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Microbiologia

Candida spp. biotypes in the oral cavity of school children from different socioeconomic categories in Piracicaba - SP, Brazil

Biotipos de Candida spp. *na cavidade oral de escolares de diferentes categorias socioeconômicas de Piracicaba - SP, Brasil*

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Two hundred and thirty-nine (239) Brazilian children, distributed into five distinct socioeconomic categories (A to E) were studied. Saliva samples were analyzed as to flow rate, pH, buffer capacity and microbial parameters. The results revealed the presence of *Candida spp.* in 47.3% of the samples. The most commonly isolated species was *C. albicans,* in all socioeconomic categories, followed by *C. tropicalis, C. krusei* and *C. parapsilosis*. There was no statistical correlation between secretion rate, buffer capacity and *Candida spp.* CFU/ml. The prevalence of *Candida spp.* did not differ substantially among the groups; however the microorganisms were more detected in categories B and C. Among all species, *C. albicans* was the most prevalent. Only 5% of the sample presented more than one species – *C. albicans* associated with *C. tropicalis*, *C. parapsilosis* or *C. krusei*. It was possible to detect a significant correlation between caries indices and the socioeconomic categories. All categories presented increased caries indices; however the studied population was considered of low caries risk. There was no positive correlation between the presence of *Candida* and caries risk in the analyzed population.

UNITERMS: Child; Socioeconomic factors; Saliva; *Candida albicans*.

INTRODUCTION

The occurrence of *Candida spp. ca*n be observed in the oral cavity; they are commensal yeasts which are part of the normal oral microbiota. Infections caused by these organisms are associated with factors such as: decreased immunity, endocrine disorders, soft tissue lesions, poor oral hygiene, long-term therapy with antibiotics, hormones and others. The variety of clinical manifestations of oral candidiasis reflects the diversity of the countless predetermining conditions.

In the strict sense, there are no essentially pathogenic yeasts[24]. However, those that are associated with human or animal diseases are capable to promote infection in healthy individuals. Alterations in the host's defense cells, physiology or normal microbiota are the factors that usually precede colonization, infection and disease produced by these organisms. The severity of the disease will depend on the severity of the alterations presented by the host, as well as on the several pathogenic properties exhibited by the fungi; the degree of debility must be considerable to allow the invasion of these microorganisms, considered saprophyte. The reasons why healthy subjects harbor such organisms are still unknown. Nutritional factors, the interaction with bacterial microbiota and the presence of antibodies in the saliva are thought to influence the incidence of those organisms[30]. The presence of yeasts in the mouth of healthy patients oscillates, depending on variables such as: collection technique, age and race of the subjects, and

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the methodology utilized to quantify and qualify the host-parasite interaction[28].

Considering that the presence of yeasts in the saliva is not always based on pathological factors and that their incidence in the oral cavity is related the homeostatic balance, it seems opportune to evaluate the frequency of *Candida* in the saliva of healthy carriers, from different socioeconomic status. Salivary samples of Brazilian schoolchildren from different socioeconomic categories, aging from 6 to 8 years, were analyzed in order to study the frequency of *Candida* in relation to other clinical and salivary parameters such as buffer capacity and salivary flow.

MATERIALS AND METHODS

Study Design

The sample comprised school children from Piracicaba - SP, southeast of Brazil, of both genders, without distinction of race or skin color, aging from 6 to 8 years. The individuals were invited to take part in this study, regardless of their oral hygienic or feeding habits. The criteria of exclusion were the presence of fixed or mobile orthodontic appliances and the treatment with systemic antibiotics or other drugs capable of altering the salivary flow or the ecological constitution of the oral microbiota, interfering, therefore, with the research.

The sample consisted of 239 children from five different social categories, who were aleatory chosen. Statistical parameters of sampling were followed, guaranteeing a good representation of the population. The classification of the school children as to their socioeconomic category was carried out according to the criteria adopted by the Brazilian Association of Advertisers and by the Brazilian Institute of Market Research (ABA/ABIPEME). A questionnaire was individually applied to each child, who was, afterwords, classified as belonging to one of the socioeconomic categories (A, B, C, D or E).

