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Prevalence of yeast species in the oral cavity and its relationship to dental caries

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ABSTRACT. The purpose of this study was to evaluate the frequency of yeasts in the oral cavity, and to determine the main yeast species present and whether there is a correlation between the presence of yeasts in the mouth and caries or dental plaque. Ninety-five healthy children and adolescents who were residents of rural villages participated in the study. They were submitted to an odontological examination for the identification of dental caries and dental plaque, as well as for yeast culture and identification. The frequency of yeasts was 33.7%, with no difference between females and males. Yeasts predominated in children (37.7%) more than in adolescents (26.5%). Caries and dental plaque were present in 70.3 and 96.8% of the sample, respectively, and *Candida albicans* was the most prevalent species (75%). The findings indicated a correlation between the presence of caries and yeasts in the mouth ($p = 0.0087$) and between yeasts and the number of carious teeth ($p < 0.05$). However, there was no correlation between yeasts and the dental plaque index ($p = 0.49$).

Keywords: yeasts, dental caries, *Candida albicans*.

RESUMO. Prevalência de leveduras na cavidade bucal e sua relação com a cárie dental. O objetivo deste estudo foi avaliar a frequência de leveduras na cavidade bucal, determinar as principais espécies de leveduras presentes e, se existe correlação entre a presença de leveduras na boca com cárie ou placa dental. Participaram deste estudo 95 crianças e adolescentes saudáveis que residiam em Vilas Rurais, na região Oeste do Estado do Paraná. Eles foram submetidos a exame odontológico para identificação de cárie e placa dental, além de pesquisa de levedura na cavidade bucal. A frequência de leveduras foi 33,7% e não houve diferença entre os gêneros femininos e masculinos. As leveduras predominaram nas crianças (37,7%) em relação aos adolescentes (26,5%). Cárie e placa dental estavam presentes, respectivamente, em 70,3 e 96,8% da amostra e *Candida albicans* foi à espécie mais prevalente (75%). Os resultados mostraram correlação entre a presença de cárie e de levedura na boca ($p = 0,0087$) e também entre a presença de leveduras e o número de dentes cariados ($p < 0,05$). Por outro lado, não houve correlação entre leveduras e índice de placa dental ($p = 0,49$).

Palavras-chave: leveduras, cárie dentária, *Candida albicans*.

Introduction

Different species of yeasts can colonize the oral mucosa under well-balanced ecosystem conditions. However, alterations in this equilibrium transform these microorganisms into infectious agents, expressing virulence factors and invading tissues (FANELLO et al., 2006; NAVARRO-GARCIA et al., 2001). The incidence of fungal infections has increased in recent decades in many countries, which has stimulated scientific studies.

In relation to oral infections, in addition to stomatitis, fungi have been found in different situations such as root canal infections, especially in the root canals of obdurate teeth in which treatment

has failed. *C. albicans* has been associated with cases of persistent root canal infections, because this yeast can be resistant to some intracanal medications (SIQUEIRA-JÚNIOR; SEN, 2004).

Dental caries is a chronic and multifactorial disease that, although avoidable, still represents an important problem in public health, since it affects approximately 90% of the population, mainly children and adolescents, compromising their quality of life and development (LOESCHE et al., 1995). The development of caries depends on the interaction of factors relating to the host, especially a diet rich in fermentable carbohydrates, and the presence of cariogenic microorganisms (THYLSTRUP, 1998). Caries are caused by a highly specific and complex

microbial process resulting from a nonspecific accumulation of acid-producing microorganisms on teeth; it should be interesting to confirm the possible involvement of other microorganisms in their formation.

Classically, the microorganisms involved in the genesis and development of caries are bacteria such as *Streptococcus mutans* and other cocci and rods (PAROLO; MALTZ, 2006). But there is evidence of the involvement of *C. albicans* in the etiology of dental caries (LOESCHE et al., 1995; NIKAWA et al., 2003b; SIQUEIRA-JÚNIOR et al., 2002; SZIEGOLEIT et al., 1999). Yeast cells are certainly acidogenic microorganisms, but the primary caries process has not yet been linked to the presence of yeast cells. Therefore, the aim of this study was to evaluate the frequency of yeasts in the oral cavity, determine the main species present, and assess the possibility of a correlation between the presence of yeasts with caries and dental plaque.

Material and methods

The population of this study was composed of 61 children ages 7 to 12, and 34 adolescents 13 to 17 years old, resident in the rural villages of Anahy, Santa Tereza do Oeste and Corbélia, located in the State of Paraná, Brazil.

All inhabitants of these locations aged between 7 and 17 (about 120) were invited, but participation in the research was voluntary and occurred after their guardians completed the Free and Informed Terms of Consent, following Resolution no. 196/96 of the National Council of Health, and previously approved by the Ethics Committee for Research of the State University of Western Paraná.

