute (CLSI) criteria⁽¹²⁾. The following antimicrobials were used: ampicillin, oxacillin, tetracycline and chloramphenicol. The diameters of incubation halos were interpreted after 24 hours of incubation at 36° C, in Müller-Hinton agar (HiMedia Laboratories Pvt Ltd, Mumbai, India). Still as control test, bacteria with phenotypes resistant to oxacillin isolated by means of the disc diffusion technique were submitted to a ciprofloxacin sensitivity test. All antimicrobials had their sensitivity tested with a *S. aureus* control strain (ATCC 25923).

Statistical analysis

Initially, a manual organization of questionnaires was done, followed by double typing into Microsoft Excel 2010, and import to the Statistical Package for the Social Sciences - SPSS for Windows (version 20.0). With this data, descriptive, univariate and bivariate analyses were performed. With regard to categorical variables, a Chi-square test was performed and the significance level was $p \leq 0.05$, with a confidence interval (CI) of 95%.

Ethical aspects

The initial research project was submitted to the appreciation of a research ethics committee and received a favorable opinion. The study performance respected all ethical precepts that rule human research.

RESULTS

A total of 138 nursing students participated in the study, with a predominance of women (113/81.9%) aged between 17 and 20 years old (52/37.7%). About 80% of the students used public transportation (Table 1), and 89.9% of participants reported they did not have recent comorbidities.

Students were distributed over nine academic terms. We chose to group them in blocks, by similarities. In the initial terms (1st to 3rd), students carried out their activities in laboratories only. From the 4th to the 6th term, the hospital internships were frequent and intensive, and in the last three terms, students performed their duties in both primary health care and in the hospital network. Most of them (88/63.8%) carried out some internship at the time, and nearly half of them were participating or had participated in an extracurricular internship. Regarding the last time they had been in a hospital setting, most of them (86.9%) reported a period shorter than 60 days (Table 2).

Cultures were submitted to confirmatory tests of catalase and coagulase. The prevalence of *Staphylococcus aureus* (positive

Table 1 – Distribution of students, regarding sociodemographic variables, Teresina, Piauí, Brazil, 2015

Variables	n(%)	\bar{X}	\pm	IC 95%	Mín.-max.
Age (in years-old)		21.4	2.5	21.0-21.8	17-34
17 to 20	52(37.7)				
21 to 25	77(55.8)				
26 to 30	8(5.8)				
Over 30	1(0.7)				
Gender					
Male	26(18.8)				
Female	112(81.2)				
Color of the skin					
White	28(20.3)				
Asian	5(3.6)				
Brown	86(62.3)				
Black	19(13.8)				
Means of transportation					
Public	110(79.7)				
Private	28(20.3)				

Score: \bar{X} = mean, \pm = standard deviation, IC 95% = confidence interval, Min-max = minimum and maximum.

Table 2 – Distribution of students, regarding training variables (N = 138), Teresina, Piauí, Brazil, 2015

Variables	n	%
Current period		
First to third	47	34.1
Fourth to sixth	49	35.5
Seventh to ninth	42	30.5
Doing an internship in the current subjects		
Yes	88	63.8
No	50	36.2
Doing or has done an extracurricular internship		
Yes	62	44.9
No	76	55.1
Last went to a hospital		
1 to 10 days ago	94	68.1
11 to 60 days ago	26	18.8
Over 60 days ago	18	12.9

coagulase) was 21.7%. Regarding the resistance profile, 24.1% of *Staphylococcus aureus* strains were resistant to oxacillin. For ampicillin, the profile was 82.8% of resistance (Table 3).

In the investigation of the statistical correlation between colonization by *Staphylococcus aureus* and other independent variables, only the block or term attended was statistically significant ($p = 0.04$) (Table 4).

Table 3 – Profile of susceptibility to antimicrobials of isolated *Staphylococcus aureus* strains in nursing students, Teresina, Piauí, Brazil, 2015

Variables	n	%
Catalase		
Positive	135	97.8
Negative	3	2.2
Coagulase		
Positive	30	21.7
Negative	108	78.3
Antibiogram		
Yes	29	21.0
No	109	79.0
Oxacillin-sensitive		
Sensitive	14	48.3
Intermediate	8	27.6
Resistant	7	24.1
Tetracycline-sensitive		
Sensitive	22	75.9
Intermediate	1	3.4
Resistant	6	20.7
Chloramphenicol-sensitive		
Sensitive	27	93.1
Intermediate	2	6.9
Resistant	-	-
Ampicillin-sensitive		
Sensitive	5	17.2
Intermediate	-	-
Resistant	24	82.8
Performed a ciprofloxacin sensitivity test		
Yes	6	20.7
No	23	79.3
Ciprofloxacin-sensitive		
Sensitive	2	28.6
Intermediate	-	-
Resistant	5	71.4

Table 4 – Distribution of results of *Staphylococcus aureus* (coagulase +/-), according to sociodemographic curricular variables, Teresina, Piauí, Brazil, 2015

	Coagulase		p value
	Positive n(%)	Negative n(%)	
Age Group			0.73
17 to 21	18(22.8)	61(77.2)	
22 and over	12(20.3)	47(79.7)	
Color of the skin			0.15
White	3(10.7)	25(89.3)	
Asian	1(20.0)	4(80.0)	
Brown	24(27.9)	62(72.1)	
Black	2(10.5)	17(89.2)	
Gender			0.16
Male	3(11.5)	23(88.5)	
Female	27(24.1)	85(75.9)	

To be continued

Table 4 (concluded)