The sampling of whole stimulated saliva was carried out between 8:00 and 9:00 a.m. in most of the children[33] an, occasionally, between 1:30 and 2:30 p.m., according to the schedule of the school[12]. Sterile glass tubes of saliva were submitted to 30 seconds of vibration in order to obtain a uniform suspension. After this procedure, the saliva was diluted in decimal series from 10^{-1} to 10^{-3} in phosphate buffer 0.05 M, pH 7.3[29]. For the cultivation of yeasts, aliquots of 25 µl of pure saliva and of each dilution were inoculated in Sabouraud Dextrose Agar medium (SDA) added with 0.1 mg/ml of chloramphenycol (Carlos Erba, Inc.), and incubated at 37ºC for 48-96 hours[26]. The counting of CFU/ml was carried out after the growth of characteristic yeast colonies. The morphological characteristics of *Candida* colonies and those of colonies with different aspects were confirmed in a stereoscopic microscope. Gram staining was utilized in order to recognize other types of cells. The colonies that presented gram-positive budding cells of yeasts were classified as suggestive of *Candida*. For more precise identification, at least 3 colonies of each sample were inoculated in 2% malt-agar medium (2 g of malt extract, 1.8 g of agar in 100 ml of distilled water). After incubation and growth, these sub-cultures had their cellular and growth characteristics analyzed in order to confirm the presence of *Candida.* These isolates were stored in a refrigerator at 4ºC and maintained until the biochemical identification of the species was done[26]. Chi-squared and Kruskal-Wallis tests, with a significance level of 5%, were used in the analysis of data, as well as the Statistical Package for the Social Science (SPSS)[18]. Clinical exams were carried out by an examiner who applied the following tooth decay indexes: DMFT, DMFS, dmft and dmfs[23]. In addition, pH and buffer capacity of the saliva were evaluated with an Ingold pH eletrod and an Orion 701 potenciometer previously calibrated[5] with patterns of pH 4 and 7. First, pH was determined in 2 ml of stimulated saliva collected in tubes. Buffer capacity was determined in 0.5 ml of saliva collected in assay tubes containing 1.5 ml of HCl 5 mM. These tubes were agitated and, after 10 minutes, the pH was measured.

RESULTS

Samples of saliva were collected and processed. The number of yeast cells CFU/ml was determined and the species were identified. Regarding the number of individuals harboring *Candida* and the incidence of those microorganisms, Table 1 presents the 239 subjects divided according to their social categories. Among the boys (116), 28.4% of the individuals belonged to category B, while 20.7%, 19%, 16.4% and 15.5% belonged to categories E, D, A and C, respectively. Among the girls (123), 23.6% belonged to category A, while 22%, 21.1%, 17.9% and 15.4% belonged to categories B, D, C and E, respectively. The distribution of *Candida*

TABLE 1 - Distribution and frequency of *Candida spp.* in the oral cavity of children from different socioeconomic categories.

Variables		Gender				Total	
		Male		Female			
		N	%	N	%	N	%
Social categories	A	19	16.4	29	23.6	48	20.1
	B	33	28.4	27	22.0	60	25.1
	C	18	15.5	22	17.9	40	16.7
	D	22	19.0	26	21.1	48	20.1
	E	24	20.7	19	15.4	43	18.0
	Total	116	100	123	100	239	100
Candida spp.	Presence	55	47.4	58	47.2	113	47.3
	Absence	61	52.6	65	52.8	126	52.7

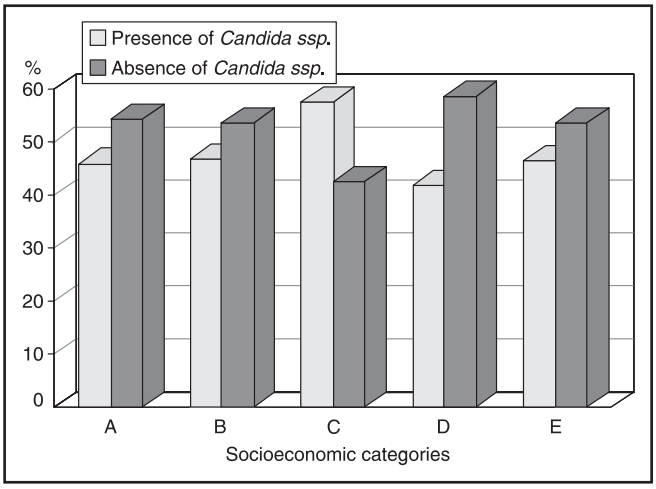

CHART 1 - Occurrence of *Candida spp.* in different socioeconomic categories.