Personal data were collected using a standard individual form containing identity information, and medical and oral-dental history. Individuals who showed factors that could modify oral microbiota, such as orthodontic appliances, the existence of a systemic disease, or the continuous use of medicines, were excluded.

The biological samples were obtained by an oral rinse in which the mouth was washed for 30 second with 10 mL of sterile water. The mouthwash liquid was deposited in conical tubes and stored in an insulated container until microbiological processing. Later, this suspension was washed three times in sterilized 0.1 M phosphate-buffered saline pH 7.4 (PBS) by centrifugation 3.000 rpm, the pellet was resuspended in 1 mL of PBS, and 10 μ L aliquots were inoculated with a bacteriological loop onto the surface of differential selective medium CHROMagar Candida (CHROMagar™ Microbiology, Paris,

France) in Petri dishes. These dishes were incubated at 25°C for 48h. Yeasts were identified by germ-tube production, micromorphology, and chlamydospore production on Tween 80-corneal agar, assimilation and fermentation tests according to Larone (1995) and complemented by Kurtzman and Fell (1998). All isolates identified as *C. albicans* were screened for their ability to grow on Sabouraud Dextrose Agar at 45°C for 48h, in order to exclude *C. dubliniensis*.

Cariou teeth were defined as proposed by Pitts et al. (1997), who considered only the cavities that affect the dentine.

The Simplified Oral Hygiene Index (OHI-S) was used to determine the presence of dental plaque. Basic fuchsin (Eviplac®, Biodinâmica, Brazil) was used as a stain to reveal the presence of plaque on tooth surfaces, which was expressed according to a pre-established scale. Oral hygiene was considered good when the final score was from 0 to 1, regular from 1.1 to 2, and poor from 2.1 to 3.

The statistical analysis was processed through the program Epi-Info v. 6.04 of the World Health Organization, using the Mantel-Haenszel Chi-square test and Fisher's exact test, with a significance level of 5%.

Results

Oral rinses were obtained from 95 subjects, 46.3% (n = 44) females and 53.7% (n = 51) males. Only 95.8% (n = 91/95) of these permitted a physical examination for the identification of caries, and none of them had used orthodontic appliances. The OHI-S was carried out with 90.5% (n = 86/95) of the subjects.

Caries were found in 70.3% (64/91) of the population, with a mean of 4.5 carious teeth per individual and a range of 1 to 17 carious teeth. The prevalence of caries was similar in females (69.8%, 30/43) and males (70.8%, 34/48), but was higher in children (79.3%, 46/58) than in adolescents (54.5%, 18/33).

Cultures were positive for yeasts in 33.7% (32/95) of cases, revealing considerable microbial density, since 46.9% (15/32) of those cultures showed a count of more than 20 colonies. *C. albicans* was the most prevalent species, and *C. dubliniensis* was not isolated (Figure 1).

Fourteen of the 32 positive cultures were obtained from female subjects (43.5%), while 18/32 were from males (56.5%). Table 1 shows the similarity of the yeast frequency between females (31.8%) and males (35.2%), and the predominance of positive cultures in children (37.7%) compared to adolescents (26.5%).

There was a correlation between the presence of caries and the isolation of yeasts ($p = 0.0087$) as seen

in Table 1. Figure 2 shows that the increase of yeasts corresponded to the increase in the number of carious lesions.

Table 1. Distribution of oral cavity cultures positive for yeasts according to gender, age and presence of caries.

Analyzed variables	Total N	Positive cultures			<i>Candida albicans</i>		
		n	%	χ^2	n	%	χ^2
Oral cavity samples (n = 95)							
Female	44	14	31.8	0.004	9	64.3	0.169
Male	51	18	35.3		14	77.8	
Children	61	23	37.7	1.524	17	73.8	0.77
Adolescents	34	9	26.5		6	66.7	
Physical examination samples (n = 91)							
Without caries	27	5	18.5	9.49	2	40.0	7.414
With caries	64	26	40.8		20	76.9	
Sample with caries (n = 64)							
Female	30	13	43.3	0.357	9	69.3	0
Male	34	13	38.2		11	84.6	
Children	46	19	41.3	0.62	15	78.9	0.009
Adolescents	18	7	38.9		5	71.4	
1-5 caries	45	16	35.6	3.764	11	68.8	5.72
6-10 caries	13	6	46.2		6	100	
10+ caries	6	4	66.7		3	75	

χ^2 = value of chi-square.