	Coagulase		p value
	Positive n(%)	Negative n(%)	
Period			0.04
First to third	12(25.5)	35(74.5)	
Fourth to sixth	10(20.4)	39(79.6)	
Seventh to ninth	8(19.1)	34(80.9)	
Means of transportation			0.94
Public (bus)	24(21.8)	86(78.2)	
Private	6(21.4)	22(78.6)	
Doing an internship in the current subjects			0.36
Yes	17(19.3)	71(80.7)	
No	13(26.0)	37(74.0)	
Doing or has done an extra-curricular internship			0.83
Yes	14(22.6)	48(77.4)	
No	16(21.0)	60.0(78.9)	
Last went to the hospital			-
1 to 60 days ago	29(23.8)	93(76.2)	
61 days ago or more	1(6.3)	15(93.8)	

Note: Confidence interval (CI) of 95%. The p-value was obtained by the Chi-square test. The significance level was defined at $p \leq 0.05$.

DISCUSSION

The results of this study highlighted the presence of multi-resistant *Staphylococcus aureus* in the nasal cavity, with a prevalence within the limits reported in the literature (20% to 40%)^(5,13).

Staphylococcus aureus is considered a persistent member of the human endogenous microbiota. It behaves as one of the main virulent agents, when it comes to infectious processes of serious repercussions, and has long been the main responsible for infections related to health care in hospitals, being increasingly found within communities with reports of multi-resistance⁽¹⁴⁾.

The nostrils are the main site for *S. aureus* colonization. Most of the time, this colonization is asymptomatic and has great clinical importance, especially when it comes to health professionals who may transmit the strains through contaminated hands or even through the air⁽¹³⁻¹⁵⁾.

Nursing students play an important role in the epidemiology and the pathogenesis of the infection and may act as a source of dissemination of the *S. aureus*, in both the community and the hospital environment, but also in the transportation of bacteria from one environment to another^(5,16).

Students of the initial terms (1st to 3rd) who have not begun their extracurricular internship in hospitals and primary health care units had a prevalence of *S. aureus* above 50%, which can indicate the presence of the pathogenic agent and probably of the community-acquired MRSA, a fact that has been pointed out in other studies as an emerging dissemination⁽¹⁷⁻¹⁸⁾.

The course attended has nine blocks or academic terms, with basic subjects taught in the initial terms which do not include the participation of students in settings of provision

of services. In the subsequent terms, the initial contact with such settings takes place, which is required by the vocational subjects, with a predominance of hospitals.

In this study, a high prevalence of colonization was observed precisely in the initial terms. From the third term on, when contact with healthcare environments begins, the prevalence increases (43.8%) and grows gradually over the subsequent terms, associated with high prevalence and resistance. When we analyze the positive samples only, 84.5% of students were doing an internship in hospitals, that is, the contact with the hospital environment had a crucial significance for positive results⁽¹⁹⁻²⁰⁾.

The mean of days of the last contact with a hospital environment among colonized individuals was 9.2 days, and among non-colonized individuals it was 41.7 days, which shows a relationship between the nearness of the last contact with hospital microbiota and the presence of colonization.

Regarding the analysis of the antimicrobials sensitivity profile, a high resistance to ampicillin was observed (91.1%), which contrasted with the high sensitivity to tetracycline and chloramphenicol (84.4%). Greater prominence was given to the resistance to oxacillin (ORSA), with 40% of resistance and 42.2% of intermediates, a significant percentage since it concerns asymptomatic individuals. A study carried out with nursing students found only 8.8% of samples which were resistant to oxacillin⁽²⁰⁻²²⁾.

Efforts must be made in order to implement rules and routines aimed at controlling the dissemination of *S. aureus* strains among students and health professionals, since eradication and control are very difficult to achieve after they have settled within a community. In this context, in view of the high morbi-mortality and the exponential growth of microbial resistance cases, the implementation of control strategies is recommended⁽²³⁾.

In this context, nasal decolonization of health professionals who are epidemiologically related to the transmission of *S. aureus*, by means of topical mupirocin, is recommended by

most guidelines of international health agencies⁽⁷⁾. However, there are few studies conducted by life sciences students that highlight the benefits of this therapy.

Limitations and contributions to nursing

The main limitations to this study are inherent to the research design. Cross-sectional studies do not allow for the follow-up of subjects, which would help assess the incidence of *S. aureus* throughout the course in the same individual. However, finding the prevalence rate by means of cross-sectional studies and its relationship with clinical and training variables may indicate the need for further follow-up studies with the purpose of investigating risk and protection factors.

Considering that most of nursing students' training is carried out in direct contact with patients and that there are still controversies regarding the benefits of antimicrobial intervention in colonized individuals, the epidemiological monitoring of incidence of *S. aureus* strains and the susceptibility profiles of isolated strains, it is essential to establish preventive and therapeutic measures.

CONCLUSIONS

The nasal cavity is an important bacterial flora of *S. aureus* and MRSA. The prevalence of colonization by the *Staphylococcus aureus* in the nasal cavity of nursing students of the higher education institution studied was 21.4%. This study recalls the importance of upper airways in the transmission and contamination of pathogenic microorganisms, and points out the need to provide a national picture of the occurrence of *S. aureus* sensitive and resistant to oxacillin in health professionals and students.

The high incidence of antimicrobial-resistant *Staphylococcus aureus*, especially to oxacillin, reinforces the event of multi-resistant strains in the community, indicating the need for studies that assess the phenotypes and genotypes responsible for this bacterial resistance.

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