Genetic similarity of *Candida albicans* isolated from the buccal cavity of children with Down's syndrome and their parents and/or caregivers

Similaridade genética de Candida albicans isolada da cavidade bucal de crianças com síndrome de Down e seus pais e/ou responsáveis

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Abstract

Objective – *Candida albicans* is the fungus most closely related to the human oral mucosa colonization. Biological characteristics of the fungus allow high adaptation to the environmental conditions presented by the human mucosas, resulting in *Candida* species often being described as colonizers and pathogens in childrens with Down's syndrome. **Methods** – The DNA extraction of buccal *C albicans* simultaneously isolated of CWDS and P and/or R it was done. RAPD was accomplished using intra-specific primers for polymorphism analysis of *C. albicans*: RSD10 5'-CCGCAGCC A-3' and RSD12 5'-GGTCCGTGTTTCAAGACG-3'. **Results** – We detected a genetic similarity between *Candida albicans* isolated from the mouth of children with Down's syndrome and those of their parents and/or caregivers, using RAPD with the primers RSD 10 and 12. Nine of the 40 (22.5%) pairs of oral *C. albicans* analyzed, had genetic homology (identical) in two conjugate pairs (2 / 9) (22.2%) (Similarity coefficient SAB 1). The other two conjugate pairs of buccal *C. albicans* (2 / 9) (22.2%) showed high relatedness (similarity coefficient SAB between 0.90 and 0.99). In other isolates (5 / 9) (55.6%), no correlation between the strains analyzed (similarity coefficient SAB <0.5) was found. **Conclusion** – The analysis of genetic similarity of the pairs of buccal *C. albicans*, isolated concomitantly from children with Down's syndrome and parents and/or caregivers, proved the intrafamilial transmission of this fungus between parents and their Down´s syndrome children, and confirmed the occurrence of isolates from other sources and possible genetic variation among these isolates.

Descriptors: Down's syndrome; *Candida albicans*; Mycology

Resumo

Objetivo – *Candida albicans* é o fungo mais relatado como colonizador da mucosa bucal humana. Características biológicas do fungo permitem elevada capacidade de adequação às condições ambientais apresentadas pelas mucosas orgânicas humanas, tornando *albicans*, a espécie de *Candida* mais descritas nos processos de colonização e patogenidade da boca de crianças com síndrome de Down. **Métodos** – O DNA de *C. albicans* bucal simultaneamente isolado de crianças com síndrome de Down e pais e/ou responsáveis foi extraído. RAPD foi realizado usando primers intraespecíficos para a análise do polimorfismo de *C. albicans*: RSD10 5'-CCGCAGCC A-3' e RSD12 5'-GGTCCGTGTTTCAAGACG-3'. **Resultados** – Foi detectada uma semelhança genética entre *Candida albicans* isoladas da boca de crianças com síndrome de Down e de seus pais e/ou responsáveis, utilizando RAPD com os primers RSD 10 e 12. Nove dos 40 (22,5%) pares de *C. albicans* orais analisadas, possuiam homologia genética (idênticos) em dois pares conjugados (09/02) (22,2%) (coeficiente de similaridade SAB 1). Os outros dois pares conjugados de *C. albicans* bucais (09/02) (22,2%) apresentaram alta afinidade (SAB coeficiente de similaridade entre 0,90 e 0,99). Em outros isolados (09/05) (55,6%), nenhuma correlação entre as cepas analisadas (coeficiente de similaridade SAB <0,5) foi encontrado. **Conclusão** – A análise de similaridade genética dos pares de *C. albicans* bucais, isolado, concomitantemente, de crianças com síndrome de Down e pais e/ou responsáveis, comprovou a transmissão intrafamiliar desse fungo entre pais e filhos com síndrome de Down, e confirmou a ocorrência de isolados provenientes de outras fontes e possível variação genética entre os isolados.

