

Original Research Article

Nasal carriage of *Staphylococcus aureus* among indigenous people of Toba Qom ethnic, Benjamín Aceval, Paraguay

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ABSTRACT

Background: *Staphylococcus aureus* are Gram positive cocci components of the normal microbiota of the skin and mucosa, but can attack other sites of the organism depending on favourable conditions. The condition of host of this bacterium colonizing the nasal mucosa is recognized as a potential risk factor for subsequent invasive infections. This research had the objective to investigate the prevalence of nasal carriers of *S. aureus* among Toba Qom indigenous ethnic group of the San Francisco de Asis community, Paraguay, and to determine the sensitivity profile to antimicrobial agents.

Methods: 141 samples of the nasal cavities were analyzed. Isolation and identification were performed by conventional microbiological methods, by the staining characteristics of the Gram method, catalase and coagulase tests, mannitol fermentation test and deoxyribonuclease test. Antimicrobial susceptibility test was performed by the agar diffusion technique according to a procedure standardized by the National Committee for Clinical Laboratory Standards.

Results: 22 of the 141 individual examined samples were colonized by *Staphylococcus aureus*, which corresponds to a prevalence of 15.6%.

Conclusions: Multidrug-resistant strains were not isolated. All samples presented good sensitivity to most antibiotics except for nalidixic acid, which all samples presented resistance.

Keywords: *Staphylococcus aureus*, Nasal carriers, Indigenous people, Sinusitis, Rinitis

INTRODUCTION

Bacteria of the genus *Staphylococcus* are described as immobile, non-spore-forming Gram-positive Cocci, configured as rounded cells and measuring about one micron in diameter. Through optical microscopy, they appear as isolated cells, in pairs or agglomerated with resemblance to a bunch of grapes. Most species are aerobic or facultative anaerobes. They multiply fast in

most culture media at 37°C, but shows better pigment formation at temperatures between 20°C and 25°C. When cultured in solid media, *Staphylococcus aureus* colonies shapes in rounded, high and bright rugged forms with gold-yellow coloration.^{1,2} Most species are benign and have symbiotic relations with their hosts; however, some species can cause suppurative lesions, abscesses, and various pyogenic infections elsewhere in the body, and may even lead to fatal generalized infection.³ Specimens of the genus *Staphylococcus* are capable of producing

hemolysis, coagulating plasma, and producing extracellular enzymes and toxins, including a thermostable enteric toxin responsible for food contamination and causing food toxoinfection.^{4,5} It is frequently found in the nasopharyngeal of healthy adults in the condition of persistent hosts, with a higher incidence among hospitalized patients, health professionals and people with eczematous diseases. This microorganism has easy spread through the ambient, being responsible for several hospital infections. Staphylococcal infections, usually superficial and mild in most patients, can become serious in newborns, surgical patients, carcinomatous, diabetic and immune-compromised. The multidrug-resistant strains (MRSA) are more frequent in hospital environments.^{6,7}

Species of the genus *Staphylococcus* are found in soil, water and animal products such as cheese, meat and milk. They are often found on the surface of the skin and in their glands and in membranous areas of mammals and birds, besides the mouth, mammary glands and in the gastrointestinal tract, upper respiratory and urinary tract in humans.⁵ The genus is classified into two categories: coagulase positive and coagulase negative. Among the coagulase positive strains, *S. aureus* is the species most related to infections in humans, both in community as in hospital. Although sensitive to the action of several antimicrobials, there are strains with resistance to a large variety of them. For this reason, adequate antibiotic therapy should be based on the antimicrobial susceptibility test. The use of benzylpenicillin in the 40's of the last century temporarily solved the problem, but in the following years resistant samples were detected. The progressive spread of these strains decreased the therapeutic value of the antibiotics previously used. Currently, only a few strains continue to show sensitivity to penicillin.^{3,8,9}

The incidence and prevalence of *S. aureus* in a determined population depends on a myriad of factors. According to Sollid et al, geographic location, age, gender, ethnicity and body niches are variables that must be considered, and factors as bacterial genomics, host genetic determinants and clinical aspects impacts the frequency of the bacterium carriage.¹⁰ We consider that lifestyle and culture habits also influences in these aspects.

Considering the importance of *S. aureus* as a pathogen, the objective of this research is to investigate the prevalence of this bacterium in the nasal cavity in indigenous people of the Toba Qom ethnic group of the San Francisco de Asis community, in the city of Benjamín Aceval, and also test the sensitivity of the found strains to antimicrobials in this population.