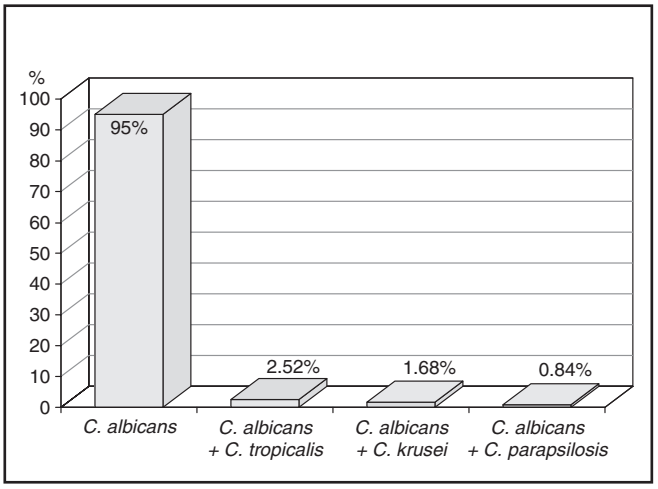

CHART 2 - Incidence of *Candida albicans* in association with other species.

spp. in all socioeconomic categor*ies is shown in Chart 1.*

Category C showed the highest percentage (60%) among all categories. Among the oral yeasts, the species *C. albicans* appeared solely in 95% of the individuals. The association of two different species occurred in 5% of the sample (Chart 2) – the presence of *Candida albicans* with other species occurred as follows: *C. albicans* + *C. tropicalis* (2.52%), *C. albicans* + *C. krusei* (1.68%) *and Candida albicans* + *C. parapsilosis* (0.84%).

Salivary factors such as buffer capacity, salivary flow and other clinical parameters were also investigated. Table 2 refers to the distribution of children regarding the frequency of *Candida*, saliva secretion rate and buffer capacity. It was observed that 46.6% (111), 22.7% (54) and 26.1% (62) of the individuals showed secretion rates of 0.1 to 0.7 ml/min, > 0.7 to 1.0 ml/min, and > 1.0 to 2.0 ml/min, respectively, and only 4.6% (11) demonstrated values above 2.0 ml/min. It could also be detected that the frequency of xerostomic children was null in the whole sampling. The results on buffer capacity showed that 47.7% (112) of the sample presented normal values of pH (between 5 and 7), and 37.4% (88) showed limiting values of pH (between 4 and 5). Among the individuals harboring *Candida*, 20.1% (48) revealed val-

TABLE 2 - CFU of *Candida spp.*/ml, buffer capacity and secretion rate in school children from different socioeconomic status.

Variables		Gender					
		Male		Female		Total	
		N	%	N	%	N	%
Secretion rate (ml/min)	0.1-0.7	50	43.1	61	50.0	111	46.6
	> 0.7-1.0	24	20.7	30	24.6	54	22.7
	> 1.0-2.0	35	30.2	27	22.1	62	26.1
	> 2.0	7	6.0	4	3.3	11	4.6
	Total	116	100	122	100	238	100
Buffer capacity	< 4	14	12.3	21	17.4	35	14.9
	> 4-5	34	29.8	54	44.6	88	37.4
	> 5-7	66	57.9	46	38.0	112	47.7
	Total	114	100	121	100	235	100
Candida spp. (CFU/ml)	None	61	52.6	65	52.8	126	52.7
	0-100	18	15.5	20	16.3	38	15.9
	> 100-400	17	14.7	10	8.1	27	11.3
	> 400	20	17.2	28	22.8	48	20.1
	Total	116	100	123	100	239	100

ues of CFU/ml > 400; 15.9% (38) showed values < 100 and 11.3% (27), values from 100 to 400. In 52.7% (126) of the samples such microorganisms were not detected. Differences between genders may be observed in relation to secretion rate and buffer capacity.

The presence of *Candida spp.* was observed in 113 individuals, which represents 47.3% of the total sample. The frequency of *Candida spp.* in relation to secretion rate and buffer capacity is shown in Table 3. Differences may be observed in secretion rate and buffer capacity in relation to the presence or absence of *Candida spp.* in the samples. Secretion rate was strongly decreased (0.1-0.70 ml/min) for the 49.6% (56) of subjects who presented with *Candida*. For values above 2 ml/min, it was observed that only 3.5% (4) of the individuals harbored *Candida.* In 52.7% (126) of the sample this microorganism was not found.

The results on the tooth decay (DMFT, DMFS, dmft and dmfs) are shown in Chart 3. The greatest averages were found in categories E and D; the values increased from category A to E. The Kruskall-Wallis test individually applied to the decay indices was significant at the level of 5%, revealing differences among all socioeconomic categories.