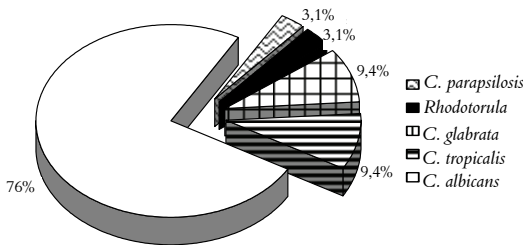


Figure 1. Species distribution of the 32 yeasts isolated from oral cavity of children and adolescents.

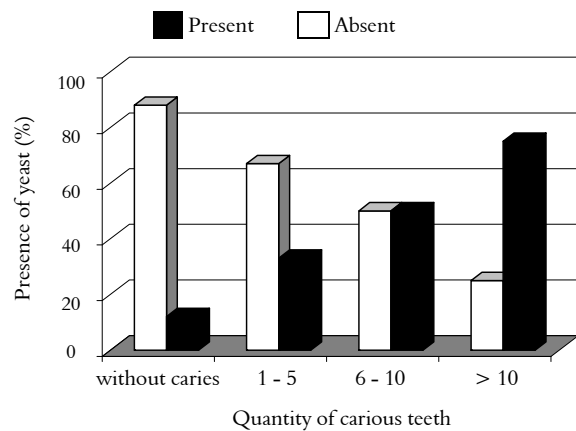


Figure 2. Frequency of yeasts in the oral cavity and the relationship to the numbers of carious teeth (n = 91).

Moreover, the isolation of *C. albicans* was proportional to the increase in the number of carious teeth (Figure 3).

The average OHI-S in the 86 analyzed subjects was 2.26, being poor in 67.5% (n = 58), regular in 30.2% (n = 26) and good in only 2.3% (n = 2). In this group, 29 out of 86 subjects (33.7%) presented yeasts and *C. albicans* was the most predominant specie, regardless of the analyzed score (Figure 4). The association between OHI-S

and the isolation of yeasts was not significant (p = 0.49).

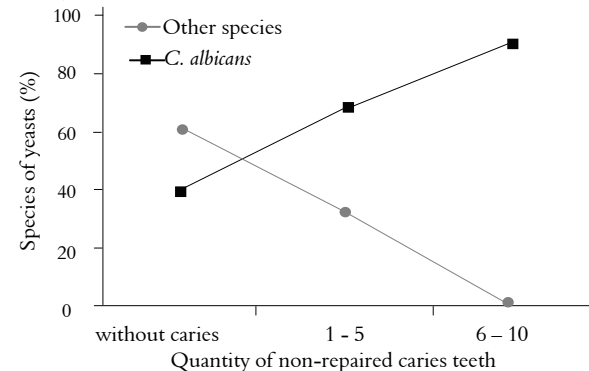


Figure 3. Frequency of isolation of *Candida albicans* and other yeast species according to the number of carious teeth.

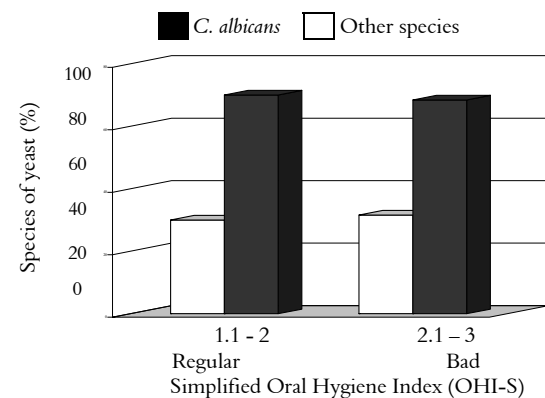


Figure 4. *Candida albicans* isolation in relation to the Oral Hygiene Index. OHI-S < 1 was found in only 2 subjects, who did not show yeast in their oral cavity.

Discussion

Isolation of yeasts from the oral cavity is common, and is influenced by variables such as age,

general state of health, and socio-economic and cultural levels. Therefore yeast colonization rates vary greatly according to populations and countries (AL-KARAAWI et al., 2002). Moreover, Kadir et al. (2005) observed that there are intrinsic differences in the role of *Candida* species among different ages and populations. Siudikiene et al. (2006) reported that diabetic youths had fewer dental caries and plaque, despite showing more frequent growth of yeasts than did non-diabetic control individuals.

In this study, the frequency of yeasts among the children was 37.7%, an intermediate value between those found in healthy American children (30%) (JABRA-RIZK et al., 2001) and Portuguese children (47%) (STARR et al., 2002). However, in immunocompromised children, this rate tends to increase, as observed in Africans with malnutrition (48.4%) and in HIV-positive Americans (50%) (JABRA-RIZK et al., 2001). Similar variations in colonization rates are also observed in individuals from other age groups and originating from different regions (ARENDORF; WALKER, 1980; LOESCHE et al., 1995; MOALIC et al., 2001; NEGRONI et al., 2002).

