Occurrence and predisposing factors associated to the genus *Candida* in children

Ocorrência e fatores predisponentes associados ao gênero *Candida* em crianças

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Abstract

The objectives of this study are: 1. to assess the frequency of the yeast *Candida* in the oral mucosa of children in relation to predisposing factors such as caries, food and oral hygiene, and 2. to identify *Candida* species in caries-active children. We adopted an exploratory and descriptive methodology within a quantitative and qualitative approach. Oral mucosa of 59 children aged 2-12, treated at the Comprehensive Dental Clinic of Maria Milza College (CLIOF-FAMAM), were sampled with swab from March to May 2014. A questionnaire was administered to the legal representatives to assess the child's behavior, and a dental clinical evaluation was performed to determine the number of active caries per child. *Candida* species were identified by means of chromogenic culture media (CHROMagar™*Candida*). Forty five point seventy six percent of the children were infected by *Candida*, being more frequent among males aged 8-10. Most of the active-caries children presented at least one *Candida* species, mainly *Candida* albicans followed by *Candida krusei*, *Candida tropicalis* and *Candida* spp. A positive association between the presence of *Candida* and caries, and poor oral hygiene was verified.

Keywords: Yeast. Risk factors. Children.

Resumo

Os objetivos do estudo foram averiguar a frequência de fungos do gênero *Candida* na mucosa oral de crianças em relação aos fatores predisponentes como cárie, alimentação e higienização bucal e identificar as espécies de *Candida* ocorrentes nas crianças com cárie ativa. O estudo possui caráter descritivo do tipo experimental com abordagem de cunho quali-quantitativo. Foram amostradas 59 crianças com idade entre dois e doze anos, atendidas na Clínica Odontológica Universitária da Faculdade Maria Milza (CLIOF-FAMAM), com coleta de amostra da mucosa com swab no período de março a maio de 2014. Foi aplicado um questionário ao responsável legal acerca dos componentes comportamentais de cada uma das crianças e avaliação clínica odontológica para verificação do número de cáries ativas. A identificação das espécies de *Candida* foi realizada utilizando-se o meio de cultura cromogênico CHROMagar™*Candida*. Das crianças avaliadas, 45,76% apresentaram crescimento de *Candida*, sendo mais frequente no sexo masculino com idades entre 8-10 anos. A maioria das crianças com cárie ativa apresentou, pelo menos, uma espécie de *Candida*, principalmente *Candida* albicans seguido por Candida *krusei*, *Candida tropicalis* e *Candida* spp. Verificou-se uma associação positiva entre a presença de *Candida* e de cárie e falta de higiene oral.

Palavras-chave: Leveduras. Fatores de Risco. Crianças.

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INTRODUCTION

The yeasts of the genus *Candida* have a widespread distribution and can be present in the environment as well as in human commensal microbiota[1]. Approximately half of the healthy adult population hosts these yeasts in the oral cavity, where they colonize the tongue, the palate, the tonsils, the labial and cheek mucosa, caries, radicular canals and periodontal pockets. They can also be found in the dental and gingival biofilm interface [2,3,4,5].

These unicellular yeasts do not generally trigger pathological processes, but under certain circumstances the biological balance can be disrupted allowing the yeasts to adhere to the epithelium, invade the connective gingival tissue, trigger virulent factors and induce inflammatory processes[5,6]. Among the factors that contribute to *Candida* virulence are the capacity of growing at 37°C, phenotypic variability, hiphae, pseudohyphae and biofilm formation, and hydrolytic enzyme production such as phospholipase, just to mention some[7].

About 80% of *Candida* isolates from oral mucosa belong to the species *Candida albicans*, but other species can be found such as *Candida parapsilosis*, *Candida tropicalis, Candida glabrata* and *Candida krusei*[8].

In adults, important predisposing factors for yeast colonization of the oral cavity are denture use, orthodontic appliances, food, tobacco, alcohol, pre-existing conditions, chemotherapy, immunologic modulation, endocrine alterations, and long-term use of antibiotics and corticoids[7,9,10]. In children, the main factors are the use of pacifiers, unhealthy diets, poor or inappropriate oral hygiene, and dental caries[6,11].

