NASAL CARRIAGE OF *STAPHYLOCOCCUS AUREUS* AND *CANDIDA* SPECIES IN IMMUNOCOMPETENT INDIVIDUALS

María L Nastri[1], Pedro Hecht[4], Natalia Nastri[1], Virginia Jewtuchowicz[1,2], Alicia Cuesta[1], Juliana Estrugo[3], Ariel Gualtieri[4], Daniel O. Sordelli[2], Alcira Rosa[1]

[1] *Department of Microbiology, School of Dentistry, University of Buenos Aires, Argentina*
[2] *Department of Microbiology, School of Medicine, University of Buenos Aires, Argentina*
[3] *Department of Periodontology, School of Dentistry, University of Buenos Aires, Argentina*
[4] *Department of Biophysics, School of Dentistry, University of Buenos Aires, Argentina*

ABSTRACT

The aim of this study was to analyze the prevalence of Staphylococcus aureus and Candida species in samples of nasal mucosa from 100 immunocompetent subjects of both sexes, aged 18-70 years, during stomatological clinical examination. Samples were taken from the mucosa of both nasal fossae using sterile swabs.Samples were observed fresh, stained with Gram and Giemsa, and cultured on selective differential media at 37ºC to isolate and identify the selected microorganisms; conventional biochemical tests and commercial equipment and molecular studies using PCR were performed.

A digital thermometer-hygrometer was used to measure room temperature at the time of sampling, which was on average 25±2º C, with relative ambient humidity 66±11%.

S. aureus was isolated from 38% of the samples; 4% of the samples were methicillin-resistant (MRSA) strains, with 2% identified genetically as community-acquired (CA-MRSA) and 2% as hospital-acquired (HA-MRSA).

Candida was identified in 23% of the samples, with prevalence of C. albicans (19%) followed by C. dubliniensis (3%) and C. krusei (1%). There was significant association between Candida and S. aureus (Chi-squared = 27.75; df = 1; (p<0.001). The nasal cavity is a reservoir and the identification of genus and species contributes to adequate epidemiological surveillance.

Key words*: Nasal cavity - Staphylococcus aureus - Candida.*

PORTACIÓN NASAL DE *STAPHYLOCOCCUS AUREUS* Y ESPECIES DE *CANDIDA* EN INDIVIDUOS INMUNOCOMPETENTES

RESUMEN

El propósito de este trabajo fue analizar la prevalencia de Staphylococcus aureus y especies de Candida en muestras de mucosa nasal de 100 individuos inmunocompetentes de ambos sexos con edades entre 18-70 años, durante el examen clínico estomatológico. Las muestras fueron obtenidas con hisopos estériles sobre la mucosa de ambas fosas nasales. Se realizó observación en fresco, tinción de Gram, Giemsa y cultivos en medios selectivos y diferenciales a 37ºC para el aislamiento e identificación de los microorganismos seleccionados, pruebas bioquímicas convencionales y equipos comerciales y estudios moleculares mediante la prueba de PCR.

Con un termo higrómetro digital se midió la temperatura ambiente cuyo promedio en el momento de la toma en el consultorio fue de 25±2 ºC y la humedad relativa ambiente fue del 66±11 %.

S aureus se aisló en el 38% de las muestras y dentro del mismo, 4% fueron meticilino resistentes (MRSA) siendo genéticamente 2 % de la Comunidad (MRSA-CA) y 2 % Hospitalarios (MRSA-HA). En el 23% de las muestras fue identificada Candida siendo la especie prevalente C. albicans: 19% y en menor proporción C. dubliniensis: 3% , C. krusei: 1%. Se registró una asociación significativa entre Candida y S. aureus (Chi-cuadrado= 27,75; gl= 1; (p< 0,001). La cavidad nasal constituye un reservorio y la identificación de género y especie contribuye a la adecuada vigilancia epidemiológica.

Palabras clave*: Cavidad nasal - Staphylococcus aureus - Candida.*

INTRODUCTION

Microorganisms in their reservoirs can act as a source of infection and spread.

The nasal fossae are an ecological niche that can be colonized by *S. aureus*, and asymptomatic *S. aureus* colonization is much more common than infection. Transmission occurs directly by contact with a carrier. Depending on the population studied, the carrier rate ranges from 25% to 50% in the general population and is higher in injection drug users, insulin-dependent patients, hemodialysis patients, patients with skin disorders, intravascular catheters or HIV/AIDS and healthcare providers[1-2]. Carriage plays a relevant role in the epidemiology and pathogenesis of infection. In healthy subjects, 20% are persistent carriers, 60% are intermittent carriers and 20% were never carriers[3].