The predominance of yeasts in the oral cavity of children compared to adolescents is in agreement with findings by Ugun-Can et al. (2007). Moalic et al. (2001) also suggested that younger people show higher rates of colonization by yeasts. This can be explained, among other factors, by the motor and cognitive immaturity shown by children. This immaturity can make it hard for children to acquire good personal hygiene habits, which favors the disequilibrium of their microbiota.

The frequency of yeasts found in this study was similar in males and females, as previously observed by Arendorf and Walker (1980) and Al-Karaawi et al. (2002), but differed from the findings of Moalic et al. (2001), in which the presence of yeasts was significantly higher among males.

As seen on Table 1 in this study, 40.6% (26/64) of the subjects who had caries harbored yeasts in their mouths, whereas in those without caries, this frequency was only 18.5% (5/27). These data indicate a correlation between the presence of yeasts in the mouth and dental caries. Correlation between the presence of yeasts in the mouth and dental caries was recently reported by others (CERQUEIRA et al., 2007; ROZKIEWICZ et al., 2006; SIGNORETTO et al., 2009). Other evidence consists of a significant reduction in the prevalence of *C. albicans* in buccal mucosa from Portuguese children after odontological treatment and oral hygiene instruction (STARR et al., 2002).

However, Moalic et al. (2001) did not find a positive correlation between the presence of caries and

yeasts in a population of young adults, but suggested a trend of association between caries and number of yeast colonies. Loesche et al. (1995) found a significant association between caries and yeasts, suggesting that these microorganisms would be good indicators of carious activity in elderly people. Recently, Signoretto et al. (2009) showed that antiseptic treatment alone for dental decay is not sufficient for the eradication of yeasts present in caries lesions. According to these authors, the oral cavity of children could act as a reservoir for fungi, and eradication of *Candida* spp. could be needed to prevent exacerbation of caries lesions, as in many cases anti-caries therapy alone is not sufficient to resolve tooth decay in children, particularly in those with extensive open decay, but need additional therapy with an antifungal mouthwash.

Majjala et al (2007) also found no evidence that *C. albicans* is important in dental caries pathology; however, these authors employed two other methods for detecting yeasts in carious tooth samples, and did not make cultures to identify the yeasts, which is a gold-standard method.

C. albicans was the predominant species found in this as in other studies (AL-KARAAWI et al., 2002; FANELLO et al., 2006; LOESCHE et al., 1995; MOALIC et al., 2001). However, *C. albicans* was identified in only 22% of positive cultures in malnourished Nigerian children (JABRA-RIZK et al., 2001), with an increase of other species, as also observed in HIV-positive populations (AL-KARAAWI et al., 2002; ERKÖSE; ERTURAN, 2007; JABRA-RIZK et al., 2001; LUQUE et al., 2009). Erköse and Erturan (2007) found similar results in HIV-positive individuals, although they found *C. albicans* as the most prevalent species and identified *C. dubliniensis* (11.3%). The latter species was not isolated in our study, but is more frequent in HIV-positive subjects.

In the oral cavity, the high prevalence of *C. albicans* has been attributed to its capacity to form germ tubes, facilitating adhesion (NIKAWA et al., 2003a), as well as that it is the most frequently occurring species in normal microbiota of the oral cavity (NAVARRO-GARCIA et al., 2001).

The isolation of *C. albicans* in proportion to the increase in the number of carious teeth (Figure 4), allied to the positive correlation found between the presence of yeast in mouth and dental caries, suggests that this yeast plays an important role in the etiology and development of caries. This suggestion is supported by the study by Nikawa et al. (2003b), who demonstrated the *in vitro* cariogenic potential of this yeast on hydroxyapatite.

In the analyzed population, a correlation was found between the presence of caries and yeasts in the mouth, in which *C. albicans* was the most prevalent species, as much for those with caries as for those with regular and poor OHI-S. Previously, Rozkiewicz et al. (2006) reported the presence of *C. albicans* in dental plaque and carious lesions in preschool and school-age children. This association is worthy of a more detailed evaluation, because the demonstration of the adherence of *C. albicans* on hydroxyapatite allows us to consider that its presence may represent a risk factor for the occurrence of caries, through the high acidogenic potential shown by the dental plaque in which this yeast is present (NIKAWA et al., 2003b).

Conclusion

In conclusion, the results of the present study reinforce those of earlier studies, implying that yeast cells are not merely passively associated with the caries process. It remains unclear whether yeasts are causative agents in caries initiation or progression, or whether high yeast counts are merely a consequence of high caries activity. This question justifies future studies that may elucidate the real role of this microorganism in the etiology of caries.

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