Dietary habits are related to the etiology of various pathologies and research shows that nutritional deficiencies and unhealthy diets represent a source of free radicals that can alter genetic material heightening the risk of pathology development[12]. According to international studies, children and teenagers with healthy dietary habits have lower chronic disease and obesity risks in adult life[13]. The National Research on School Children´s Health defined beans, fruit, vegetables and milk as healthy diet indicators that should be ingested at least five days a week. Unhealthy diet indicators such as fried foods, sausages, crackers, cookies and soda should be ingested in small quantities[14].

Oral hygiene is very important and should start during breastfeeding. After the first deciduous tooth eruption twice-a-day tooth brushing is recommended, especially just before bedtime. Parents should supervise the procedure until the child acquires complete fine movement control which coincides with the acquisition of writing skills. The objective of tooth brushing and flossing is to eliminate or disorganize the bacterial plaque that is constantly formed on tooth surface[15]. According to Barros et al.[16] teeth should be brushed three times a day: in the morning, after lunch and after dinner or before bedtime including every tooth surface and the tongue.

Dental caries is considered a transmissible infectious disease caused by bacteria of the Streptococcus mutans species and affecting the whole population, especially children. This pathology is the consequence of propagation on the enamel surface of microorganisms that produce acid as a result of fermenting carbohydrate metabolism[11]. The fixed acidity caused by the presence of sugar leads to calcium phosphate dissolution on the superficial layers of the enamel structure and liberation into the oral cavity. The mineral loss can result in the development of a cavity and its evolution can lead to complete crown destruction[17]. Caries lesions modify the oral environment and create new sites for biofilm retention. Various studies have tried to relate *Candida* proliferation to dental caries lesions since biofilm retention is an ideal habitat for microorganism growth[6].

Based on the social relevance of the subject, the aim of the this study is to assess the relation between *Candida* and the predisposing factors, such as dental caries, diet and oral hygiene, as well as identifying the species that can be found in the presence and absence of caries.

METHODOLOGY

For this research we adopted an exploratory and descriptive methodology within a quantitative and qualitative approach. The study was performed with children treated

at the Comprehensive Dental Clinic of Maria Milza College (CLIOF-FAMAM) in Cruz das Almas, Bahia, Brazil, from March to May 2014. The inclusion criteria were: age between 2 and 12 years old, being a CLIOF-FAMAM patient, agreement of the child's legal representative to participate in the research, and signature of the Informed Consent Form (ICF). The use of orthodontic appliances or the presence of chronic diseases were exclusion criteria.

Patients and their legal representatives were addressed during the screen tests and asked to sign two copies of the ICF after receiving detailed information of the nature of the research and its objectives. The second step consisted in the administration of a questionnaire with objective and discursive questions in order to construct the child's behavioral profile, which comprises eating habits and oral hygiene.

Fruit, greens and vegetable intake four times a week, and fat and fried food intake less than three times a week were considered a healthy diet. When unhealthy food intake occurred more than four times a week and healthy food intake happened less than three times a week, the diet was considered unhealthy[14].

A clinical dental evaluation was performed in order to assess the number of active caries and to collect oral mucosa samples with sterile swaps.

The samples were inoculated on CHROMagar™*Candida* and the Petri dishes incubated in a stove at 37ºC for 3 to 4 days. After identification the samples were stored in a refrigerator.

CHROMagar*Candida* is a differential culture medium used to isolate yeasts. Through the addition of chromogenic substrate to the medium the C. *albicans*, C. *tropicalis* and C. *krusei* colonies grow into different colors, which allow for quick identification in the isolation plaque. The medium produces green colonies of C. *Albicans*, blue colonies of C. *tropicalis* and light pink colonies of C. krusei. Other yeast species develop into creamy or beige coloring, depending on the manufacturer.

The results were compared using the non-parametric chi-square (X2) test at a significance level of 5% ($p < 0.05$).

The samples were collected after approval of the Maria Milza College Ethics Committee (CEP-FAMAM 50/2013), in accordance with the guidelines and standards regulating research involving human beings (resolution 466/12 of the National Health Council).

RESULTS AND DISCUSSION

Presence of the genus *Candida* in children

Of the 59 children studied, 27 (45.76%) presented yeasts of the genus *Candida*, being more frequent among males aged 8-10.

