

Nasal colonization by *Staphylococcus* sp. in inpatients

Colonização nasal por *Staphylococcus* sp. em pacientes internados

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Abstract

Objective: To analyze nasal colonization by *Staphylococcus* sp. its resistance to methicillin, and associated factors in inpatients.

Methods: Nasal sample collection, antimicrobial susceptibility tests, and analysis of medical records of inpatients (n=71) were performed, and a questionnaire was applied. Data were analyzed by descriptive and inferential statistics using the chi-square, Student's t, and Mann-Whitney tests ($\alpha=5\%$).

Results: Nearly half (44.4%) of the patients who were significantly associated with prolonged antibiotic treatment ($p=0.02$) was infected with methicillin-resistant *Staphylococcus* sp.. A significant association was observed between patients with sensitive strains and absence of antibiotic treatment prior to sample collection ($p=0.02$) or absence of wounds ($p=0.003$).

Conclusion: Strains of methicillin-resistant *Staphylococcus* sp. were found, and there was no significant difference between the *S. aureus* species and the coagulase-negative *Staphylococci* groups, which indicates the degree of spread of methicillin resistance among different species of *Staphylococcus*.

Resumo

Objetivo: Analisar a colonização nasal por *Staphylococcus* sp., sua resistência à meticilina e fatores associados em pacientes internados.

Métodos: Foram realizados coleta de amostra nasal, testes de susceptibilidade antimicrobiana e análise de prontuários médicos de pacientes internados (n=71), e foi aplicado um questionário. Os dados foram analisados por meio de estatística descritiva e inferencial usando os testes χ^2 , t de Student e Mann-Whitney ($\alpha=5\%$).

Resultados: Cerca de metade (44,4%) dos pacientes, significativamente associados ao tratamento antibiótico prolongado ($p=0,02$) estavam infectados por *Staphylococcus* sp resistentes à meticilina. Observou-se uma associação significativa entre pacientes com cepas sensíveis e ausência de tratamento com antibiótico antes da coleta ($p=0,02$) ou ausência de feridas ($p=0,003$).

Conclusão: Foram encontradas cepas de *Staphylococcus* sp. resistentes à meticilina e não houve diferença significativa entre a espécie *S. aureus* e os grupos de *estafilococos coagulase* negativos, o que indica o grau de disseminação da resistência à meticilina entre diferentes espécies de *Staphylococcus*.

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Introduction

Excessive use of antimicrobial agents or inappropriate empirical treatment have contributed to the growing number of infections by multi-resistant microorganisms in both the community and hospital environment.⁽¹⁾ As a result, the treatment of patients with these infections is becoming more complex, greatly increasing the costs of both hospitalization and treatment of these patients in public hospitals.⁽²⁾ *Staphylococcus sp.*, mainly *S. aureus*, is commonly found in the skin and mucosa of humans (especially in the anterior region of the nasal passages), being among the microorganisms most resistant to antibiotics.⁽³⁾ It is one of the main pathogens that colonizes healthy individuals in the community, leading to infection in patients admitted in hospitals.^(4,5)

In this context, it is worth emphasizing the worldwide increase in the prevalence of methicillin-resistant *S. aureus* (MRSA).⁽⁶⁾ This agent causes serious infections, whether in hospitalized individuals or otherwise, emphasizing the importance of epidemiological vigilance in detecting development of resistance in both the community and in health care services.⁽⁶⁾ In addition, *coagulase-negative Staphylococci* (CoNS) act as a reservoir of resistance genes, although these microorganism are less virulent. The presence of methicillin-resistant *coagulase-negative Staphylococci* (MRCoNS) in hospital environments can lead to the emergence of MRSA.^(7,8)

Presence of MRSA or MRCoNS in asymptomatic patients is a major source of contamination. Their early identification can reduce the risk of colonization of patients and cross-transmission between patients and health professionals, especially in hospital environment.^(7,9) Although this theme is important, it has been little studied in the northeast of Brazil, especially in interior municipalities, which are characterized by marked social and economic inequality and where a large part of the population live in conditions of social deprivation. Therefore, studies in this region are necessary to support implementation and monitoring of measures of control to both minimize the potential spread of this microorganism and subsequently reduce the risk of hospital infections.⁽¹⁰⁾

Thus, the aim of this study was to describe nasal colonization by *Staphylococcus sp.*, especially *S. aureus*, their respective sensitivity to methicillin, and associated factors in patients in a referral hospital in the interior of northeast Brazil.