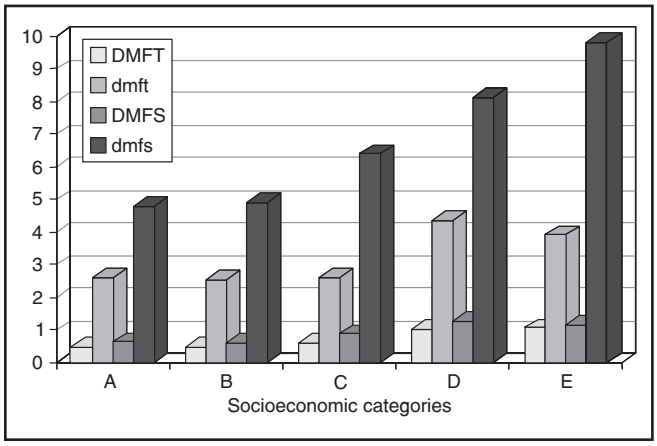

CHART 3 - Indices of caries in different socioeconomic categories.

DISCUSSION

The species and frequency of *Candida* in school children from different socioeconomic status, in association with clinical and salivary parameters, were investigated. The frequency of *Candida spp.*

TABLE 3 - CFU of *Candida spp.*/ml, buffer capacity and secretion rate in school children from different socioeconomic status.

Variables		*Candida spp.*					
		Presence		Absence		Total	
		N	%	N	%	N	%
Secretion rate (ml/min)	0.1-0.7	56	49.6	55	44.0	111	46.6
	> 0.7-1.0	28	24.8	26	20.8	54	22.7
	> 1.0-2.0	25	22.1	37	29.6	62	26.1
	> 2.0	4	3.5	7	5.6	11	4.6
	Total	113	100	125	100	238	100
Buffer capacity	< 4	22	19.8	13	10.5	35	14.9
	> 4-5	43	38.7	45	36.3	88	37.4
	> 5-7	46	41.4	66	53.2	112	47.7
	Total	111	100	124	100	235	100
Candida spp. (CFU/ml)	None	–	–	126	100	126	52.7
	0-100	38	33.6	–	–	38	15.9
	> 100-400	27	23.9	–	–	27	11.3
	> 400	48	42.5	–	–	48	20.1
	Total	113	100	126	100	238	100

in the oral cavity (*Tab*le 1) revealed that 47.3% of the sample presented with the microorganism regardless of the gender. Those results are in accordance with those of ODDS[19], which showed the existence of oral *Candida* in at least 50% of the healthy population, but the values were higher than those obtained by JORGE *et al.*[10], who found a percentage of 41.55% in healthy children aging 3 to 14 years. The causes of the presence of *C. albicans* in healthy carriers are not clearly defined[30]. In normal conditions, factors such as diet, poor oral hygiene, alterations in the salivary flow and systemic and localized disorders seem to collaborate with the appearance of such microorganisms.

Table 2 shows the results of salivary rate tests, buffer capacity and *Candida* CFU/ml. Regarding salivary flow rate, most subjects (of both genders) presented values of 0.1 to 0.7 ml/min, which is considered strongly decreased. Among girls, the most frequent buffer capacity corresponded to pH 4 to 5, while, among boys, there was greater prevalence of the interval 5 to 7, which is considered normal[14]. It could also be verified that as the values of salivary flow increased, the number of children decreased, proportionally. The percentage of xerostomic children was null. These data are in accordance with those obtained by KLOCK; KRASSE[12], who showed that there were no differences in buffer capacity and salivary flow when they were analyzed in relation to gender.

The important role played by salivary flow as a factor that extenuates dental caries has been investigated[4]. Various authors are unanimous in affirming that the absence of saliva (xerostomia) can result in a pronounced increase in caries risk[8,14]. One can not foresee the extent of the interaction of countless biological variables that influence the prevalence of caries[31]. Although it is obvious that an extreme variation of a determinant factor (e.g. xerostomia) can significantly influence the risk of development of caries, the mere hyposalivation (also a contributive factor) can not by itself explain the increase of the disease. The significant correlation observed between salivary and microbiological factors and caries in school children submitted or not to preventive programs does not allow to consider such factors as predictors of the development of caries due to the lack of consistency of the phenomenon[7]. Those pieces of

information, when analyzed together, indicate the importance of the interaction of other variables as predictors of the development of caries.

