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PRESENCE OF *STAPHYLOCOCCUS* SPP. AND *CANDIDA* SPP. IN THE HUMAN ORAL CAVITY

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ABSTRACT

The presence of yeasts and staphylococci in the oral cavity is important because they can act as supplementary microbiota and in certain situations can cause oral or systemic diseases. The aim of this work was to study the prevalence of *Candida* spp. and *Staphylococcus* spp. in the human oral cavity. Oral rinses were collected from sixty-eight individuals according to the technique described by Samaranayake and MacFarlane and then cultured on Sabouraud medium supplemented with chloramphenicol and Baird-Parker agar. After the incubation period, the microorganisms were isolated and identified through biochemical tests. The data obtained were statistically analysed by ANOVA. *Candida* spp. were isolated from 61.76% of the examined individuals and *C. albicans* was the more frequently isolated specie. *Staphylococcus* spp. were isolated from 95.60% of the individuals and 41 strains were coagulase negative (63%). Among the coagulase positive strains, nine were *S. aureus*, 11 *S. hyicus* and 4 *S. schleiferi* subspecie *coagulans*. No correlation was observed between the counts (cfu) of the isolated *Candida* spp. and *Staphylococcus* spp.

Key words: *Staphylococcus*, *Candida*, phospholipase, proteinase, killer system, oral cavity.

INTRODUCTION

Candida spp. and *Staphylococcus* spp. are usual in the human oral microbiota. However, in some situations, as in patients with periodontitis under systemic administration of penicillin and erythromycin, they can act as opportunistic microorganims and produce superinfection (7).

C. albicans is considered the main etiologic agent of candidosis, an opportunistic infection, related with local and systemic predisposing factors (2,4,15). Candidosis is commonly found in the palate of total denture users, median rhomboid glossitis, immunocompromised patients and those in treatment with antibiotics (3,26). *Staphylococcus aureus* is an important human pathogen and produce several diseases, including fatal systemic and opportunistic diseases and urinary tract infections (28). Other species of *Staphylococcus* are also related to several opportunistic infections in man and other animals.

The aim of this work was to study the prevalence of *Candida* spp. and *Staphylococcus* spp. in the human oral cavity, and to identify the species of *Candida* and coagulase positive *Staphylococcus*. Proteinase and phospholipase activities evaluation and the classification of *Candida* spp. strains through killer system were also performed.

MATERIALS AND METHODS

Sixty-eight individuals aged between 25 to 55 years old (34.45 ± 7.93), from the dental clinics of the School of Dentistry São José dos Campos/UNESP, were studied. They were informed about the aim of the research and about the oral rinses collection. All the patients signed an authorization letter. This project was submitted and approved by the Bioethic Commitee of the School of Dentistry São José dos Campos/UNESP, SP, Brazil. Each patient was examined for just one examiner.

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Individuals included those that did not present caries lesions or periodontal diseases and also those without orthodontics appliances and total or partial dentures. These patients did not relate any systemic disease or use of antibiotics during the six months that preceeded the collection of the oral rinses.

Oral rinses (60 seconds) were collected in 10 ml of sterile phosphate-buffered saline (PBS, 0.1M, pH 7.2) supplied in universal containers. In a maximum period of 3 hours after sampling, oral rinses were centrifuged at 3.000 xg and the supernatant was discharged. The final suspension was obtained by resuspending the deposit in 2.5 ml of PBS (0.1M, pH 7.2). Then, aliquotes of 0.1 ml of this sample were cultured in duplicates on Sabouraud agar (Difco, Detroit, USA) supplemented with chloramphenicol (0.1mg/ml) and on Baird-Parker agar (Difco, Detroid, USA). Plates were incubated at 37ºC for 48 hours. The samples were also cultured on CHROMagar *Candida* (CHROMagar, Paris, France).

After the incubation period, the cfu (colony forming units) were counted. Microscopic confirmation was performed from each plate. Five yeasts colonies were subcultured on Sabouraud agar and five colonies of staphylococci were subcultured on TS agar (Triptic Soy agar, Difco, Detroit, USA).