Methods

A cross-sectional study was performed in the Hospital Regional do Seridó, in Caicó, Rio Grande do Norte (RN), a municipality in northeast Brazil. This hospital is a referral institution in the interior of RN, where patients from more than 14 municipalities are treated. Due to many reasons, there is a lack of research studies in hospital in the interior of northeast Brazil. Furthermore, this region has some of the lowest social and economic indicator scores in the country,⁽¹¹⁾ making investigation more difficult, especially if it requires laboratory and infrastructure support.

Patients admitted to the medical and surgical clinics, and Intensive Care Unit (ICU) of the Hospital Regional do Seridó participated in the study. The subjects were enrolled in parallel with another study, in which the investigators aimed to identify *Staphylococcus aureus* in wounds of patients. This study included individuals with skin sores or wounds on the day of collection, or those without wounds who were hospitalized within 12 hours prior to collection. Individuals without wounds were accompanied during hospitalization to verify if pressure ulcers or post-surgical infections developed while they were in the hospital.

Nasal collection was performed in patients included in the study described above in order to verify if there was colonization by *Staphylococcus sp.* Only the first 30 patients were considered for calculation of sample size. In the first analysis, 74% of *Staphylococcus sp.* isolated in the nostrils of patients hospitalized were found. We assumed a margin of error of 15%, a design effect of 1%, and a non-response rate of 20% to estimate the sample size (71 patients).

Therefore, subjects were included until the sample size was complete (n=71) to characterize nasal

colonization by *Staphylococcus sp.*. Data were collected during the first semester of 2012.

Medical records were consulted in order to describe the factors related to the hospitalization/antibiotic treatment of the studied population. In addition, the patients completed a questionnaire containing questions related to age, gender, municipality of origin, presence of comorbidities or systemic impairment, and use of antibiotics prior to hospitalization, among other factors that could influence the frequency of methicillin-resistant *Staphylococcus sp.*.

Samples from the nasal mucosa of patients were collected using a sterile swab soaked in 0.85% saline. For each patient, the swab was inserted into both nasal cavities and the sample was then placed in sterile tubes containing Brain Heart Infusion (BHI) broth (with 7.5% NaCl), which were then packed in styrofoam boxes with crushed ice and transported to the microbiology laboratory of the university. The samples were incubated in the laboratory (37 °C; 24 h). After this period, the samples were inoculated in mannitol salt agar medium and grown in a bacteriological incubator (37 °C; 48 h). The staphylococcal colonies were then subjected to Gram stain, catalase, and free coagulase tests. Samples positive for Gram, catalase, and coagulase were classified as *Staphylococcus aureus*; samples negative for coagulase test were classified as coagulase-negative *Staphylococcus* (CoNS). All samples identified as *Staphylococcus sp.* were submitted to antibiogram analysis using the disk diffusion method to verify their resistance to methicillin.

The Chi-square or Fisher's exact test were utilized to verify the association between dependent (resistance or sensitivity to methicillin and presence of *S. aureus* or CoNS) and qualitative independent variables. The Prevalence Ratio (PR) was utilized to analyze the degree of association. The Student's t-test was utilized to ascertain whether there was a significant difference between the groups of dependent variables in relation to the patients' age. The other quantitative independent variables (number of days of antibiotic use prior to sample collection, number of days of hospitalization, and number of

hospitalizations in the last year) were analyzed using the Mann-Whitney test. A significance level of 5% was used with the Stata 10.0 statistical software.

The development of the study met the national and international standards of ethics in research involving human beings.

Results

A total of 38 (53.5%) patients were female and 33 (46.5%) were male. Patients had a mean age of 63±21 (standard deviation, sd) and time of formal education of 3.8±3.7 years (sd). A total of 40 (56.3%) patients were in the medical clinic, 20 (28.2%) patients were in the surgical clinic, and 11 (15.5%) in the ICU. Wounds (n=23; 32.4%), fractures or surgery (n=8; 11.3%), and renal or post-surgical infections (n=8; 11.3%) were the most frequent reasons for hospitalization.