The most frequent number of *Candida* CFU/ml (Table 3) was 400 CFU/ml, for both genders, which is considered as an accentuated value[22]. Our results differ from those that indicate a greater frequency of *Candida* among females[15]. However, we must consider the predisponent factors that contribute to this variability, as well as the studied age groups, which were not the same.

In the last decades, the association between the salivary levels of *Candida* and some pathologic conditions of the oral cavity has been investigated. Individuals with 400 CFU/ml of saliva showed accentuated activity of caries[22]. *Candida albicans* is the etiological agent of most clinical forms of candidiasis and, in some less common conditions, this and other species of *Candida* are part of the microflora of the digestive tract and mucocutaneous areas, however, without any pathogenic effect. In the same way, OLSEN; STENDERUP[21] reported the presence of acute or chronic candidosis in patients with more than 400 *Candida* CFU/ml. However, in most of the *Candida* carriers showing values greater than 400 CFU/ml (suggestive of oral candidiasis), manifestations were not observed, at least, at the moment of sampling. Similarly, no clinical relationship was observed between oral candidosis and such a level of yeasts when the cariogenic and fungal microbiota of oral breathers was evaluated[13]. Results of that nature still need to be further investigated in large populations that should include various ethnic origins, age groups, socioeconomic levels, with or without predisposing factors.

Table 2 correlates the presence of yeasts with salivary parameters such as salivary rate and buffer capacity. It can be verified that carriers and noncarriers of *Candida* show similar results: strongly decreased salivary flow and normal buffer capacity. Therefore, the salivary parameters analyzed in this population were not necessarily associated with the occurrence or with the levels of *Candida* in the oral cavity.

Regarding the distribution of *Candida* among the socioeconomic categories (A to E), Chart 1 demonstrates that the percentage of *Candida* in category C is slightly higher than those of other categories, although the difference was not statistically significant. Such results suggest that the presence of yeasts is not determined by social factors. The lack of researches on the presence of yeasts in different social strata in our country, in a certain way, limits further speculations concerning this matter.

The morphologic and biochemical characterization of *Candida* yeasts[17,26] made possible the identification of 293 *Candida* strains belonging to several species. Table 3 and Chart 2 show the distribution of those species in association with other species. Most of the isolated strains were identified as *C. albicans* (95%). This frequency is higher than that previously observed in the oral cavity – 60 to 70% [30]. The presence of *C. albicans* in 73.75% of healthy individuals has also been observed[9]. The methodology used for the collection of clinical material from the oral cavity – swab, mouthrinse, imprint and saliva collects – can promote variations in the found frequencies[1]. The *C. albicans* species prevailed in all investigated social categories; the observed values might be than those found in previous studies. Regarding the isolation of multiple species, the frequency of association between *C. albicans* and other species was 5%; it was found in association with: *C. tropicalis*, *C. krusei*, and *C. parapsilosis*. There was no isolated occurrence of any of these species. These data suggest that the studied age group may be a restrictive factor for the diversity of species, along with other predisposing factors previously mentioned and excluded from the sample, such as orthodontic appliances, antibiotic treatment and systemic pathologies. The association of the mentioned species may not be determined by well-known mechanisms or processes – it takes place occasionally and depends on microbiological factors such as commensalism, symbiosis, synergism or amphybiosis. As to the multicolonized individuals, it is still imprecise to affirm that there is predominance of a certain gender or social category. The results of the present study, as well as those available in the literature, are still inconsistent and do not allow for further considerations.

In relation to the clinical parameters analyzed, the results plotted in Chart 3 express the data on caries indices (DMFT, DMFS, dmft and dmfs); higher values were registered in categories D and E, which was confirmed through statistical analysis. Differences in the activity of caries among socioeconomically diverse populations seem to be a fact in several investigations. That suggests that preventive and prophylactic measures should be applied and that healthy dietary habits, access to dental care and continuous education in that sense – which certainly favor the maintenance of oral

health – should not be a privilege of the wealthiest social groups. The disparities regarding the prevalence of caries in children from different socioeconomic status submitted to private care have been demonstrated: children who attend public schools present with more caries than those who attend private schools[6]. While students of public schools show higher values of DMFT due to several non-treated ca ries, in students of private schools a greater number of restored teeth is detected. The results indicate that social inequalities exist; they are present in our country and might influence the development of dental caries. Those data show the need for public health programs that emphasize preventive – and not only healing – measures in dentistry.