Our results are in accordance with a number of studies which reported that the presence of yeast in the oral cavity of healthy individuals could vary from 2 to 71%, with an average of 34% depending on the population and sample sensitivity18. Odds[19] reported the existence of oral *Candida* in at least 50% of the healthy adult population; Akpan and Morgan[20] found that [20] to 75 % of healthy individuals hosted *Candida* as a commensal in the oral cavity; Menezes et al.[21] observed that approximately 25 to 30% of the individuals have C. *albicans* in the oral cavity, while for Matos et al.[22] the percentage varies between 30 and 70%.

Similar values were reported by other researchers, as for example Jorge et al.[23] who selected 142 children aged 3-14 and found *Candida* in 41.55% of the population; and Moreira et al.[24], who observed that the yeast was present in saliva samples of 47.3% healthy children of different socioeconomic levels. Lower values were reported by Barbieri[11] who found that 12 out of 38 children (31.57%) hosted yeasts of the genus *Candida*, and Komiyama et al.[25] observed the presence of Candida spp. in the oral microbiota of 30% of the healthy children he evaluated. Menezes et al.[21] analyzed the oral mucosa of 364 children between 1 and 5 years old at a municipality day care in Fortaleza and found that just 67 (18%) were colonized by *Candida*.

Prevalence of yeasts in males was observed by Al-hebshi et al.[26] who reported that from the 60.3% children with *Candida*, 70% were males and 56% females. On the other hand, Martins et al.[27] did not find differences between the sexes (35.2% in females and 31.8% in males) when

studying 61 children aged 7-12 and teenagers aged 13-17. The authors also reported higher yeast occurrence in children (37.7%) than in adolescents (26.5%). According to Moalic et al.[28] young individuals show higher levels of yeasts because of their neuromotor and cognitive immaturity which leads to poor oral hygiene and microbiota imbalance.

Notwithstanding, the results of a study performed by Rigo et al.[29] with 89 children aged 8-11 showed that girls had higher chances of developing *Candida* than boys (38.5 and 16%, respectively). According to Hofling et al.[30], prevalence studies of *Candida* in the oral microbiota are important to understand colonization frequency of opportunistic organisms capable of biofilm formation in order to identify reservoirs of potential infectious agents.

Candida and Active Caries

In the present study, 33 (44.07%) out of 59 children presented active caries, while 26 (55.93%) did not. Of the 33 children with caries, 21 had at least one *Candida* species and 13 had no yeast growth. In the control sample (without caries) just 05 of the individuals showed *Candida* growth while in 20 the microorganism did not develop (Table 1).

The Chi-square test indicated that the differences observed in children with and without caries were significant at $p<0.05$, with a calculated value of $11.48 > x^2$ tag= 10.83. This result indicates that the presence of the yeast is nonrandom and consequently children with active caries would be subject to *Candida* colonization.

The relation between yeast and oral caries has been reported by several authors[31,32,33]. Gouvêa-Mondin and Hofling[6] reported a higher prevalence of *Candida* in children with caries, especially in deciduous teeth. However, after repair treatment the occurrence of *Candida* was reduced in 90%. Mardegan[17] states that many caries lesions can be a risk factor for yeast proliferation in the oral cavity of children. According to the author, despite the frequent presence of yeast in the saliva and dental biofilm of children with caries, there is still no evidence of the role of *Candida* as being their cause. Besides, both authors state that children are more liable to opportunistic microorganism infections due to their immature immune system and the incomplete establishment of skin and mucosa resident microbiota. For Cortelli et al.[34] dental caries is a multifactorial disease that worsens with a diet poor in water, proteins and vitamins, and rich in sugar.

Table 1. *Candida* presence in children with and without active caries, data obtained between March to May of in Cruz das Almas, Bahia, Brazil from March to May 2014.

	Without *Candida*	With *Candida*
Without Caries	20 (60.60%)	05 (19.23%)
With Caries	13 (39.40%)	21 (80.77%)
Total	33 (100%)	26 (100%)

Candida albicans identification

In the present study *Candida albicans* was the species with higher prevalence in both groups: it was found in 16 children with caries and in 04 children in the control sample.

The predominance of *C. albicans* is often reported in the literature, and according to Ferreira[35] although the genus has 163 species, approximately 60% of the infections are caused by *C. albicans* which is the most frequent species found in the oral cavity.

