

Asymptomatic Nasal Carriers of *Staphylococcus Aureus* Among Indigenous People of Xerente Ethnic, Tocantinia City, Province of Tocantins, Brazil

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ABSTRACT

Staphylococcus aureus are Gram positive cocci with ubiquitous presence in humans as a constituent of the normal flora of the skin and mucous membranes of most mammals, and can infect various sites of the body when conditions are favorable. Objective: Determine the prevalence of nasal carriers of *S. aureus* among Xerente ethnic group and determine the sensitivity profile for antimicrobials. Methods: 122 samples of nasal cavity were examined. The isolation and identification were carried by conventional methods such as microbial catalase test, coagulase, mannitol fermentation test and deoxyribonuclease. The antibiograms were performed by diffusion technique in agar according to the standard procedure by the National Committee for Clinical Laboratory Standards. Results: A total of 122 indigenous people were examined and 15 were colonized with *S. aureus*, which showed a prevalence of 12.3%. Conclusions: Multidrug-resistant strains were not isolated. All studied samples showed good sensitivity for most of the tested antibiotics except for Nalidixic Acid, which the strains demonstrated 100% of resistance.

Keywords: *Staphylococcus Aureus*, Nasal Carriers, Indigenous People

I. INTRODUCTION

Bacteria of the genus *Staphylococcus* are described as Gram-positive, immobile, non-spore-forming cocci which rounded cells, which measures approximately one micron in diameter. Under optical microscopy, they appear as single cells, in pairs or agglomerates, resembling a bunch of grapes. Most species are aerobic or facultative anaerobes. They multiply fast in most artificial culture media at a temperature of 37°C, but have better pigment formation, which is characteristic of them, at a temperature between 20°C and 25°C. When seeded in solid media, the colonies form rounded shapes and appear high and bright rugged,

yellow-gold-colored (Brooks et al., 2009; Levinson, 2010). Most species are benign or establish host-type symbiosis (Trabulsi and Alterthum, 2014), but some species can cause suppurations, abscesses and various pyogenic infections elsewhere in the human body, and even fatal sepsis. Species of the genus *Staphylococcus* are capable of producing hemolysis, plasma coagulation or produce extracellular enzymes and toxins. A thermostable enterotoxin produced by *S. aureus* is responsible for food contamination and causes food toxoinfection (Coura, 2013; Veronesi and Focaccia, 2015). It is frequently found in the nasopharyngeal region of healthy adults at the condition of persistent carriers (approximately 25%),

with a higher incidence among hospitalized patients, health-care professionals and people with eczematous diseases. Its dispersion is easy, being responsible for several hospital infections. Staphylococcal infections, usually superficial and mild for the most of the patients, may become serious in newborns, surgical patients, carcinomatous, diabetic and immunocompromised people. Multidrug-resistant strains (MRSA) are more common in hospital environments (Porth and Matfin, 2010; Norberg et al., 2002).

Species of the genus *Staphylococcus* are found in soil, water, and animal derived products such as cheese, meat and milk. Frequently inhabit the skin, skin glands and membranous regions of mammals and birds, being often found in the mouth, mammary glands and gastrointestinal tract, urinary tract and respiratory tract. Among healthy people, three models of interaction with *S. aureus* may be observed: persistent carrier, intermittent carrier and non-carrier (Veronesi and Focaccia, 2015). 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 human infections, both in community and hospital infections. Although sensitive to several antibiotics, there are strains with notorious resistance to most of them, reason why 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. Subsequently, resistant strains were reported. The progressive spread of this strain reduced the therapeutic value of the antibiotics used until then. Currently, only a few strains are sensitive to penicillin (Paiano and Bedendo, 2009; Murray et al, 2012; Trabulsi and Alterthum, 2014). These authors corroborated the indications affirming the high adaptability of these bacteria which become resistant to new antibiotics and demonstrated the positive correlation between antimicrobial use and the prevalence of resistant strains.

Considering the importance of this bacteria, this research has the objective of investigating the prevalence of *S. aureus* in the nasal cavity of individuals of the Xerente ethnic group and test the sensitivity of the bacteria to the antimicrobials in order to know the prevalence of *S. aureus* in this population, as well as determine the biological characteristics of this agent against antibiotics..

II. METHODS AND MATERIAL

Studied population characteristics

Indigenous peoples of the Xerente ethnic group have been in contact with Brazilian general society for more than two centuries, when they acquired, added and merged their traditional knowledge with the non-indigenous world culture without losing their identity, particular culture and linguistics (Moi, 2007). In this way, the Xerente people, in order to keep their native language in their culture, only speak Portuguese with people from outside the community. Many children have difficulty expressing themselves in Portuguese because the literacy process in the early ages is only on their native language, and only later Portuguese language is taught (Freire, 2013; Rocha et al., 2015). The indigenous people of the Xerente and Xavante ethnic groups belongs to the linguistic branch Macro-Jê, and call themselves Akwë, which means, according to the people themselves, "something that is above all things," "the most remarkable" or just "people". At the end of 19th century, the separation between them took place. The Xavante migrated to the Province of Mato Grosso and the Xerente remained on the banks of the Tocantins River (Rodrigues, 2014, quoted by Rocha et al., 2015). Currently the Xerente are classified as people of the Central-Jê linguistic branch, and are located in the city of Tocantinia, 76 km from Palmas, Province of Tocantins. They occupy two reserves: Porteira and Funil, where there are 60 villages and 3350 inhabitants are established, 1689 (50.42%) of the male gender and 1661 (49.58%) of the female gender (Rocha et al., 2015). This research was