Descritores: Síndrome de Down; *Candida albicans*; Micologia

Introduction

Candida is a diploid fungus present in mouth microbiota throughout the lifetime of the individual[1-2]. This yeast can be detected in the mouth of newborns from 6 to 10 hours after childbirth and from 14 to 21 days in every gastrointestinal tract, and likewise in individuals with Down's syndrome[3-5].

The presence of *Candida* species in the buccal cavity can be due to contact of the fetus with vaginal secretion harboring *Candida* at the time of childbirth, or through cutaneous contamination from health professionals, contact with the skin of the mother's breast during breast-feeding or from affectionate exchanges between parents and children[3,6,7-9].

In children with Down's syndrome (CwDS), the buccal anatomic and physiological alterations (macroglossia, tongue fissures, gingival and smaller hard palate, tongue protrusion, crusade and open bite and fissures at the corners of the mouth) induced by this chromosomal abnormality act as additional factors conducive to the fungal colonization process[10-12]. These factors can be exacerbated by the difficulty in maintaining good oral hygiene, a diet rich in carbohydrates and immune system compromised[1,3-4,7,10,13].

Candida presents virulence characteristics: adherence, morphologic dimorphism, genetic variability (switching), exoenzyme production: aspartil proteinases and phospholipases and toxins. These fungal characteristics allow high adaptation to the environmental conditions presented by the human mucosas, resulting in *Candida* species often being described as colonizers and pathogens in CwDS [1,3-5,14-15].

The understanding of buccal transmission mechanisms of *Candida* between CwDS and parents and/or responsible caregivers (P and/or C) is necessary for the isolation and identification of this microorganism, in view of the frequent reports of candidiasis in the buccal mucosa of this pediatric group[1,4-7,15-17].

RAPD (random amplification of polymorphic DNA) is commonly employed, as it is a fast and sensitive method for analyses and detection of genetic polymorphisms in several dispersed locus for the genome of different organisms and microorganisms. In this test, oligonucletides (pri-

mers) with an arbitrary sequence are used to enhance different areas of DNA for PCR, allowing determination of the level of genetic similarity among the microbiological strains submitted to analysis[14,17-20].

The purpose of this study was to detect genetic similarity, using RAPD, among buccal *Candida albicans* isolated simultaneously from children with Down's syndrome and parents and/or responsible caregivers.

Methods

***Candida* strains**. The strains were isolated from the oral cavity of CwDS aged from newborn to eleven years old who attended the School of Dentistry at the Federal University of Goiás – Goiânia/Goiás State, Brazil. Simultaneously, samples were collected from P and/or C (mean age of 39.5 years old) of these children. The pairs of *Candida* (CwDS and P and/or C) isolates were collected from patients who had not used antibiotics for at least one month before treatment, and whose oral mucosa showed no clinical signs of disease. Morphological and biochemical tests identified the presence of *C. albicans* in nine pairs of isolates.

Extraction of DNA. The 9/40 (22.5%) pairs of *C. albicans* isolated simultaneously from the mouths of CwDS and P and/or C, were identified according to Kreeger-Van-Rij[21] (1984) and cultivated in YEPD (Yeast Extract Peptone Dextrose) medium at 37°C for 24 to 48 h. The genomic DNA extraction used the method described by Del Poeta *et al.*[22] (1999) and modified by Casali *et al.*[23] (2003).

RAPD analysis. Were used for the RAPD analysis 2µL (100 ng/µL) of *Candida*-DNA, 5 µL 10X PCR 10X buffer (200 mM Tris/HCl, pH 8.4, 500mM KCl), 200 µM dNTPs, 25 mM MgCl2, 1 µM primer and 1.5U Taq Polimerase (Invitrogen). The primers used in the reaction were RSD10 5'-CCGCAGCCA-3' and RSD12 5'-GGTCCGTGT TTCAAGAC G-3' (IDT Technologies)[19]. The DNA was denatured for 5 min at 94°C added for 5 cycles including 30s denaturation at 94°C, 2 min annealing at 52°C and 2min primer-extension at 72°C. The reaction was maintained at 72°C for 15 min. Negative controls were included in each run and reproducibility was checked for the reaction[17,20]. The PCR products were separated in agarose gels (1-2%) and electrophoresis was performed at room temperature in TBE buffer (89 nM Tris/HCl, 89 mM boric acid, 2.5 mM EDTA, pH 8.0), products stained with ethidium bromide and viewed under UV light.