The yeasts strains were identified by growth on CHROMagar and by germ tube test, hyphae/pseudohyphae and chlamidospores growth, carbohydrate fermentation and assimilation according to Samaranayake and MacFarlane (26) and Sandvén (27). After the identification, proteinase and phospholipase activities were evaluated. Samples were also classified according to killer system (22).

Phospholipase production was evaluated according to Price *et al.* (23). Proteinase production test was performed according to Rüchel *et al.* (24).

Proteinase and phospholipase activities were determined by measuring the colony and the precipitation zone according to Price *et al.* (23).

Staphylococci isolates were identified biochemically through coagulase, Voges Proskauer, D-threalose fermentation and beta-galactosidase tests according to Forbes *et al.* (5). *S. aureus* ATCC 6538 and *E. coli* ATCC 8739 beta-galactosidase positive were used as positive control.

The data were analysed statistically using ANOVA. Differences were considered statistically significant when $p \leq 0.05$.

RESULTS

The distribution of the patients positive to *Candida* spp. and *Staphylococcus* spp. according to their age is presented in Table 1.

Candida spp. were isolated from 42 individuals (61.76%). *C. albicans* was the most frequently isolated specie, followed by *C. tropicalis* (20.42%), *C. glabrata* (6.12%) and *C. kefyr* (2.04%) (Table 2).

Staphylococcus spp. were isolated from 65 individuals (95.60%). Among these isolates 41 (63%) were coagulase negative. From the 24 coagulase positive isolates nine were *S. aureus*, 11 *S. hyicus* and 4 *S. schleiferi* subspecie *coagulans* (Table 3).

No correlation (R^2=1.2%) between the cfu of *Candida* spp. and *Staphylococcus* spp. was observed (Fig. 1).

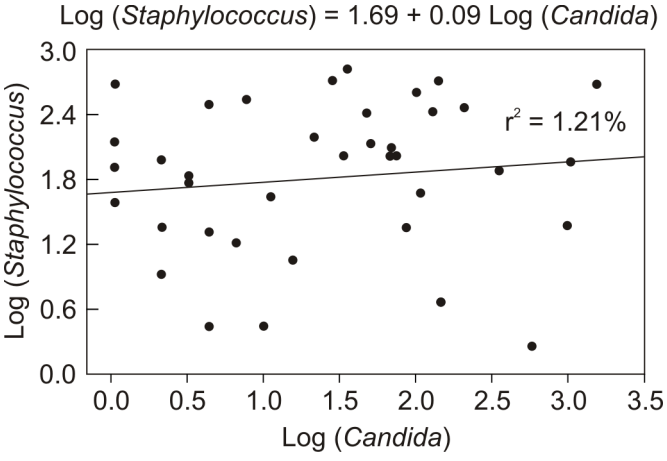

Figure 1. Dispersion diagram, regression line and correlation coeficient between the logarithm of cfu values of *Staphylococcus* and *Candida* genus isolated from human oral rinses.
Log = logarithm cfu (logarithm colonies forming units).

Table 1. Distribution of the patients positive to *Candida* spp. and *Staphylococus* spp. according to their ages.

Age	Total of patients (n)	Number and % *Candida* spp. positive patients in relation to the total of patients	% of *Candida* ssp. positive patients according to the ages	Number and % *Staphylococcus* spp. positive patients in relation to the total of patients	% of *Staphylococcus* ssp. positive patients according to the ages
25-35	45	29 (42.65%)	64.44	43 (63.24%)	95.56
36-45	20	10 (14.70%)	50	19 (27.94%)	95
46-55	3	3 (4.41%)	100	3 (4.41%)	100
Total of patients	68	42 (61.66%)		65 (95.59%)	

Table 2. Number (n) and percentage (%) of the species of *Candida* isolated from the studied individuals.

Species	n	%
C. albicans	30	61.22
C. tropicalis	10	20.42
C. glabrata	03	6.12
C. kefyr	01	2.04
Candida spp.	05	10.20
Total of species	49	100

Table 3. Number (n) and percentage (%) of the species of *Staphylococcus* isolated from the studied individuals.

Species	n	%
S. aureus	09	13.9
S. hyicus	11	17.0
*S. schleiferi***	04	6.1
Staphylococcus spp.*	41	63.0
Total	65	100

* Coagulase negative; ** subspecie *coagulans*.