guided by all ethical procedures, respect for cultural diversity, preservation of the health and integrity of the patient and maintenance of the anonymity, validated by the precepts of the Declarations of Helsinki of the 18th World Medical Assembly, ILO Convention 169 on Indigenous and Tribal Peoples and other regulations adopted by the Health Ministry of Brazil.

Design and study field

The research has a cross-sectional, descriptive and observational design, with a representative sample, carried out with Xerente indigenous people from Salto community, Tocantinia city, Province of Tocantins. Volunteers of both genders, with ages ranging from 1 to 72 years participated of this research by expontaneous demand after informed about the proceedings, importance of the research and, after the results, informed individually about the results and possible treatment.

Samples, instruments and laboratory proceedings

The research was performed in January 2016. The inclusion criteria to the sample universe was: to belong to the ethnic group Xerente, to be permanent resident in the Salto community, not to have attended a hospital environment, not to be user of antibiotics, to be a volunteer as a member of the research. The group was formed by 122 people aged from 1 to 72 years old. From each volunteer or those responsible, in the case of children, authorization was requested for the collection of material and the use of the data for scientific purposes without personal identification of the participant. The informed consent was after the explanation of the technique, the disease and its possible complications, and the importance of the research. Nasal secretions were collected with sterile swabs. Each collected sample unit was identified with the name, gender and age, and conditioned in tubes containing transport medium, where it was kept until processing in the Laboratory of Microbiology of the Uniabeu University Center. The material was sown in

Brewer's culture media, and then spiked to the Blood-agar and Mannitol-salt-agar media. The media were incubated into a bacteriological oven (37°C) for 24 hours. The primary identification was performed by the morphotinctorial characteristics, face to the Gram method, catalase tests, presence of hemolysis in blood-agar, fermentation of mannitol, test of coagulase in tube and deoxyribonuclease test.

The standard strain of *S. aureus* ATCC 95923 was used as a control pattern for the antimicrobial susceptibility test. The antibiograms were performed by the agar diffusion technique according to the procedure standardized by the National Committee for Clinical Laboratory Standards (NCCLS, 1993). From the growth of *S. aureus*, cultures were transferred to 5 mL of the Brain-Hearth-Infusion (BHI) medium for five hours. The suspension was prepared with density adjusted to 0.5 of McFarland's turbidity, which corresponds to the concentration of approximately 10^8 Colony Forming Units/mL. With the aid of sterile swabs, the suspensions were seeded on the surface of Mueller-Hinton-agar, in order to obtain confluent growth. Cefar brand disks impregnated with antibiotics were applied on the seeded surface; the plates were incubated at 37°C for 24 hours (Kloss and Bannermann, 1999). 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, Cephalotin, Cefoclor, Cephalexin, Cefotaxime. Samples with zones of inhibition with less than 10mm were considered resistant, and the occurrence of any growth within the halo was interpreted as evidence of a resistant subpopulation.

III. RESULTS AND DISCUSSION

The population was compound by 122 Xerente indigenous people examined, and 15 of them were

colonized by *Staphylococcus aureus*, corresponding to a prevalence of 12.3%. No multidrug resistant strains were isolated from the nasal cavities. All researched samples showed sensitivity to most of the tested antibiotics, except for Nalidixic Acid, which presented 100% resistance for all samples (Table 1).

Table 1. *Staphylococcus aureus* strains isolated from the nasal secretion of healthy carriers among Xerente natives and respective sensitivity to tested antibiotics.

Tested antibiotics	Sensible strains	% Sensible strains	Resistant strains	% Resistant strains
Ampicilin	12	80	3	20
Vancomycin	15	100	0	0
Penicilin	11	73,3	4	26,7
Eritromicin	9	60	6	40
Clindamicin	10	66,7	5	33,3
Oxacilin	12	80	3	20
Rifampicin	15	100	0	0
Gentamicin	15	100	0	0
Ciprofloxacin	15	100	0	0
Nalidixic acid	0	0	15	100
Norfloxacin	15	100	0	0
Cephalotin	15	100	0	0
Cefoclor	15	100	0	0
Cephalexin	15	100	0	0
Cefotaxime	13	86,7	2	13,3

The research was developed following the protocol of Koneman and Stephen (2001) and Levinson (2010), who used the morphofunctional characterization of bacterial cells and classic tests in the process of diagnosis and identification of the genus and species of the bacterium. *Staphylococcus aureus* was already

known as a pathogen before the antibiotic era, being one of the most frequent causes of hospital infections, a fact that indicates the importance of prophylactic measures. In the epidemiological and pathogenic view of these infections, healthy carriers are considered important links in the chain of transmission, and there is high variation about the prevalence and incidence among different groups, fact also verified in our research.

