

# Presence of *Staphylococcus aureus* in the Nasal Cavity of Children Attending a Public Daycare Center in Francisco Beltrão—Paraná—Brazil\*

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## ABSTRACT

The objective of this study was to evaluate the presence of *Staphylococcus aureus* in the nasal cavity of children attending a daycare center in the town of Francisco Beltrão—PR, by comparing the frequency between age groups and genders. Antimicrobial susceptibility testing with different drugs was also carried out in the bacterial isolates. A prospective study involving 200 children aged 2 and 4 years, grouped by gender, was carried out. Samples were collected from the nasal vestibules of children, incubated and identified. Antimicrobial susceptibility testing was carried out in 30% of the *S. aureus* colonies. The results showed that 35% of children (10% female and 25% male) were colonized by *S. aureus*. The frequency of colonization in both male age groups was similar, with  $201.93 \pm 13.10$  CFU/plate for 2-year-old and  $266 \pm 12.60$  CFU/plate for 4-year-old. However, colonization averages for females were different, with  $30.43 \pm 1.17$  CFU/plate for 2-year-old and  $394.38 \pm 10.70$  CFU/plate for 4-year-old. In the antimicrobial susceptibility testing, some strains were resistant to oxacillin, erythromycin and tetracycline, in addition to showing intermediate susceptibility to cephalothin.

**Keywords:** Nasal Cavity; Frequency; Susceptibility; Antimicrobials

## 1. Introduction

Humans interact with the many microorganisms, because they are a definitive and intermediate host of various species. The main interaction occurs with endogenous microorganisms, mostly bacteria of the normal human microbiota [1]. The human body represents several environmental niches for the bacteria, providing warmth, moisture and food necessary for their development.

One of the most important bacteria in the normal human microbiota is *Staphylococcus* sp., commonly transmitted from person to person through direct or indirect contact [2]. The transmission depends on a source (carriers or patients) and the rate of microorganisms released, as well as the microorganism survival ability, pathogenicity and frequency of contact between healthy and infected individuals [3].

*Staphylococcus* sp. are usually found in the human

skin and mucous membranes; however, the main species *Staphylococcus aureus* has been isolated from various body parts, including hands and mouth [4]. The nasal cavity is an ecosystem suitable for the growth and inter-relationship of this microorganism, showing colonization with different phagotypes [5]. The nostrils provide an environment in which *S. aureus* can propagate and remain for extended periods [6]. *S. aureus* has a prevalence of 20% to 40% in the nasal cavity of adults [4], but in children this percentage can reach 100% by the age of four and declines thereafter [7]. However, carriage is a long-term condition, with its incidence decreasing from the age of one, ranging between 10% and 40% for both immunocompromised and healthy children. Carriage is a transient condition variable for each patient [8], which plays a key role as a risk factor for infections in hemodialysis and surgery patients, as well as those with catheters, intravascular devices and infected by HIV [9].

This species is extremely important in the nasal cavity,

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because it can act as secondary microbiota and acquire pathogenic character, overcoming physical and chemical barriers, as well as the host's immune system, to cause infections [5]. *Staphylococcal* infections can be superficial, affecting the skin and subcutaneous tissue, or deep, which originate from the focus of the superficial infections [4]. The consequences of *S. aureus* infections can be serious [10], because the pathogen is considered the main cause of surgical and skin infections (pimples and boils), being also responsible for the toxic shock syndrome and severe illnesses, including pneumonia, meningitis, endocarditis and septicemia [11]. Furthermore, it is considered an important pathogen in children for commonly causing pharyngitis [12], because of its pathogenic mechanisms, such as the production of virulence factors.

Colonization by *S. aureus* is currently a concern due to the increasing number of carriers and colonized patients in the general population, as infections can result in additional complications, thus requiring antimicrobial therapy [13]. Children who attend daycare centers and other community care premises can carry strains resistant to multiple antimicrobials, which cause frequent infections in immune immature individuals [14].

However, this profile is most commonly found in hospitalized children, because the strains do not have their growth affected by the maximum level of antibacterial tolerated by the host [15]. The main resistance developed by *S. aureus* is to methicillin. This is currently becoming a serious problem because of the related high morbidity and mortality indices, as well as a high prevalence in community-acquired infections [16].

The microorganism has developed resistance to this beta-lactam antibiotic after having acquired resistance to penicillin. Methicillin-resistant strains, also known as MRSA, are not inhibited by any of the beta-lactam antibiotics [17]. This can be related to the presence of the protein PBP2 or  $\beta$ -lactamases, which are enzymes produced by *S. aureus* capable of hydrolyzing the beta-lactam ring of some antibiotics. The production of  $\beta$ -lactamases is mediated by plasmids, which confer resistance to such antibiotics by breaking the beta-lactam ring, and thus inactivating the drug [18].

In recent decades, MRSA has become a serious public health problem because of the threat of developing resistance to vancomycin, the only antimicrobial currently effective against MRSA. CA-MRSA or community-acquired MRSA is also a cause of concern, as it can cause fatal infections in individuals who are not in the risk-group for *S. aureus*, such as healthy children, teenagers and adults [19,20].