Table 4. Frequency of killer biotypes and enzymatic activity of the *Candida* spp. isolates studied.

Species	Biotypes killer	n	Phospholipase activity			Proteinase activity		
			1	2	3	1	2	3
C. albicans	111	18	0	8	10	0	5	13
	114	03	2	0	1	0	1	2
	116	01	0	0	1	0	1	0
	411	03	1	0	2	0	0	3
	443	01	0	0	1	0	0	1
	888	04	1	1	2	1	1	2
Total		30	4	9	17	1	8	21
C. tropicalis	111	05	1	2	2	1	3	2
	114	03	1	1	1	1	1	1
	888	02	1	0	1	1	0	1
Total		10	4	3	4	3	4	4
C. glabrata	111	02	0	1	1	0	2	0
	114	01	0	0	1	1	0	0
Total		03	0	1	2	1	2	0
C. kefyr	111	01	1	0	0	1	0	0
Candida spp.	111	01	4	0	0	0	1	0
	114	04	1	0	0	4	0	0
Total		05	5	0	0	4	1	0
Total of species		49						

Enzymatic activities: 1= negative, 2 = positive, 3 = strongly positive.

The most frequently isolated killer biotype observed among the studied strains was the 111 (60% of the strains) followed by the biotype 114 (10%) (Table 4). Eighteen (60%) of the *C. albicans* isolates produced phospholipase and 18 (60%) showed proteinase activity. *C. kefyr* strain did not produce phospholipase and proteinase.

Among the 68 individuals, 39 (57.35%) presented both studied microorganisms in the oral cavity. Five individuals (7.14%) presented *C. albicans* and *S. aureus* in the oral rinses but no correlation between the ufc of these species was observed.

DISCUSSION

Öhman and Jontell (16) observed an association between *C. albicans* and *S. aureus* in a case of angular cheilitis. In the present study, no correlation between the presence of the two genus was observed. However, in this study no individuals presented angular cheilitis or other oral lesions suggesting candidosis or staphylococci infection.

The literature describes frequent colonization of the oral cavity in healthy individuals without lesions, varying from 20% to 55% and in some cases 80% (2,3,6,9). In the present study, 71.42% of the individuals presented *Candida* and these data are in accordance with previous studies.

From the 49 strains isolated, 30 (61.22%) were *C. albicans*, followed by *C. tropicalis* (20.42%), *C. glabrata* (6.12%) and *C. kefyr* (2.04%). Jorge *et al.* (8) reported the presence of *C. albicans* in saliva of 27.6% of the 428 studied individuals (17 to 23 years old) and in 33.1% of children from 3 to 14 years old. In the present study, the number of individuals with *Candida* spp. was higher in relation to all the groups described by these authors. The highest percentage of *Candida* spp. isolation in this study can be mainly related to the collection method used in this study (oral rinses).

C. tropicalis can cause invasive and nosocomial candidosis (14) and is considered an opportunistic pathogen in immunocompromised patients (6). *C. tropicalis* can be found in routine cultures from the nose, throat, skin, vagina and digestive tract (10). In this work, *C. tropicalis* was the second most frequently isolated specie. Paula *et al.* (19) reported that 16% of the isolates from the oral cavity of patients with cancer during radiotherapy belonging to the genus *Candida* were identified as *C. tropicalis.* This result is similar to the value obtained in this study (20.42%), despite the absence of such predisposing factor among the patients. Three strains (6.12%) were identified as *C. glabrata*. According to Larone (12), approximately 7% of the oral isolates are *C. glabrata*. *C. glabrata* is also related with endocarditis and systemic fungemia (10).

Among the studied isolates only one (2.04%) was identified as *C. kefyr*. Five strains isolated from the oral rinses could not be identified through biochemical tests and were considered *Candida* spp.

Among the *Candida* spp. virulence factors, proteinase and phospholipase activities have been extensively studied as they can play an important role in the pathogenesis of the candidosis. The determination of the enzymatic activity of *C. albicans* and other species isolated from different anatomic sites was observed by several authors (17,19,21,24). These studies showed a variation from 60 to 100% of isolates with proteinase activity and 50 to 100% for phospholipase activity. The results of the present work are in accordance with the percentages, since 60% of *C. albicans* strains produced phospholipase or presented proteinase activity.