As shown in table 1, 63 (88.8%) patients had *Staphylococcus sp.* (either *S. aureus* or CoNS) in their nostrils. Among the 11 patients who died, four (36.4%) had *S. aureus* and five (45.4%) had CoNS in their nostrils; in the samples from two (18.2%) of them, no bacteria grew or was identified as staphylococci. Among the resistant strains, the antibiogram for *Staphylococcus sp.* showed that seven (25%) were *S. aureus* and 21 (75%) were CoNS. However, this difference was not statistically significant (p=0.45). The general descriptive results for resistance/sensitivity of *Staphylococcus sp.* to methicillin, without specifying species or group, is shown in table 1.

Table 1. Absolute and percent distribution, identification, and behavior of nasal *Staphylococcus sp.* relative to methicillin

Dependent variables	n(%)
Presence of nasal <i>Staphylococcus sp.</i>	
<i>S. aureus</i>	20(28.8)
Coagulase-negative <i>Staphylococcus</i>	43(60.6)
No growth of <i>Staphylococcus</i> or bacteria	8(11.3)
Behavior of <i>Staphylococcus sp.</i> relative to methicillin	
Resistant	28(44.4)
Susceptible	35(55.6)
Behavior of <i>S. aureus</i> relative to methicillin	
Resistant	7(9.9)
Susceptible	64(90.1)

Of the 7 samples of nasal MRSA, 71.4% were found in patients from the medical clinic and 28.6% in patients from the surgical clinic or ICU. The patients with MRSA had some kind of systemic impairment (71.4%), diabetes (28.6%), suffered from cancer (28.6%), and had been hospitalized in the previous year (57.1%). Most patients (57.1%) were from a municipality with more than 60,000 inhabitants and 28.6% had died.

Data in table 2 allow identifying an association between dependent and independent variables. A statistically significant association was found only with antibiotic resistance, prior use of antibiotics, and presence of wounds.

The difference between groups of dependent variables and quantitative variables can be seen in table 3. A statistically significant difference was found only between antibiotic resistance and number of days of use of antibiotics prior to sample collection.

Table 2. Patient characteristics associated with presence of *Staphylococci* (CoNS and *S. aureus*) into the nostril and their susceptibility to methicillin

Characteristics	Susceptibility to methicillin					<i>Staphylococcus</i> sp				
	Resistant n(%)	No resistant n(%)	PR	95%CI	p-value	<i>S. aureus</i> n(%)	CoNS n(%)	PR	95%CI	p-value
Gender										
Male	13(44.8)	16(55.2)				11(37.9)	18(62.1)			
Female	15(44.1)	19(55.9)	1.02	0.58-1.77	0.95	9(26.5)	25(73.5)	1.43	0.69-2.97	0.33
Use of antibiotics prior to sample collection										
Yes	20(57.1)	15(42.9)				10(28.6)	25(71.4)			
No	8(28.6)	20(71.4)	2.00	1.04-3.84	0.02	10(35.7)	18(64.3)	0.80	0.39-1.65	0.54
Hospitalization in the last year										
Yes	15(45.5)	18(54.5)				9(27.3)	24(72.7)			
No	13(43.3)	17(56.7)	1.05	0.60-1.83	0.87	11(36.7)	19(63.3)	0.74	0.36-1.54	0.42
Presence of wound										
Yes	25(56.8)	19(43.2)				13(29.5)	31(70.5)			
No	3(15.8)	16(84.2)	3.60	1.23-10.49	0.003	7(36.8)	12(63.2)	0.80	0.38-1.69	0.57
City										
Caicó	14(37.8)	23(62.2)				13(35.1)	24(64.9)			
Other city	14(53.8)	12(46.2)	0.70	0.41-1.21	0.21	7(26.9)	19(73.1)	1.30	0.60-2.82	0.49
Systemic impairment										
Yes	25(49.0)	26(51.0)				14(27.5)	37(72.5)			
No	3(25.0)	9(75.0)	1.96	0.71-5.43	0.13	6(50.0)	6(50.0)	0.55	0.27-1.13	0.17
Clinic										
ICU	4(40.0)	6(60.0)	1			3(13.0)	7(70.0)	1		
Medical	19(51.4)	18(48.6)	0.78	0.34-1.77		13(35.1)	24(64.9)	0.85	0.30-2.42	
Surgical	5(31.3)	11(68.8)	1.28	0.45-3.66	0.38	4(25.0)	12(75.0)	1.20	0.34-4.28	0.76
Diabetes										
Yes	17(51.5)	16(48.5)				9(27.3)	24(72.7)			
No	11(36.7)	19(63.3)	1.40	0.79-2.50	0.24	11(36.7)	19(63.3)	0.74	0.36-1.54	0.42
Cardiovascular disorders										
Yes	8(33.3)	16(66.7)				6(25.0)	18(75.0)			
No	20(51.3)	19(48.7)	0.65	0.34-1.24	0.16	14(35.9)	25(64.1)	0.70	0.31-1.56	0.37
Cancer										
Yes	4(66.7)	2(33.3)				3(50.0)	3(50.0)			
No	24(42.1)	33(57.9)	1.583	0.83-3.01	0.39	17(29.8)	40(70.2)	1.68	0.69-4.10	0.37