Clustering analysis: Amplified standard DNA fragment bands were used to build up a binary matrix by means of the Unweighted Pair-Group Method using Arithmetic Averages (UPGMA), to generate dendograms for identification of clusters of related isolates. The genetic relatedness was measured by using the Jaccard Coefficient test based on band positions. In accordance with Dassanayake and Samaranayake[24] (2003), the strains were classified in terms of similarity correlation coefficient, as follows: 1.0 or 100% = identical strains; 0.9 or 90% = highly related strains; 0.8 or 80% = moderately related samples; and ≤ 0.7 or 70% = unrelated strains.

The Ethics Committee of Medical Human and Animal Research of Clinical Hospital of the Federal University of Goiás (HC / UFG) approved this research protocol (CEPMHA / HC / UFG ° N 035/2002) and parents or caregivers (P and / or C) by children with Down's syndrome (CwDS) gave informed consent for this study.

Results

Nine (9/40) (22.5%) pairs of *C. albicans* concomitantly isolated from mouths of CwDS and P and/or C, were analyzed. Two primers (RSD10 and RSD12) were used to distinguish the different genotypes of the *Candida* yeasts and a profile of bands with high resolution for RSD10 and for RSD12 (Figures 1 and 2) were obtained.

The amplification products profile of RAPD, using dendograms, allowed identification of the occurrence of identical strains (similarity coefficient SAB = 1 or 100%) or highly related (similarity coefficient SAB > 0.9 or 90%) and suggested the occurrence of distinctly related strains (similarity coefficient SAB < 0.5 or 50%) of *C. albicans* (Figures 1, 2 and Graph 1). The determination of genetic similarity among the nine positive cases in the P and/or C group with their respective children demonstrated genetic homology (identical strains) of the fungus in two (2/9) (22.2%) conjugated pairs (16-16RF and 37-37RF) and highly related similarity (SAB > 0.9) in another two (2/9) conjugated pairs (08-08RF and 22-22RF). Genomic similarity was not found in the rest of the cases (5/9) (55.5%) of *Candida* from P and/or C and the respective CwDS (Table 1). Analysis of the independent individuals of the pairs (children and adult responsible) demonstrated that 72% (13/18) possessed genetic similarity with a coefficient greater than 0.9. However, five individuals (04RF, 14RF, 19RF, 27RF and 33RF) presented different (or unrelated) strains to most of the isolated strains.

Table 1. Similarity relationship among the isolated pairs obtained from children with Down's syndrome (CwDS) and parents and/or caregiver (P and/or C) (FR-Familial Relationship)

Isolated pairs (CwDS and P and/or C)	Similarity coefficient	Percentage similarity
04 – 04FR	0.45	45%
08 – 08FR	0.95	95%
14 – 14FR	0.45	45%
16 – 16FR	1.0	100%
19 – 19FR	0.45	45%
22 – 22FR	0.95	95%
27 – 27FR	0.45	45%
33 – 33FR	0.45	45%
37 – 37FR	1.0	100%
Mean	0.68	68%