The increasing multi-resistance of *S. aureus* to antibiotics is a major issue[1].

Staphylococcus aureus (Sa) is a bacterial species often isolated from patients both with hospital-acquired infections (HA-MRSA) and community-acquired infections (CA-MRSA). It has become one of the hospital and community pathogens of great epidemiological interest, and causes mild to life-threatening infections such endocarditis or septicemias. *S. aureus* in nasal carriers can also colonize other locations such as intact skin[4].

Our group carried out an analysis of MSRA carriage in a healthy population of patients with gingival-periodontal disease, unrelated to infected patients[5].

Infections of the paranasal sinuses (maxillary, sphenoid, ethmoid and frontal) and sinusitis due to *Candida* spp. may be due to translocation by which the fungus has propagated from the nasal cavity. They may also occur as a result of treatment with cytotoxic drugs or processes associated to leukemia, multiple myeloma and others[6].

Studies by Ponikau *et al.*[7] and Braun *et al.*[8] show high incidence of fungal colonization (91 to 96%) in subjects with chronic nasal sinus pathology, compared to previous studies which had established little relationship between these conditions.

The aim of this study was to determine the prevalence of *S. aureus* (Sa) and identify methicillin resistant species (MSRA) and *Candida* species (Ca) in samples of nasal mucosa from immunocompetent patients.

MATERIALS AND METHODS

Study population

The study was performed on samples of nasal mucosa from 100 immunocompetent adults of both sexes, aged 18 to 70 years, mean age 43.3±15.4 (54.9% female and 45.1% male) who received care at the School of Dentistry of Buenos Aires University during 2005-2009. Their evaluation included medical-dental history and clinical examination. Patient participation was voluntary and all participants signed informed consent after the purpose of the study had been explained to them.

Exclusion criteria

Pregnant women, subjects with systemic diseases who had taken antibiotics, non-steroidal anti-inflammatory drugs and/or corticoids or antifungal medication within six months prior to the sampling were excluded from the study.

Temperature and humidity measurement

A digital thermometer-hygrometer was used to record the temperature and humidity in the room where the nasal mucosa was sampled.

Sample collection

To prevent contamination, the outer area on the nostrils was rubbed with sterile gauze soaked in sterile distilled water, which was then discarded.

Nasal mucosa was sampled by rotating a sterile swab on the mucosa in both nasal fossae. The material collected was placed in a sterile tube with a stopper and sent to the laboratory within 2 hours.

Sample processing: Microbiological studies.

Microscopic studies were performed by: a) observation of fresh samples, b) smears stained using Gram and Giemsa techniques. The clinical samples were cultured on selective differential media to isolate the selected microorganisms, and incubated under specific atmospheric conditions. Quality control was performed previously on the culture mediums by using ATCC reference strains, following Cuesta[9]. Isolate phenotypes were identified by using conventional biochemical assays and commercial equipment to verify the presence of morphological and stain characteristics for the genus.

Staphylococcus species were identified by using mannitol salt agar and CHROMagar™ *Staph aureus* (Sa) (CHROMagar® Company, Paris, France) following Cuesta[9] Platzer [10] and Fowler[11].

Sensitivity to antimicrobials was tested according to NCCLS and CLSI (Clinical Laboratory Standards Institute).[12].

Molecular studies

The MRSA strains isolated were subjected to PCR (Polymerase Chain Reaction) in a thermal cycler (Mini Cycler™, MJ Research Inc.) using Taq polymerase from Invitrogen[13]. We investigated the mecA gene and LukF-PV and LukS PV genes, two contiguous genes co-transcribing and coding for Panton Valentine leukocidin (PVL), which represent one of the genetic markers associated to CA-MRSA[9].

The PCR products were analyzed by gel electrophoresis in 2% agarose with 0.5ug/ml ethidium bromide, following Jewtuchowicz[14] and Von Eiff[15] ATCC positive controls were used for the PCR of methicillin-resistant and PVL carrying strains, following Cuesta [9].

Candida was identified by using CHROMagar Candida® medium (CHROMagar Company, Paris, France). Yeast species were identified according to the color of colonies, following Odds.[16], observation of micromorphology in milk-Tween 80 1% agar and carbohydrate assimilation profile using commercial system Api ID 32D (â BioMérieux, France)[17 -18].

Statistical analysis

Frequency distribution and arithmetic mean were performed for the microorganisms analyzed. Standard deviation and 95% confidence interval for temperature and humidity, and Chi-squared (c^2) test for comparison of frequencies in contingency tables, with Yates correction for continuity (significance level $p< 0.05$).

RESULTS

Average room temperature during sampling was 25 ± 2ºC and relative humidity was 66 ± 11% (Table 1).