Investigating streptococci biotypes and lactobacilli in various populations, in association with clinical and salivary parameters, showed that individuals belonging to less favored social groups are frequently the ones who present the greatest levels of cariogenic microorganisms[30], which confirms our previous considerations. The anamnesis carried out at the time of the obtainment of clinical and salivary data from our sample showed that the individuals who belonged to the less favored socioeconomic categories (D and E) presented levels of oral hygiene incompatible with oral health and reported a smaller number of daily toothbrushings. Such a behavior, due to economic factors (which is less probable) or to the lack of constant preventive education, jointly with frequent ingestion of carbohydrates, accentuates the cariogenic process, facilitating the development of dental caries in larger proportion and in shorter periods of time. The utilization of a common family toothbrush was reported by several school children who belonged to less favored categories. That suggests the transmission of microorganisms through the toothbrush and consequent amplification of the disease.

An evaluation of caries risk in school children with mixed dentition showed that the average index of caries for deciduous teeth (dmfs) was 4.94, which is considered relatively low for such populations[7]. The data that we obtained, however, showed greater values: dmfs index of 6.82. The values of DMFT in the socioeconomic categories (A to E) ranged from 0.48 to 1.07, which is considered as a low prevalence of caries by PINTO[23] and KLOCK *et al.*[11]. These data are in accordance with other results recently obtained[2], which revealed DMFT of 0.66 in children aging 6 to 7 years. That means that recently erupted permanent teeth were not severely affected by caries. In our study, it was not possible to establish a positive correlation between *C. albicans* and dental caries because the greatest values of DMFT were observed in the less favored categories (D and E), while the occurrence of *Candida* was regularly distributed among categories A to E. Therefore, one cannot ensure that the presence of yeasts in the oral cavity is inherent to the populations that show higher prevalence of caries.

Economic factors – particularly the level of education of families – directly influence dietary and hygienic habits and, consequently, the oral health of individuals[3]. In the Third World countries and in some developed countries it has been verified that the less favored socioeconomic classes are the most affected by dental caries[32] – the highest prevalence of caries is observed in those who possess the smallest purchasing power. In Saudi Arabia, the prevalence of caries was also evaluated in preschool children from different socioeconomic categories: the highest values were verified in children who belonged to the lowest socioeconomic categories, showing that dental caries in children seem to be associated with the social stratum, when it is particularly characterized by the level of the parents' occupation[27]. The association between dental caries and several factors such as dietary habit, utilization of fluorine, socioeconomic and cultural factors, as well as the access to dental care has been clearly demonstrated in several investigations[8,16].

C. albicans presents acidogenic and heterofermentative characteristics, particularly in environments rich in carbohydrates, and can, thus, take part in the process of dental caries[25]. Yeasts and oral lactobacilli are present in the oral cavity due to the great number of retentive sites[20]. As well as lactobacilli, yeasts are acid-tolerant, which might justify their effective participation in the cariogenic process. The presence of yeasts and *Lactobacillus* in the oral cavity has been the subject of studies that aim at elucidating possible interactions between those microorganisms and their relationship with the complexity of the development of caries, with particular emphasis on caries risk. Although that was not the specific subject of our study, it is a field of research that should be explored, since it is a matter of scientific interest.

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Duzentos e trinta e nove crianças brasileiras foram estudadas, distribuídas em cinco categorias socioeconômicas distintas (A a E). As amostras de saliva foram analisadas avaliando-se o fluxo salivar, pH, capacidade tampão e parâmetros microbianos. Os resultados mostraram *Candida* em 47,3% da amostra total. A espécie mais encontrada foi *C. albicans* em todas as categorias socioeconômicas seguidas por *C. tropicalis*, *C. krusei* e *C. parapsilosis*. A saliva e os parâmetros de investigacão clínicos como a proporcão de secreção, capacidade tampão e CFU/ml de *Candida* não mostraram nenhuma correlação estatística entre estes parâmetros. A freqüência de *Candida* não diferiu substancialmente entre os grupos; no entanto elas foram mais acentuadas entre as categorias B e C. Entre todas as espécies, *C. albicans* foi a mais prevalente. Somente 5% das amostras mostraram mais de uma espécie e revelaram a presença de *C. albicans* associada com *C. tropicalis*, *C. Parapsilosis* ou *C. krusei*. Foi possível detectar uma correlação significante entre os índices de cárie e as categorias socioeconômicas. As categorias A e E mostraram índices de cárie aumentados; no entanto, a amostra da população foi considerada de risco de cárie baixo. Não havia nenhuma correlação positiva entre *Candida* e risco de cárie.

UNITERMOS: Criança; Fatores socioeconômicos; Saliva; *Candida albicans*.

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