METHODS

This research has a cross-sectional, descriptive and observational design, with a representative sample of the population of the Toba Qom ethnic group from the São

Francisco de Asis community, located in the city of Benjamín Aceval, in the Chaco region of Paraguay. Through random demand, volunteers of both genders, ranging from 1 to 71 years old, were participants in this research, which was carried out in September 2017. The adopted inclusion criteria for the sample universe: to belong to the ethnic Toba Qom, to be resident in the community of San Francisco de Asis, not to be a user of antibiotics, to be a volunteer to the research. The eligible participants group was formed by 141 people. From each volunteer, or from their parents in case of children, express authorization was requested for the material collection and for the use of the data for scientific dissemination without personal identification of each participant. Before permission, all participants were informed about the technique, the importance of the research, the disease and the purpose of the research. All procedures were implemented with respect to cultural diversity, national laws and the International Labour Organization Convention 169 on Indigenous and Tribal Peoples. Sterile cotton wool swabs were used to obtain the samples from the nasal cavities. Each collected sample was packed into tubes containing the Stuart transport medium, where it was kept until processing at the Microbiology Research Laboratory of UNIABEU University Center. The material was seeded in Brewer's medium and the obtained growth was spiked on blood-agar and agar-hypertonic-agar media and then incubated in a bacteriological oven at 37°C for 24 hours. The primary identification was made by the staining characteristics of the Gram method, catalase tests, hemolysis in blood-agar, mannitol fermentation test, coagulase test and deoxyribonuclease test. Sensitivity tests were performed by the agar diffusion technique according to the procedure standardized by the National Committee for Clinical Laboratory Standards.¹¹ Samples from the growth of *S. aureus* cultures were transplanted to 5 ml of the Brain-Heart-Infusion medium (HBI) for 5 hours. The suspension was prepared with density adjusted to 0.5 of McFarland's turbidity scale, which corresponds to the concentration of approximately 10⁸ Colony Forming Units/ml. With the use sterile swabs, the suspensions were seeded on the Mueller-Hinton agar surface in order to obtain confluent growth. Cefar brand discs impregnated with antibiotics were applied to the seeded surface and then the dishes were incubated at 37°C for 24 hours.¹² After the incubation period, the diameter of growth inhibition halos was measured. The following antibiotics were tested: ampicillin, vancomycin, penicillin, erythromycin, clindamycin, oxacillin, rifampicin, gentamicin, ciprofloxacin, nalidixic acid, norfloxacin, cefalotin, cefoclor, cephalixin, cefotaxime and sulfamethoxazole-trimethoprim. Samples which showed zones of inhibition with less than 10 mm were considered resistant, and the occurrence of any growth within the halo was interpreted as an evidence of a resistant subpopulation.

RESULTS

Out of 141 Toba Qom ethnic people examined 22 were colonized by *S. aureus*, with prevalence of 15.6%. No

multidrug resistant strains were isolated. All samples showed good sensitivity to most of the antibiotics tested,

except for nalidixic acid, which showed a resistance of 100% (Table 1).

Table 1: Strains of *S. aureus* isolated from the nasal secretion of healthy carriers among Toba Qom ethnic group of the San Francisco de Asis and antimicrobial susceptibility.

Tested antibiotics	Sensible strains	% sensible strains	Resistant strains	% Resistant strains
Ampicillin	20	90,90	2	9,10
Vancomycin	22	100	0	0
Penicillin	18	81,82	4	18,18
Erythromycin	17	77,27	5	22,73
Clindamycin	20	90,90	2	9,10
Oxacillin	22	100	0	0
Rifampicin	22	100	0	0
Gentamicin	22	100	0	0
Ciprofloxacin	22	100	0	0
Nalidixic acid	0	0	22	100
Norfloxacin	22	100	0	0
Cephalotin	22	100	0	0
Cefoclor	22	100	0	0
Cephalexin	22	100	0	0
Cefotaxime	21	95,45	1	4,55
Sulfamethoxazole-trimethoprim	20	90,90	2	9,10

DISCUSSION

This research followed the protocols of Konneman and Stephen and Trabulsi and Alterthum, who recommend the staining characterization of bacterial cells and the application of classical methods for the diagnosis and identification of the genus and species of these bacteria.^{3,13} Before the antibiotic era, *S. aureus* was already known as a pathogen, and is currently one of the most frequent bacteria causing hospital infections, a reason that indicates the need for rigorous prophylaxis measures. From an epidemiological and pathogenic point-of-view of these infections, healthy carriers are considered important links in the epidemiological chain, with a high prevalence and incidence variation among different groups.

Ayepola et al studied nasal carriers of *S. aureus* and associated risk factors of transmission among students of a university in Nigeria.¹⁴ Among the 227 students examined, 56.7% were colonized by *S. aureus*. Sensitivity tests showed high resistance to the following tested antibiotics: cloxacillin (91%), ceftazidime (71%), cotrimoxazole (23%), erythromycin (20%), oxacillin (16%). These high resistance rates were not recorded among strains isolated from the Toba Qom ethnic group, where all 22 isolated samples were susceptible to oxacillin and erythromycin. These authors considered that students with nasal colonization of *S. aureus* are important transmitters of this bacterium for people hospitalized in the Hospital of the University of Nigeria.