Therefore, the main concern lies with the healthy carrier, who is considered the most silent, yet the most dan-

gerous source of infection-causing microorganisms [21]. The pathogen can become a serious health problem especially for children living in collective environments, such as daycare and community centers, because transmission can occur by close contact, generating pathological events [12] due to the immature immune system of these individuals, consequently making them more susceptible to the bacteria [21].

However, it is suggested that in the absence of risk factors, colonization does not characterize a worse prognosis. Still, effective strategies to prevent transmission through nasal carriage are important measures against *S. aureus* infections [11]. It is necessary to highlight the value of identification and determination of this microorganism susceptibility to indicate the first-line empiric therapy to assist in choosing an effective alternative antimicrobial.

In this context, the present study aimed to evaluate the presence of *S. aureus* in the nasal cavity of 2 and 4-year-old children who attend a public daycare center in the town of Francisco Beltrão—PR.

## 2. Material and Methods

The study was carried out from May to July 2010, with 200 children divided into two groups. Group 1 had a total of 100 females (50 aged 2 years and 50 aged 4 years) whereas group 2 was formed by 100 males (50 aged 2 years and 50 aged 4 years). Children attended the Delfo Fregonese public daycare center in Francisco Beltrão-PR.

Inclusion criteria were children of both genders who regularly attended the daycare center, aged 2 and 4 years, with no obvious signs of airway infection.

A total of three collections were made from each group. Each sampling was performed in triplicate, generating 300 plates per group at each collection and, thus, totaling 900 plates per group. Sample collection was performed with a sterile swab, with 15-day intervals between collections. The material was collected from both nostrils of each individual, then inoculated onto Petri plates containing Baird Parker medium. The plates were incubated at 36°C for 48 h.

After incubation, colonies with *S. aureus* characteristics were stained by the Gram method and evaluated in micromorphological tests. Strains staining as gram-positive cocci were subjected to identification through the catalase and coagulase tests.

Colonies with *S. aureus* characteristic morphology were selected and suspended in a 0.85% NaCl solution to reach a 0.5 McFarland turbidity standard, corresponding to approximately  $1.5 \times 10^8$  CFU. Inoculation was then carried out on Mueller-Hinton agar (Merck) using a sterile swab. After, paper disks with the following antimicrobials were applied: oxacillin (1  $\mu$ g), ciprofloxacin (5

µg), erythromycin (15 µg), gentamicin (10 µg), cephalothin (30 µg) and vancomycin (30 µg). Plates were incubated at 35°C for 24 h. Susceptibility was determined by measuring the growth inhibition zone (mm). The results were interpreted according to the CLSI [22].

After the antimicrobial susceptibility testing, strains resistant to oxacillin were analyzed for the presence of β-lactamase through the iodometric method, which consists of iodine tapes that acquire a light coloring when reacting with the enzyme.

The obtained data underwent statistical evaluations following the Analysis of Variance (ANOVA) rules, the mean and standard deviation were determined and compared by the Tukey's test at 5% significance.

This study was conducted in compliance with the provisions of Resolution 196/96 of the National Committee for Ethics in Research at the Brazilian Ministry of Health, and it was approved by the Human Research Ethics Committee. It is noteworthy that the children involved did not suffer any kind of risk, and an informed term of consent was signed by the parents and the institution responsible.

### 3. Results and Discussion

It was found that 70 children were colonized by *S. aureus* strains, as follows: 30 children aged 2 years and 40 children aged 4 years, of which 20 were females and 50 males. Results related to the quantification of *S. aureus* found in the nasal cavity of these children are presented in **Table 1**.

**Table 1** shows that *S. aureus* frequency was different for females from both age groups, because 2-year-old had an average of 30.43 ± 11.17 CFU/plate, whereas 4-year-old had an average of 394.38 ± 10.70 CFU/plate, thus being considered the most colonized group, with significant differences between the two groups.

Both age groups showed different colonization frequencies for males: 2-year-old had an average of 201.93 ± 13.10 CFU/plate, whereas 4-year-old had an average 266 ± 12.60 CFU/plate.

The colonies isolated and identified as *S. aureus* were evaluated for antimicrobial resistance. Of the total colonies observed in the male and female groups, 30% were randomly selected: 10 and 118 colonies from the female group, aged 2 and 4 years, respectively; and 60 and 80 colonies from the male group, aged 2 and 4 years, respectively. The results are summarized in **Tables 2** and **3**.

**Table 2** shows a difference in antimicrobial susceptibility between the female age groups (Group 1). Erythromycin-resistant strains were found in three isolated colonies from the 2-year-old group. Remaining colonies from the same group were susceptible to other antimicrobials.

**Table 1. Comparison of *Staphylococcus aureus* CFU/plate across three samplings from both genders, aged 2 and 4 years.**

Females (Group 1)		Males (Group 2)	
2 years	4 years	2 years	4 years
30.43 ± 11.1d	394.38 ± 10.7a	201.93 ± 13.1c	266 ± 12.6b

Averages followed by the same letter horizontally do not differ by the Tukey's test at 5% significance.