S. aureus was isolated from 38% of the samples; and 4% were methicillin-resistant (MRSA). Genetically, we identified 2 % community-acquired (CA-MRSA) and 2% hospital-acquired (HA-MRSA) (Fig.1). The four MRSA in the total sample were found in males.

Table 1: Descriptive statistical parameters for temperature and relative humidity

Parameter (sample size: N=100)	Room temperature (°C)	Relative humidity (%)
Mean	25	66
Standard deviation (SD)	2	11
95% confidence interval of observations (CI95%)	(21; 29)	(44; 88)
Standard error (SE)	0.2	1.1
Maximum	29	90
Minimum	18	48
Median	25	60

Table 2: Contingency of *Staphylococcus aureus* and *Candida* spp

		Presence of *Staphylococcus aureus*	
		Positive	Negative
Presence of *Candida* spp	Positive	20	3
	Negative	18	59

X^2 (Chi-squared test): p<0.001

Candida was identified in 23% of the samples. The prevalent species were *C. albicans* (19%), *C. dubliniensis* (3%) and *C. krusei* (1%) (Fig 2).

Significant association was found between *Candida* and *S. aureus* (Chi-squared = 27.75; df = 1; (p<0.001) (Table 2).

DISCUSSION

The appearance of new species related to human health calls for the development of simple, quick, efficient methods for their identification at clinical microbiology laboratories.

The relationship between colonization and infection has not been fully elucidated, although it has been associated to intrinsic factors of the host as well as factors of the microorganism. There is controversy regarding whether *S. aureus* is considered normal microbiota, as some authors have studied

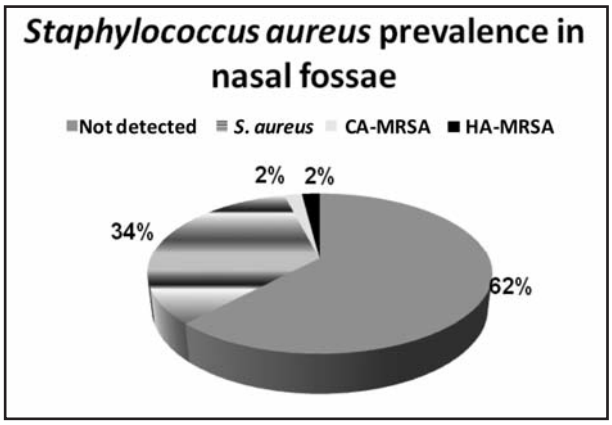

Fig. 1: Distribution of Staphylococcus aureus and methicillin-resistant Staphylococcus aureus (MRSA) and its genotypic detection as CA-MRSA (community acquired) and HA-MRSA (hospital-acquired), isolated from nasal fossae.

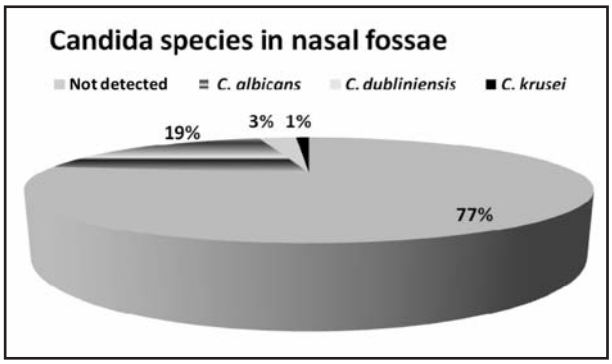

Fig. 2: Distribution of Candida species isolated from nasal fossae.

the correlation between *S. aureus* nasal carriage and the presence of *S. aureus* bacteremia, having found clonality between the two strains (nasal and blood) in 80% of the cases, whereby nasal carriage of *S. aureus* might be considered as a factor associated to bacteremia[15].

Moreover, the nasal colonization rate by *S. aureus* varies according to the population studied. In healthy adults, three types of nasal carriage can be distinguished, associated to host and microorganism factors. These are: a) intermittent carriers (60% of individuals), b) persistent carriers (20%) and c) non-carriers (20%).

Longitudinal studies have shown that colonization is transient rather than permanent in a given individual, and may change over time[19,20].

Archer[1] reports that there is nasal colonization by *Staphylococcus aureus* in 20%-30% of the general population and it does not imply the presence of true infection.

Kuehnert[21] analyzed *S. aureus* nasal colonization and MRSA in 9622 persons one year of age or older as part of a National Health and Nutrition Examination Survey, 2001-2002 in USA, and found that estimated prevalence and colonization of *S. aureus* and MRSA were 32.4% and 0.8%, respectively, and may vary according to demographics and the organism.