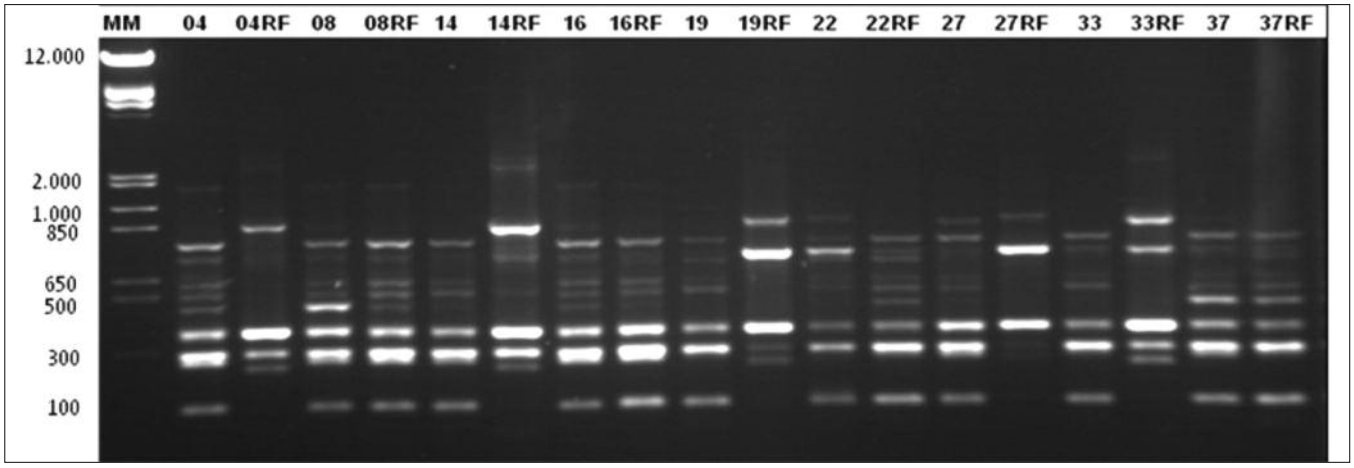


Figure 1. Electrophoretic profile of DNA fragments of simultaneously isolated *C. albicans* from the mouth of CwDS and P and/or C , by PCR using primer RSD 10 (MM – molecular marker and FR – familial relationship – P and/or C)