Russomando et al conducted a research of the bacterial flora of the oral cavity of children attended at a dental clinic in Paraguay.¹⁵ The sample consisted of 112 children aged from 2 to 5 years old. The collected material from the oral cavity was seeded in selective *Staphylococcus* culture medium. The isolated strains were resistant to several antibiotics: penicillin (89%), chloramphenicol (16.2%), oxacillin (10.8%), erythromycin (8%), clindamycin (8%), gentamycin (5.4%). Among the found strains, 4 presented resistance to multiple antibiotics and were carriers of the *mecA* gene. Our results with the Toba Qom population differ substantially from the results of these authors.

Odorizzi et al performed an epidemiological research on asymptomatic carriers of *S. aureus* in the nasal cavity among Xerente indigenous ethnic group in the city of Tocantínia, Province of Tocantins, Brazil.¹⁶ The researchers analyzed 122 samples from nasal cavities. Bacteria isolation and identification were performed by conventional methods. Among the 122 individuals examined, 15 (12.3%) were colonized by *S. aureus*. The authors did not found multidrug-resistant strains and all samples presented good sensitivity to most of the tested antibiotics, except for nalidixic acid, which had 100% resistance. Similar to our results, the strains found in the Xerente population were susceptible to most antibiotics, coinciding in the resistance to nalidixic acid, which strains had 100% resistance both in Xerente and Toba Qom population groups.

The investigators Antón et al searched for pathogenic bacteria in nasal secretion samples of 49 indigenous

children of indigenous Warao ethnic group, María López community, Province of Sucre, Venezuela.¹⁷ They isolated *S. aureus* in 9 (14.3%) children and all strains showed sensitivity to the tested antibiotics: cefotaxime, ciprofloxacin, gentamicin, vancomycin, penicillin, erythromycin, tetracycline, clindamycin and sulfamethoxazole-trimethoprim. Our results with 22 positive samples of Toba Qom revealed strains resistant to some of these tested antibiotics (Table 1), probably due to the contact with the non-indigenous population and the acquisition of varieties of this bacterium already resistant to these antimicrobials, fact that may be not occurred with the indigenous Warao ethnic group. The prevalence rate among Toba Qom (15.6%) was approximated to that recorded by the authors among the Warao (14.3%). We also agree with the authors' opinion that the colonization by *S. aureus* and other pathogens in the nasopharynx constitutes the normal flora at the moment of the research, but may represents future risks for the development of severe infections of the respiratory tract and in other sites of the organism.

Coimbra-Jr et al performed an epidemiological research on nasal carriers of *S. aureus* among indigenous populations of the Suruí and Karitiana ethnic groups in the Province of Rondônia, Brazil.¹⁸ Among the Suruí, these scientists found 26.3% of positivity, and among the Karitiana population, 36.2%. The antimicrobial tests revealed that most of the strains had sensitivity to most antibiotics, and no multidrug-resistant strains were isolated. The rates of carriers of the bacteria in question were notably higher than those found in our research with Toba Qom but, as well as among the Suruí and Karitiana, no multidrug-resistant strains were found in the population of the community of San Francisco de Asís.

The researchers Abraão et al studied the bacterial flora of the nasal and oral cavities, mainly *S. aureus* among 328 indigenous in communities located at the cities of Feijó and Mâncio-Lima, Province of Acre, Brazil.¹⁹ The results revealed that 55.8% inhabitants of these communities were colonized by *S. aureus*. The investigators concluded that the prevalence of MRSA at the Amazonian indigenous population should be around 0.6%. Our research with nasal material of the Toba Qom did not find any multidrug-resistant strain, and differs substantially from the results obtained by the researchers Abraão et al both in the prevalence rates as in the resistance profile of the *S. aureus* among nasal carriers.¹⁹ Geographic, environmental and cultural differences may explain this divergence.