Epidemiological studies on nasal carriers of *S. aureus* were performed by Coimbra-Jr et al. (1985) among people of the Suruí and Karitiana ethnic groups in the Province of Rondonia. Among the Suruí they found 26.3% of positivity, and among the Karitiana population, 36.2%. The antimicrobial sensitivity tests used showed that the majority of the strains tested were sensitive to most antibiotics, and the researchers did not isolate multidrug resistant strains. The rates of *S. aureus* carriers were higher than those found in our research with Xerente people, where the prevalence of asymptomatic carriers was lower, but, as well as verified among the Suruí and the Karitiana, no multidrug resistant strains were found.

Anton et al. (2011) investigated pathogenic bacteria in samples of nasal secretion of 49 indigenous children of the Warao ethnic group, María Lopez community, in the city of Benítez, Province of Sucre, Venezuela. Among the bacterial species identified, *S. aureus* was found in nine (14.3%) of the children, and all the isolated strains showed sensitivity to the following antibiotics: Trimethoprim-Sulfamethoxazole, Clindamycin, Erythromycin, Tetracycline, Penicillin, Vancomycin, Ciprofloxacin, Gentamicin and Cefotaxime. Our results, with 15 positive samples of *S. aureus* isolated from the Xerente, showed resistance to some of the tested antibiotics, probably due to the frequent contact of the Xerente with the non-indigenous population, already carrying strains resistant to some of the tested antibiotics, and perhaps this fact do not occur with the indigenous Warao

ethnic group. The prevalence index found in our research was similar to that found in the study of the authors, with a positive result of 12.3% in nasal colonization. We also agree with the opinion of the authors that the colonization by *S. aureus* and other pathogens of the nasopharynx constitutes the normal flora at the moment, but represent risks for the development of severe infections of the respiratory tract and in other sites of the organism.

The bacterial flora of the nasal cavity of 259 indigenous people of the Terena ethnic group from Moreira community in the city of Miranda, Province of Mato Grosso do Sul, Brazil, was studied by Bastos et al. (2013). They found a positivity of 12.4% for *S. aureus*. Antibiotic sensitivity tests showed resistance to Penicillin (97%), Ampicillin (97%) and Erythromycin (25%). The authors considered that this fact can be explained by the frequent use of these antibiotics by the health agents who attend to that community. They also found a strain of *S. aureus* (0.4%) sensitive to all tested antibiotics. This result contrasts with the obtained by Kluytman et al. (1997), which indicated a 5% penicillin resistance in the general population, as well as those verified in our research, where 4 (26.7%) of the 15 positive samples were resistant to this antibiotic.

According to Bastos et al. (2013), the presence of *S. aureus* in healthy adult carriers highlights the possibility of this bacterium colonizing other sites of the organism. We agree with the cited authors, as we agree with Norberg et al. (2002) who considered that *S. aureus* carriers are more likely to infect with the existing strains in their nasal cavities and to transmit them to other people, especially in hospital environment.

Abraao et al. (2015) studied the bacterial flora of the nasal and oral cavity of 328 indigenous people living in Feijo and Mâncio Lima cities, in the Brazilian Amazonian Province of Acre. The results of the

research revealed that 55.8% of the population had *S. aureus*. They concluded that the prevalence of MRSA in the Amazonian indigenous population was 0.6%. Our research with nasal material of the Xerente revealed positivity of 12.3% of *S. aureus*, a rate significantly lower than that found by the authors, and we did not find any multidrug resistant strains.

Bowen et al. (2014) researched the biological characteristics of *S. aureus* isolated from 508 Australian aboriginal children. They performed antimicrobial susceptibility tests and did not detect multidrug resistant strains, as well as in our research with the same bacterium isolated from the nasal secretion of Xerente people in the Province of Tocantins.

Our research with the Xerente people revealed a significantly lower prevalence of *S. aureus* carriers when compared to the other studies conducted with indigenous people in other regions of the country. Coimbra-Jr et al. (1985) considers that inter-human contact is relevant and, beyond the contact with other to nasal carriers, other factors facilitate transmission among the traditional populations: the continuous use of clothing without frequent washing, clothing sharing among individuals, use of shared hammocks, the construction of traditional dwellings with insufficient ventilation, which make them damp and humid. The authors considered that these factors facilitates the survival of staphylococci and other pathogens outside the human body that constitute a source of contamination. Although these conditions were also found in the Xerente community, there is a lower prevalence rate among this studied group.

IV. CONCLUSION

No multidrug resistant strains of *S. aureus* were isolated from the nasal cavity of the Xerente people examined in the Salto community. All the isolated samples showed sensitivity to the antimicrobials tested, 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 population can be explained by the infrequent use of antibiotics to the detriment of the use of traditional phytotherapeutic resources of the Xerente culture.

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