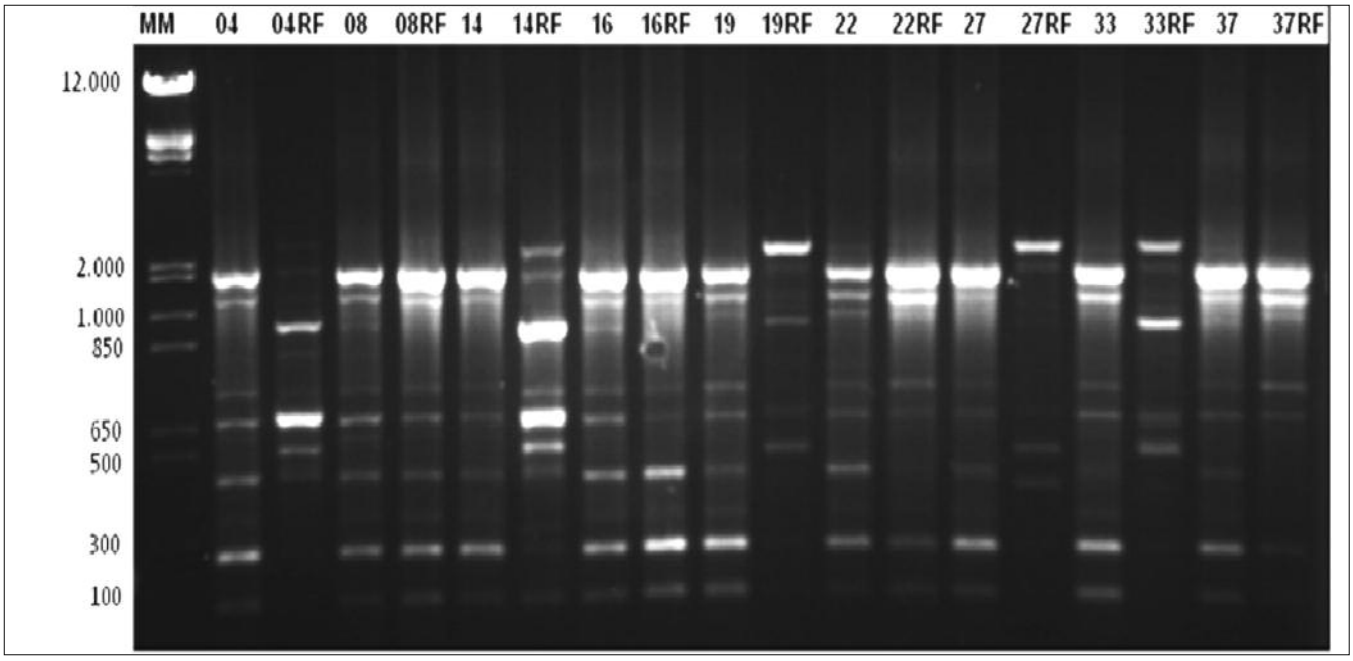
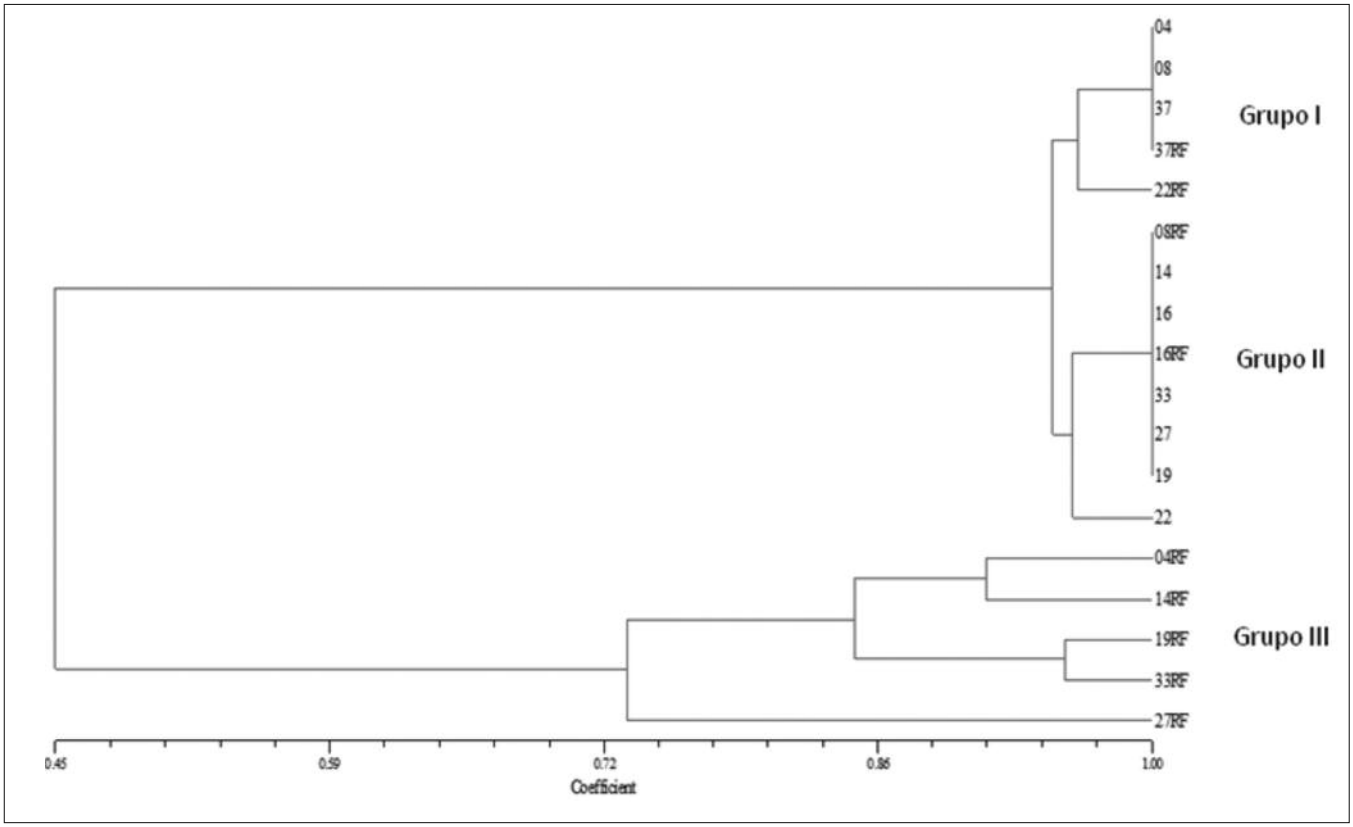