Almeida (1) reported the production of phospholipase by 20% of *C. guilliermondii* strains. According to this author, *C. krusei*, *C. parapsilosis* and *C. tropicalis* did not produce phospholipase. In this study, among the 10 isolates of *C. tropicalis*, four presented strong phospholipase activity.

In the present study, six different killer biotypes could be obtained (111, 114, 116, 411, 443, 888). The biotype 111 was the most frequent. Polonelli *et al.* (23) obtained 25 different biotypes among 100 strains, and the biotype 111 was also the most prevalent. Magaró *et al.* (13) related 3 different killer biotypes (111, 214 and 411).

In this work, six strains were classified as the killer biotype 888. This biotype is resistant to all the killer proteins. These data are very useful and interesting for epidemiological purposes as this biotype was isolated from the same institution and anatomical site.

Several studies reported that the anterior nares are the most frequent site where *Staphylococcus* spp. can be found (18). According to Suzuki *et al.* (28), the oral cavity can be a reservoir of *S. aureus*. Knighton (09) studied the presence of coagulase-positive staphylococci in the oral cavity and nose in Dental students and detected this microorganism in the saliva of 47.50% of the individuals and in the nasal fossae of 41.7% of them. According to this author, this correlation suggests that pathogenic staphylococci reservoir in the oral cavity are as important as those in the nasal fossae. Piochi and Zelante (20) detected *S. aureus* in 35% of the salivary samples and emphasized the importance of the oral cavity as a reservoir of pathogenic staphylococci. *S. aureus* was isolated from 13.9% of the oral rinses in this study.

The isolation of *S. hyicus* (17%) and *S. schleiferi* subspecie *coagulans* (6.1%) was interesting, since there is no study in the literature describing the presence of these species in the human oral cavity.

Coagulase-negative staphylococci were found in 63% of the oral cavities. Although, being considered microorganisms with little clinical importance for many years, nowadays they are associated to several human diseases (11), such as urinary infections, endocarditis, cardiac valvula infections, osteomyelitis, endolphtalmitis and nosocomial infections (25). In the last ten years, the incidence of bacteremia caused by coagulase-negative staphylococci has increased dramatically. Approximately half of the deaths associated with bacteremia are caused by coagulase-negative staphylococci.

Candida spp. and *Staphylococcus* spp. have been increasingly reported as potential opportunistic pathogens, involved in superinfection cases. In this way, more studies on their virulence, pathogenicity and correlation with other pathogens are very important.

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RESUMO

Presença de *Staphylococcus* spp. e *Candida* spp. na cavidade bucal humana

A presença de leveduras do gênero *Candida* e *Staphylococcus* na cavidade bucal humana é de extrema importância, pois podem atuar como microbiota suplementar e em determinadas situações causar doença bucal ou sistêmica. O objetivo do presente trabalho foi estudar a prevalência de *Candida* spp. e *Staphylococcus* spp. na cavidade bucal humana. Enxagüe bucal foi coletado de 68 indivíduos segundo a técnica proposta por Samaranayake e MacFarlane e a seguir semeados em ágar Sabouraud dextrose com cloranfenicol e ágar Baird-Parker. Após crescimento, os microrganismos foram isolados e identificados através de provas bioquímicas. Os dados foram analisados através de análise de variância (ANOVA). Leveduras do gênero *Candida* foram encontradas em 61,76% dos indivíduos examinados, sendo *C. albicans* a mais frequentemente isolada. *Staphylococcus* spp. foram isolados em 95,60% das cavidades bucais, sendo 41 cepas (63%) coagulase-negativas. Das cepas coagulase-positivas, nove eram *S. aureus*, 11 *S. hyicus*, e quatro *S. schleiferi* subespécie *coagulans*. Não foi observada correlação entre as contagens (UFC) de *Candida* spp. e *Staphylococcus* spp. encontradas nos enxagües bucais dos indivíduos examinados.

Palavras-chave: *Staphylococcus*, *Candida*, fosfolipase, proteinase, sistema *killer*, cavidade bucal.

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