The same group[22] analyzed nasal colonization by *S. aureus* from 2001 to 2004 and found that the prevalence of *S. aureus* colonization decreased from 32.4% in 2001-2002 to 28.6% in 2003-2004 ($P < .01$), while the prevalence of colonization by MRSA increased from 0.8% to 1.5% ($p < .05$), concluding that nasal colonization by MRSA has increased in the United States, despite an overall reduction in nasal colonization by *S. aureus.*

Our study found higher *Staphylococcus aureus* and MRSA prevalence than Kuehnert[21] and Gorwitz [22]. Platzer [10] detected lower proportions of *Staphylococcus aureus* nasal carriage (22.7 %) in a healthy population in the city Santiago de Chile.

Further studies should be undertaken in larger numbers of patients, and there should be longitudinal studies to analyze nasal colonization by *S. aureus* and MRSA, to ascertain whether they are persistent or intermittent carriers, as suggested by Kluytman[3] and epidemiological surveillance of community transmission should be performed.

Candida infection is considered endogenous, caused by commensal species previously present in the subject, with *C. albicans* being the species with highest incidence. These species should be defined as opportunistic within the general concept of host-parasite relations[23-25].

Colonization by the genus *Candida* in humans usually remains as such due to the different defense mechanisms of the human body against any agent. The intervention of non-specific natural barriers, such as external barriers (skin and mucosa) is considered important[16].

We agree with Torres-Rodriguez[26] regarding the use of cultures in chromogenic media for the primary isolation of *Candida*, because it facilitates species identification.

Ponikau et al.[7] and Marple[27] both report that nasal sinus fungal colonization is often sub-diagnosed, and they have created consensuses based on applying diagnostic methods with the aim of detecting fungi and determining their role in the etiopathogenesis of nasal sinus diseases. A range of precise diagnostic methods is currently available for identifying the fungi. However, culture in specific media is the standard of choice for the purpose[7,27]. Our study used these media for identifying Candida species.

The oral cavity has been proved to be a reservoir and source of dissemination to other parts of the body and a source of transmission to other persons, foods and objects[26].

Nasal fossae can also be an ecological niche for colonization by the genus Candida. It is necessary to identify not only the genus but also the species in the nasal fossae for proper epidemiological surveillance, as they are reservoirs of opportunistic microorganisms[27].

Our study found *Candida albicans* 19%, C. *dubliniensis 3%,* and *C.krusei* 1% in nasal mucosa, and did not identify C. *parapsilosis.* However, according to reports published by certain European currents, it is not possible to specify whether it is only colonization by co-habitation or whether the presence of these microorganisms is a determining factor of chronic disease[8, 28].

We agree with Rodero[29] that it is not surprising to isolate *C. albicans,* since it is the yeast most often associated to colonization of skin and gastrointestinal tract. Nevertheless, in recent decades, there has been a change in the spectrum of infections by yeasts and an increase in the appearance of other non-*C. albicans* yeasts.

The results of the study by Martin[30] on microorganisms causing sepsis in the United States during the past twenty years show that the incidence of Gram-positive bacteria has increased more than that of Gram-negative bacteria, and fungal infections have increased exponentially.

Our study detected a significant association between the presence of *Candida* and *Staphylococcus aureus* in nasal mucosa. Noverr[4] and Peters [31] suggest that *Candida albicans* acts as a scaffolding for *Staphylococcus aureus*, forming microcolonies and polymicrobial biofilm. Further studies are needed to interpret this association.

Temperature and moisture were recorded at the room where nasal mucosa was sampled in order to detect possible exogenous variables in the transportation and isolation of the microorganisms that were found. Further studies on larger numbers of patients will shed light the matter.

It would be interesting to perform epidemiological studies in different geographical areas across Argentina in order to carry out surveillance of nasal carriage of these pathogens and the way they relate to other human pathologies.

CONCLUSION

The following were recorded for nasal mucosa samples from immunocompetent patients:

- 38% nasal carriage of *Staphylococcus aureus*, which included 4% genetically identified as community-acquired methicillin-resistant strains (CA-MRSA) and hospital-acquired methicillin-resistant strains (HA-MRSA);
- the most frequently identified Candida species was *C. albicans*, followed by *C. dubliniensis* and *C. krusei;*
- a significant association between *Staphylococcus aureus* and *Candida.*

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CORRESPONDENCE

Dra. Alcira Cristina ROSA
Cátedra de Microbiología y Parasitología
Facultad de Odontología - Universidad de Buenos Aires
M.T. de Alvear 2142 - (C1122AAH) Buenos Aires, Argentina
E-mail: flianastri@yahoo.com

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