Figure 2. Distribution of DNA fragments of buccal *C. albicans* of CwDS and P and/or C in electrophoretic profile using the primer RSD 12 for RAPD. (MM – molecular marker and FR – familial relationship – P and/or C)



Graph 1. Dendrogram of genetic similarity (coefficient of Jaccard) among the conjugated pairs of buccal *C. albicans* of CwDS and P and/or C, obtained using the primers RSD 10 and RSD 12 (Groups I, II and III were divided according to genetic similarity)

Dendograms showed *C. albicans* isolate distribution into three groups containing five (Group I), eight (Group II) and five (Group III) individuals. In Group I, four individuals presented homology (coefficient 1), while in Group II there were eight genetically homologous individuals. Groups I and II were highly correlated (coefficient of similarity 0.95); however, Group III was not correlated to a significant degree with Groups I or II (coefficient of similarity 0.45). These results suggest genetic diversity among the members of Group III compared with Groups I and II.

Discussion

The genetic homology of the pairs, 16-16RF and 37-37RF of *C. albicans* concomitantly isolated from the mouths of CwDS and P

and/or C, and analyzed by RAPD, showed intrafamilial transmission (Figures 1, 2 and Graph 1). Samaranayake *et al.*[19] (2003), using the same primers for RAPD in *C. albicans* samples from HIV patients with and without buccal candidiasis, found intraspecific polymorphism among the yeasts of this same fungus species. The homologous genetic aspect of *C. albicans* was also seen in horizontal transmission of mother and newborn during an episode of candidaemia[25].

The difficulty in finding primers for intraspecific analysis of yeasts is a factor hampering homology detection among the pairs of *C. albicans* analyzed[19].

The genetic likeness of two pairs (16-16RF and 37-37RF) of *C. albicans* from the mouths of CwDS and P and/or C demonstrated by the RAPD technique and dendograms suggest the intrafamilial transmission of this yeast (Graph 1).

The genetic similarity between the pairs of *C. albicans* isolated concomitantly from the mouths of CwDS and P and/or R in this study (Table 1) demonstrated *Candida* samples of the buccal microbiota of each pair of individuals of the intrafamilial relationship analyzed. There may also be other *Candida* stains present at the time of collection of saliva samples, as occurs with vaginal transmission of microorganisms between mother and infant during childbirth [5,7,9]. The need for more frequent medical and laboratorial care in CwDS from birth onwards, exposes them to other sources of *Candida* transmission, besides those presented by health professionals [3,6-7,15]. The microbiota of the mouths these children is thus in contact with *Candida* from numerous sources[1,6-9,11,26].