Bastos et al investigated the bacterial flora of the nasal cavity of 259 indigenous people of Terena ethnic who inhabits the Moreira community at the city of Miranda, Province of Mato Grosso do Sul, Brazil.²⁰ The researchers found a prevalence of 12.4% positivity for *S. aureus*. The antibiotic sensitivity tests tested showed a high rate of resistance to penicillin (97%), ampicillin (97%) and erythromycin (25%). The authors considered

that this fact can be explained by the frequent prescription of these specific antibiotics by the health professionals who attend that community. They also found a strain of *S. aureus* (0.4%) with sensibility to all the tested antibiotics. This result contrasts with those verified by Kluitman et al, which pointed to a 5% penicillin resistance in the general population, as well as those verified in our research, where 4 (18.18%) of the 22 samples were resistant to penicillin.²¹ According to Bastos et al, the presence of *S. aureus* in healthy adult carriers highlights the possibility of the bacterium colonizing other sites of the organism.²⁰ We agree with this conclusion, considering that *S. aureus* carriers are more likely to become infected with the existing strains in their nostrils and to transmit them to other people. Prevalence rate approach to the result found among Toba Qom ethnic people, although resistance profile to antibiotics shows significant differences.

The biological characteristic of *S. aureus* among Australian aboriginal children was studied by Bowen et al.²² The antimicrobial tests of the strains isolated from the nasal cavity did not encountered multidrug-resistant strains, a fact also observed in our research with the same microorganism isolated from the nasal cavity of the Toba Qom indigenous people in the city of Benjamín Aceval, Paraguay.

Calderini et al considered infections caused by multidrug-resistant *S. aureus* (MRSA) at the present a worrisome health problem.²³ This bacterium can implant itself in several parts of the organism causing primary infections and occasionally can lead to the generalized infection. The authors' research was performed examining the nasal secretions from patients hospitalized at the Institute of Tropical Medicine in the city of Asunción, Paraguay. From the nasal cultures of 86 patients, 28 (38%) were positive for *S. aureus* and the analysis of sensitivity to antibiotics revealed that 50% of the samples showed resistance to oxacillin. In the results of our research with 22 *S. aureus* positive samples among 141 examined people, all strains were sensitive to most of the antibiotics (Table 1), including oxacillin, which sensitivity was 100%, thus demonstrating that the community of San Francisco de Asís is still free of multidrug-resistant strains of *S. aureus* already disseminated in the country.

A pilot study focusing the molecular characterization of *S. aureus* from 28 hemodialyzed patients at the Clinic Hospital of Paraguay was carried out by Isidre et al.²⁴ The frequency of nasal carriers in this group was 50% (14 positives), being 93% of the strains (13 samples) methicillin resistant and 57% (6 samples) gentamicin resistant. A worrisome 30% of the positive samples were multidrug-resistant strains. The periodical frequency that hemodialyzed patients attend to the hospital environment may explain why this population presents a high prevalence of multidrug-resistant *Staphylococcus* colonizing the nasal mucosa.

The prevalence of nasal carriers of *S. aureus* in children with physical disability and the resistance profile of this bacterium to antimicrobials was investigated by Queste et al in Asunción, Paraguay.²⁵ The researchers collected 80 nasal samples of children with physical disabilities attended at the National Bureau of Human Rights. The identification and sensitivity of the bacteria were investigated by conventional tests. 18 positive samples of *S. aureus* were found, equivalent to a prevalence rate of 22.5%. Among the 18 isolates, 4 were resistant to methicillin, therefore classified as MRSA. The presence of pathogenic bacteria at the nasopharyngeal level in the infant population represents a risk for the development of severe infections of the respiratory tract and other sites of the organism. Differently from the studies of Calderini et al, Isidro et al and Queste et al, we did not find multidrug-resistant strains among the Toba Qom indigenous people, which characterize a distinct resistance profile among the strains that affects this community face to other populations of Paraguay.²³⁻²⁵

According to Kluitmans et al and Chambers, several studies worldwide point to an average the prevalence of asymptomatic carriers of *S. aureus* in the general population around 37%, ranging from averages of 25% to 50%.^{21,26} A recent systematic review conducted by Sollid et al exemplify the heterogeneity of the nasal carriage prevalence rates: 23,1% in China, 41,7% to 57,8% in French Guyana, 29% in Gabon, 29,4% in India, 9,1% in Indonesia, 36,4% in Switzerland, 13% in Tunisia and 30,4% in United States.¹⁰ The results of our research among the Toba Qom ethnic group revealed the existence of 15.6% prevalence of nasal carriers of *S. aureus*, therefore below the international averages and also lower than the specific groups studied in Paraguay by Calderini et al, Isidro et al and Queste et al, whose results revealed prevalence rates reaching, respectively, 38%, 50% and 22.5%.²³⁻²⁵

CONCLUSION

S. aureus multidrug-resistant strains were not isolated from the nasal cavities of the indigenous people Toba Qom ethnic of the San Francisco de Asis community. All the *S. aureus* samples presented good sensitivity to the tested antibiotics, except for nalidixic acid, to which all samples were resistant. We consider that the low frequency of resistance of *S. aureus* to antibiotics in this specific population can be explained by cultural habits, as the infrequent use of antibiotics to the detriment of the use of traditional phytotherapeutic resources of the Toba Qom tradition.

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