Likewise, Rozkiewicz et al.[31], when studying the presence of *C. albicans* in oral samples of 102 healthy children aged 4-7, observed that the occurrence of the yeast was significantly higher (64%) in children with dental caries than in children without caries lesions (43.7%).

Martins et al.[27] reported a positive correlation between dental caries and the presence of yeasts in the oral cavity, i.e. there was a higher isolation of *C. albicans* when the number of teeth with caries increased. This fact suggests that the yeast has an important role in caries

etiology and development as also reported by Nikawa et al.[2] who showed the cariogenic potential of the yeast in vitro.

A similar correlation between caries and yeast was observed by Akdeniz et al.[36] although they could not find significant differences in *C. albicans* isolation associated to diet, use of pacifiers or bottles, and salivary ph.

Non albicans species identification

In the present research *C. krusei* was observed in 05 children with caries, and *C. tropicalis* was found in 01 child in each group. Non identified species, *Candida* spp., was found in 10 caries bearing children and in 01 child without caries. Co-occurrence of more than one species was observed in some cases: *C. albicans* and *Candida* spp. were isolated in 07 children with caries and in 01 child from the control sample. *C. albicans* and *C. krusei* were confirmed in 04 children with active caries.

According to Jorge et al.[23] other species besides C. albicans can be found in the oral cavity of healthy individuals, as for example *C. tropicalis* and *C. glabrata*. In normal circumstances these microorganisms do not unleash inflammatory processes, but can become pathological in specific situations such as oral mucosa or immunological alterations that can lead to infections.

Crocco et al.[37] stated that *C. krusei* can be isolated in approximately 19% of the lesions in patients with *Candida* spp. Bouchara et al.[38] highlighted the relevance of identifying *C. krusei* because of its resistance to fluconazole.

Dal Vesco et al.[4] used chromogenic medium and also reported the prevalence of *C. albicans* (70%) followed by *C. tropicalis*, *C. glabrata* and *C. krusei* in 2 and 3 year-old children. In 4 year old children the pattern was the same except for the absence of *C. krusei*.

Yucesoy and Marol[39] obtained a higher number of *C. albicans* colonies, followed by *C. tropicalis*. Menezes et al.[21] also observed the prevalence of *C. albicans*, which was isolated in 30 samples (45%) while C. tropicalis, *C. guilliermondi*, *C. glabrata* and *C. stellatoiea* were isolated in 31%, 17%, 4.5% and 1.5% of the samples, respectively.

Al-hebshi et al.[26] emphasized the high frequency of *C. albicans* which was isolated in 68% of the samples, followed by *C. tropicalis* (11.8%), *C. glabrata* (5.5%), *C. krusei* (2.3%), and *Candida* spp. (11.4%). The authors reported the co-occurrence of two or more *Candida* species in 25 % of the individuals tested and suggest there could be synergistic or antagonistic interactions between two or more yeast species, as is the case of *C. tropicalis* and *C. glabrata* that are mutually exclusive, while *C. albicans* favors the presence of *C. glabrata* and other no identified species. However, more research should be undertaken to prove this theory.

Barbieri[11] observed that among the identified *Candida* species, 26 (83.8%) were *C. albicans* and 05 (16.12%) were non-albicans *Candida such* as *C. tropicalis* (9.6%); *C. glabrata* (3.2%), and *C. guilliermondii* (3.2%). According to the author, *C. tropicalis* is the non-albicans yeast more often related to candidemia, especially in immunosuppressed patients. Moreover, the multicolonization, genotypes and distribution of the found *Candida* species seem to be related to the development of dental caries. Still according to Barbieri[11], children bearing non-albicans species had more caries, mainly those colonized by *C. tropicalis*. The author suggests that the non-albicans species might be related to microbiota changes that increase the risk of caries. Furthermore, the fact that all the children were colonized by *C. albicans* can imply the participation of this species in potentially cariogenic biofilm formation.

In a study carried out by Rigo et al.[29] with 89 children between 9 and 11 years old the researchers observed that of the 23 samples with *Candida,* 18 were *C. albicans* and 5 *C. krusei*. In the present study, Candida was observed in 28.5 % of the children with caries.

Candida and dietary habits

In the present research a negative association between the presence of *Candida* and the ingestion of healthy food (fruits and vegetables) more than three times a week was observed. The Chi-square test indicated a not significant

association at p<0.05, with a calculated value of 0.17 < x^2 tag = 10.83 (Table 2).