A higher degree of genomic similarity of *Candida sp.* has been found in interspecific analysis of species of yeast samples. Pinto[27] (2003), Resende *et al.*[2] (2004), and Valério *et al.*[28] (2004), analyzed samples of *Candida* species from clinical and hospital sources, finding interspecific differentiation of the genome polymorphism of the species of yeasts, using RAPD and several interspecific primers (M13 (F/R), OPA, OPA01, OPA02, OPA03, OPA08, OPA09, RP1-4, RP-2 RP4-2 and SOY). The intraspecific genetic similarity among *C. albicans* samples from several nosocomial specimens has been found to range from 49 to 91% among isolates analyzed[13]. Profiles of RAPD demonstrating up to 85% similarity have been detected among *C. albicans* strains in the buccal cavity of immunodeficient individuals[18], diabetics, dental prosthesis users and hemodialyzed patients[29]. Our results were similar results to those of the cited study, where we found genetic similarity between buccal *C. albicans* yeasts of CwDS and P and/or C ranging from 45 to 100% (Figure 1). The finding of five isolates of *C. albicans*, genetically different from the others isolated, belonging to P and/or C (04RF, 14RF, 19RF, 27PF and 33RF) indicates the possibility of genotypic shuffling or genetic drift of the present yeasts in the buccal cavity of these individuals. Samaranayake *et al.*[19] (2003) found genetic drift in isolated strains of *C. albicans* from HIV-positive patients in sequential collections in a 12-month study

Conclusion

The analysis of genetic similarity of the pairs of buccal *C. albicans*, isolated concomitantly from children with Down's syndrome and parents and/or caregivers, proved the intrafamilial transmission of this fungus between parents and their Down´s syndrome children. Additionally, some isolates showed high genetic variability indicating the possibility of genetic drift in *C. albicans* from Down's syndrome children and their parents and/or caregivers.

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References

1. Linossier A, Vargas A, Villegas R, Chimenos E. Quantitative relationship between salivary level of *S. mutans* and *C. albicans* in children with Down's syndrome. Med Oral. 2002; 7:284-92.

2. Resende JCP, Franco GR, Rosa CA, Hahn RC, Hamdan JS. Phenotypic and genotypic identification of *Candida* spp. Isolated from hospitalized patients. Rev Iberoamer Micol. 2004;21:24-8.

3. Lacaz CS, Porto E, Martins JEC. Micologia médica. São Paulo: Sarvier; 1991.

4. Pardi G, Cardoso EI. Algunas consideraciones sobre *C. albicans* como agente etiológico de candidiasis bucal. Acta Odontol Venez. [periódico on-line]. 2002; 40 [acesso 5 ago 2006]. Disponível em: http://www.actaodontologica.com

5. Ribeiro EL. Leveduras de *Candida* isoladas da boca de crianças com e sem síndrome de Down: aspectos feno-genotípicos, relação intrafamilial e perfil de imunoglobulinas. [tese de doutorado] Brasília, DF: Faculdade de Ciências da Saúde, Universidade de Brasília; 2008.

6. Amorim STSP, Moreira H, Carraro TE. Amamentação em crianças com síndrome de Down: a percepção das mães sobre a atuação dos profissionais de saúde. Rev Nutr. 1999;12:91-101.

7. Carvalho FG, Parisotto TM, Hebling J, Spolidorio LC, Spolidorio DMP. Presence of *Candida* spp in infants oral cavity and its association with early childhood caries. Braz J Oral Sci 2007;6:1249-53.

8. Menezes EA, Guerra ACP, Rodrigues RCB, Peixoto MML, Lima LS, Cunha FA. Isolamento de *Candid*a spp. no mamilo de lactantes do Banco de Leite Humano da Universidade Federal do Ceará e teste de suscetibilidade a antifúngicos. J Bras Patol Med Lab. 2004;40:299-305.

9. Scherma AP, Santos DVO, Jorge AOC, Rocha FC. Presença de *Candida* spp na cavidade bucal de lactantes durante os primeiros quatro meses de vida. Ciênc Odontol Bras. 2004;7:79-86.

10. Coelho CRZ, Loevy, HT. Aspectos odontológicos da Síndrome de Down, ARS Cvr Odontol. 1982;8:9-16.

11. Roncari AM, Rodrigues AB, Elias MS. Síndrome de Down e Odontologia. Investigação. Rev Cient Univ Franca. 2002;4(6):70-4.