CoNS – Coagulase-negative *Staphylococcus*; ICU – Intensive Care Unit; PR – Prevalence Ratio; 95%CI – 95% Confidence Interval

Table 3. Descriptive and inferential statistics between dependent and independent quantitative variables

Dependent variables	Quantitative independent variables							
	Age		N° days with antibiotic prior to collection		N° days hospitalized in a hospital		N° hospitalizations in the last year	
	Mean (standard deviation)	p-value	Median (quartile 25-quartile 75)	p-value	Median (quartile 25-quartile 75)	p-value	Median (quartile 25-quartile 75)	p-value
<i>Staphylococcus sp.</i> resistance to methicillin								
MRSA	65.7(19.5)	0.48	3.5(0.2-9.7)	0.02	5.4(3.0-11.2)	0.05	1(0.0-1.7)	0.89
MSSA	61.8(23.0)		0(0.0-3.5)		3(2.0-5.4)		1(0.0-1.0)	
<i>Staphylococcus sp.</i>								
<i>S. aureus</i>	59.5(24.1)	0.31	0.5(0.0-3.0)	0.15	3(2.2-5.4)	0.20	1(0.0-1.0)	0.57
CoNS	65.4(20.1)		2.0(0.0-9.0)		5.4(2.0-10.0)		1(0.0-2.0)	

CoNS – Coagulase-negative *Staphylococcus*; MRSA – methicillin-resistant *S. aureus*; MSSA – methicillin-sensitive *S. aureus*

Discussion

Although *Staphylococcus sp.* is part of the resident microbiota, being mainly found in the nasal cavities,⁽¹²⁾ they are also frequently found in hospital environment as the main agent of various infections.⁽¹³⁾ In the present study, almost 90% of patients had *Staphylococcus sp.* in their nostrils, and most were CoNS rather than *S. aureus*. Although MRSA is more frequently studied, the *mecA* gene, responsible for its resistance to methicillin, can also be found in strains of methicillin-resistant coagulase-negative *Staphylococcus* (MR-CoNS).^(7,8,14,15) The results of present study are in agreement with others^(4,11,16) in which *S. aureus* was not the most prevalent. The nasal vestibules of about 20% of the healthy population were colonized by *S. aureus*,^(4,5) an important pathogen that can spread throughout the community and has a high resistance potential.^(14,17) However, the pathogen-host interactions are partially understood, and this is our reason for investigating this subject.⁽⁵⁾

Advanced age, prior hospitalization, use of intravascular catheter, prior MRSA colonization, presence of wounds and/or ulcers, prolonged use of antibiotics, and severity of disease were considered risk factors for hospital MRSA. It should be stated that the bacteria must colonize a patient who has not been recently hospitalized, used antimicrobial agents, or had a catheter implanted to be considered a community MRSA.^(18,19) In this sense, most patients involved in our study were admitted to the hospital for treatment of an injury, bed sore, diabetic foot, or erysipela. Additionally, most of them were subject to risk factors related to hospital admission,

which favored colonization by hospital MRSA. Furthermore, there was a history of prior hospitalization in more than half of the cases in the study with MRSA and most patients had systemic impairment.