Despite our results, many researchers claim that children whose diet is based on healthy food are less vulnerable to pathological processes caused by *Candida*. On the other hand, children with diets reach in fats tend to have more infections caused by this yeast. Batchelor e Sheiham[40] report that the ingestion of food rich in sucrose can cause changes in the oral microflora that include the increase in the saliva of acidogenic and aciduric microorganisms, especially during the transition from deciduous to adult permanent teeth when there is an increase in retention surface. Pizzo et al.[41] studied the effects of carbohydrates intake on *C. albicans*, *C. tropicalis* and *C. krusei* adhesion and observed that the frequent ingestion of sucrose, glucose, maltose or fructose could be a risk factor for oral colonization by *Candida* and infections such as candidosis.

Marchioni[42] considers that healthy dietary habits are related to the risk reduction of oral cancer in children and that a diet based on rice, beans, fruits, vegetables and moderate amounts of meat might protect the oral mucosa.

According to Stenderup[43], nutritional factors, interaction with the bacterial microbiota and the presence of antibodies in the saliva affect the occurrence of these microorganisms. However, not always the presence of yeasts in the saliva is related to pathologies and their presence in the oral cavity depends on the organism homeostatic balance.

Table 2. Relation between *Candida* and diet in children, data obtained between March to May 2014 in Cruz das Almas, Bahia, Brazil

	Without *Candida*	With *Candida*
Healthy Diet	09 (27.27%)	05 (23.81%)
Unhealthy diet	29 (87.87%)	16 (76.19%)
Total	38 (100%)	21 (100%)

Candida and Oral Hygiene

In regard to oral hygiene, a positive association between *Candida* occurrence and poor oral hygiene was observed with a higher calculated x^2 (20.25) than the tabulated value (x^2 tag= 10.83) (Table 3).

Our results are in accordance with those obtained by Martins Neto et al.[44] who consider oral hygiene of great importance to prevent candidiasis and recommend the use of a soft bristled toothbrush to remove the bacterial plaque and food residues from the tongue, gums and palate. The authors also approve the use of mouthwash to improve oral asepsis. Starr et al.[45] also observed a diminished prevalence of *C. albicans* in the oral mucosa of children after dental treatment and adequate oral hygiene instruction.

Local predisposing factors for *Candida* infections are related to mechanical, thermal and chemical stimuli in the oral cavity that result from inherent physiological activity. Thus the oral cavity tends to suffer alterations caused by systemic modifications (metabolic or other) that lead to an imbalance between the microbial population and the host[9].

Rigo et al.[29] analyzed socioeconomic variables such as housing, number of people living in the same house, quantity of rooms, cars, access to dental care, auto perception in terms of oral treatment and daily tooth brushing frequency, but did not obtain significant association of any of them and the presence of *Candida*.

Table 3. Relation between *Candida* and oral hygiene in children, data obtained between March to May 2014 in Cruz das Almas, Bahia, Brazil

	Without *Candida*	With *Candida*
Good Oral Hygiene*	30 (83.33%)	02 (8.69%)
Poor Oral Hygien **	06 (16.66%)	21 (91.30%)
Total	36 (100%)	23 (100%)

*tooth brushing occurs three or more times a day; ** tooth brushing occurs zero to two times a day

CONCLUSION

Of the 59 children studied, 27 (45.76%) presented yeasts of the genus *Candida*, being more frequent among males aged 8-10.

Eighty point seventy seven percent of the children with active caries hosted at least one *Candida* species, while only 19.23% of the control sample (without caries) were colonized by the yeast.

Candida albicans growth was higher in the group with caries than in the control sample.

The non-albicans species identified in caries-bearing children were *C. tropicalis* and *C. krusei*. *Candida* spp. was found in 10 of children with caries and in 01 child without caries.

In terms of predisposing factors, the yeast was positively associated to dental caries and poor oral hygiene.

Dental treatment is a priority to achieve good life quality; hence access to dental care should be available since early childhood. Parents should be informed about the needs and abilities of their children, and their awareness about the importance of healthy diet, thorough oral hygiene and periodical visits to the dentist should be raised. Those attitudes might improve the child's oral health and reduce *Candida* infections.

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