12. Mustacchi Z; Rozone G. Síndrome de Down: aspectos clínicos e odontológicos. São Paulo: 1990. p.52-98.

13. Duran EL, Mujica MT, Jewtuchowicz VM, Finquelievich JL, Pinoni MV, Iovannitti CA. Estudio de la variabilidade genetic entre aislamientos clínicos de *C. albicans* formadores de biopelícula. Rev Iberoamer Micol. 2007;24:266-71.

14. Magee PT, Magee BB. Recent advances in the genomic analysis of *C. albicans*. Rev Iberoamer Micol. 2005;22:187-93.

15. Ribeiro EL, Guimarães RI, Inácio MCC, Ferreira WM, Cardoso CG, Dias SMS, *et al*. Aspectos das leveduras de *Candida* vinculadas às infecções nosocomiais. Newslab. 2004;64:106-28.

16. Carlstedt K, Krekmanova, L, Dahllof G, Ericsson B, Braathen G, Odeer. T. Oral carriage of *Candida* species in children and adolescents with Down's syndrome. Int J Paediatr Dent. 1996;6:95-100.

17. Lehmann PF, Lin D, Lasker BA. Genotypic identification and characterization of species and strains within the genus *C. albicans* in children with Down's syndrome. Med Oral. 2002;7:284-92.

18. González H, Silva I, Reyes E. Caracterización molecular de cepas de *C. albicans* aisladas de candidiasis oral em personas HIV positivo. Cienc Trab. 2006; 22:172-6.

19. Samaranayake YH, Samaranayake LP, Dassanayake RS, Yan JYY, Tsang WK, Cheung BPK *et al*. Genotypic shuffling of sequential clones of *C. albicans* in HIV-infected individuals with and without symptomatic oral candidiasis. J Med Microbiol. 2003;52:349-59.

20. Steffan P, Vazquez IH, Bolkow D, Xu C, Sobel JD, Alkins RA. Identification of *Candida* species by RAPD-PCR fingerprinting of colony lysates. J Clin Microbiol. 1997;35:2031-9.

21. Kreeger-Van Rij NJW. The yeast: a taxonomic study. Amsterdam: Elsevier; 1984.

22. Del Poeta M, Toffaletti DL, Rude TH, Dykstra CC, Heitman J, Perfect JR. Topoisimerase I is essential in *C. neoformans* role in pathology and as an antifungal target. Genetics. 1999;152:176-8.

23. Casali AK, Goulart L, Silva LKR, Ribeiro AM, Amaral AA, Alves SH. Molecular typing of clinical and environmental *C. neoformans* isolates in the Brazilian state Rio Grande do Sul. FEMS Yeast Res. 2003;33C:405-15.

24. Dassanayake RS, Samaranayake LP. Amplification-based nucleic acid scanning techniques to assess genetic polymorphism in *Candida*. Crit Rev Microbiol. 2003;29:1-24.

25. Tiraboschi IN, Carnovale S, Benetucci A, Fernández N, Kurlat I, Focolli MY, *et al*. Brote de candidemia por *C. albicans* em neonatologia. Rev Iberoamer Micol. 2007;24:263-7.

26. Pimenta FC, Marin JM, Uzeda M, Ito Y. Prevalência de estreptococos do grupo mutans e leveduras na saliva de 93 membros de seis famílias. Rev Patol Trop. 2001;30:15-22.

27. Pinto MP. Caracterização fenotípica e análise da variabilidade de genética de espécies do gênero *Candida* isoladas de pacientes portadores e não portadores de doença de base. [tese de doutorado]. Belo Horizonte: Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais; 2003.

28. Valério HM, Weikert-Oliveira RCB, Resende MA. Differentiation of *Candida* obtained from nosocomial candidaemia using RAPD-PCR technique. Rev Soc Bras Med Trop. 2006;39:174-8.

29. Pirez-Gonçalvez RH, Miranda ET, Baeza LC, Matsumoto MT, Zaia JE, Mendes-Giannini MJS. Genetic relatedness of conmensal strains of *C. albicans* carried in the oral cavity of patients dental prosthesis user in Brazil. Mycopathologia. 2007; 164:255-63.

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