Many studies have reported the presence of MRSA strains in the nasal mucosa, even at low levels,^(2,4-6,10,20) associated with greater morbidity and mortality. In the present study, the prevalence of MRSA was almost 10%, a value similar to that obtained in other studies involving hospitalized patients or health professionals.^(15,21) There are reports of nasal colonization of healthy individuals by MRSA (1-8%), which represent a potential risk factor for subsequent infection by *S. aureus*.^(15,19)

Although much attention has been directed to the resistance of *S. aureus*, CoNS should also be studied due to an increase in the resistant strains. In the present study, the level of resistance to methicillin was relatively high, with similar values for both *S. aureus* and CoNS. Among nursing students,⁽³⁾ all samples of *S. aureus* isolated in their nostrils were sensitive to oxacillin, whereas 79 samples of CoNS were resistant to it; 10 of these samples were resistant to both oxacillin and cefoxitin. Similarly, almost 50% of the CoNS were resistant to methicillin among pharmacy students.⁽⁷⁾ To verify this relationship among health workers, a study with health professionals found that more than 50% of *S. epidermidis* isolated from their nasal mucosae were resistant and positive for the *mecA* gene.⁽⁸⁾ In this context, we found that resistance for both *S. aureus* and CoNS among hospitalized patients is equivalent, bringing a greater concern with cross contamination in the hospital environment by resistant strains of *Staphylococcus*.

Presence of methicillin-sensitive *Staphylococcus* sp. was significantly associated with non-use by patients of antibiotic prior to sample collection, as well as absence of wounds on their bodies. On the other hand, methicillin-resistant *Staphylococcus* sp. exhibited a significant statistical difference between the number of days under antibiotic therapy prior to data collection and the resistant strains subject for more days to antimicrobial therapy. In a study with hospital patients in Madagascar, presence of *S. aureus* in their nostrils was significantly associated with prior use of antimicrobial agents and prior hospitalization, whereas prior use of antibiotics was significantly associated with presence of MRSA.⁽¹⁹⁾

Another factor described in the literature, which is significantly associated with the presence of MRSA, is prior hospitalization of the patient.⁽¹⁹⁾ In the present study this was however not evident, since hospitalization in the previous year was not significant for the presence of either resistant *Staphylococcus* sp. or *S. aureus*. Nevertheless, more than half of the patients with MRSA in the study were admitted to the hospital in the previous year.

There was no significant association between *Staphylococcus* sp. (*S. aureus* or CoNS) in the nostrils and any of the independent variables of the study, whether sociodemographic or relative to the medical profile of the patient. Regarding the CoNS, a study in French Guiana⁽¹²⁾ also did not find any association between the transport of methicillin-resistant CoNS and sociodemographic characteristics and those relative to health. Thus, the high frequency of colonization by methicillin-resistant CoNS probably depends on the overall prevalence of transport of these strains in the community and not on individual characteristics.⁽¹²⁾ This fact is relevant since it indicates the importance of the upper air waves in the acquisition and transmission of microorganisms, as literature indicates that nasal colonization is responsible for the colonization of the cutaneous surface of the body.⁽⁶⁾ Control measures for routine application require continued education, periodic bacteriological surveillance of those who work in the hospital environment, and application of best practices in infection control while they take care of patients.

Conclusion

Strains of methicillin-resistant *Staphylococcus* sp. were found among patients in the hospital where the study was conducted. However, no significant difference was found between the *S. aureus* species and the CoNS group, showing the scale of the spread of methicillin resistance among different species of *Staphylococcus*. In this perspective, association of bacterial resistance with prior use of antibiotics for a long period, indicates that their indiscriminate use is dangerous.

Collaborations

Almeida GCM; Lima NGM; Santos MM; Melo MCN and Lima KC contributed to the project design, analysis, and interpretation of data, drafting the article, critical revision, and final approval of the manuscript.

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