**Table 2. Antimicrobial susceptibility testing in *S. aureus* strains isolated from the nasal cavity of females aged 2 and 4 years.**

Antimicrobials	Females					
	2 years 10 colonies			4 years 118 colonies		
	S	I	R	S	I	R
Oxacillin (1 µg)	10	-	-	86	20	12
Ciprofloxacin (5 µg)	10	-	-	118	-	-
Erythromycin (15 µg)	7	-	03	100	05	13
Gentamicin (10 µg)	10	-	-	118	-	-
Vancomycin (30 µg)	10	-	-	118	-	-
Cephalotin (30 µg)	10	-	-	92	16	10

Profiles; S = susceptible; I = intermediate; R = resistant.

However, the 4-year-old group had 12 oxacillin-resistant strains and 20 colonies of intermediate susceptibility, five erythromycin-resistant and 13 intermediate colonies, and 10 cephalothin-resistant and 16 intermediate colonies. The remaining colonies showed susceptibility to the other antimicrobials evaluated.

**Table 3** shows differences in antimicrobial susceptibility between the two male age groups, with 2-year-old having 5 erythromycin-resistant strains and 15 intermediaries, whereas the remaining colonies proved susceptible to the other antibiotics. However, the 4-year-old group had eight oxacillin-resistant colonies and 22 intermediate, eight erythromycin-resistant colonies and 12 intermediate, and two oxacillin-resistant colonies and 13 intermediate. The remaining colonies are susceptible to the other antibiotics.

It was verified that all *S. aureus* colonies (268) were susceptible to ciprofloxacin, gentamicin and vancomycin. The 20 colonies that showed resistance to oxacillin, from Groups 1 and 2, age Group 4 years, were tested for the production of β-lactamase, which resulted negative.

In recent years, there has been an increased concern with healthy nasal *S. aureus* carriers, especially children, regarding the high rate of infections and acquired resistance to this pathogen in the community. Considering the epidemiology, the profile of children in this study is similar to other studies conducted with the same age group, involving children who attended a community en-

**Table 3. Antimicrobial susceptibility testing in *S. aureus* strains isolated from the nasal cavity of males aged 2 and 4 years.**

Antimicrobials	Males					
	2 years 60 colonies			4 years 80 colonies		
	S	I	R	S	I	R
Oxacillin (1 µg)	60	-	-	50	22	08
Ciprofloxacin (5 µg)	60	-	-	80	-	-
Erythromycin (15 µg)	40	15	05	60	12	8
Gentamicin (10 µg)	60	-	-	70	-	-
Vancomycin (30 µg)	60	-	-	70	-	-
Cephalotin (30 µg)	60	-	-	65	13	02

Profiles; S = susceptible; I = intermediate; R = resistant.

vironment [14,23,24].

In the study, *S. aureus* nasal colonization was observed in 7 (35%) children. Other authors have reported higher incidence of *S. aureus* in the nasal cavity of healthy children [14,25]. The highest prevalence was found in males, being this predominance also common in other research with children [26] and adults [27,28].

The difference between the colonization average in samples collected from both groups is observed in **Table 1**. The data obtained show that 4-year-old had a higher frequency of colonization than 2-year-old. These inequalities can be attributed to characteristics, such as gender and age, as well as immunity, risk factors, genetics, among others.

Several authors have reported that antimicrobial resistance in *S. aureus* strains over the years as a worrying fact [19,20,29]. This argument could be confirmed through the susceptibility tests conducted in this research, as strains resistant to different antibiotics were found. According to the literature [30], this characteristic can be attributed mostly to the excessive and indiscriminate use of antimicrobials.

The results found in this work show that only colonies from 4-year-old, in both groups, were resistant to oxacillin. This profile was also observed in a study with healthy newborns [7] and in a research on community-acquired infections in children [24].

In a general analysis, the use of antibiotics like ciprofloxacin and gentamicin for infections caused by this organism in the community continue to be the most effective method, because of antimicrobial susceptibility found in both age groups and genders. Vancomycin is an alternative only when *S. aureus* strains are not susceptible to any other antimicrobial, often being used in hospital treatments.

The iodometric test performed on oxacillin-resistant strains for the assessment of  $\beta$ -lactamase resulted negative for the presence of this enzyme. Considering this

resistance pattern, it can be suggested that these strains have a different resistance mechanism, such as the production of PBP2 protein, which generates a low affinity for this beta-lactam antimicrobial [18].

The results obtained in this study can contribute in the creation of programs for the development of healthy children and strategies to prevent *S. aureus* transmission, considering the increased presence of community-acquired resistant strains.

#### 4. Conclusions

With the results obtained in this study, it was concluded that *S. aureus* strains are frequently found in the nasal cavity of children aged 2 to 4 years, both males and females. However, higher frequency is found among 4-year-old males.

Oxacillin-resistant *S. aureus* strains were only found among 4-year-old from both groups, but they were considered  $\beta$ -lactamase negative. All *S. aureus* strains from the 268 colonies evaluated were susceptible to ciprofloxacin, gentamicin and vancomycin.

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