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 children 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

**Introduction**

*Candida* is a diploid fungus present in mouth microbiota throughout the lifetime of the individual¹-². 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 syndrome³-⁵.

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 children¹,²,⁶-⁹.

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 functional colonization process¹⁰-¹². These factors can be exacerbated by the difficulty in maintaining good oral hygiene, a diet rich in carbohydrates and immune system compromised¹³-⁴,⁷,¹⁰,¹¹.

*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.¹³-⁵,¹⁰,¹¹,¹⁴-¹⁵

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 group¹³-⁴,¹⁰,¹¹,¹⁴-¹⁵.

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 loci for the genome of different organisms and microorganisms. In this test, oligonucleotides (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.\textsuperscript{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\textsuperscript{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.\textsuperscript{22} (1999) and modified by Casali et al.\textsuperscript{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 \textsuperscript{19} 5’-CCGCCAGCCA-3’ and RSD12 \textsuperscript{19} 5’-GGTCCGTGTTTCAAGAC G-3’ (IDT Technologies).\textsuperscript{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.\textsuperscript{17,20} The PCR products were separated in agarose gels (1-2%) and electrophoresis was performed at room temperature in TBE buffer (89 mM 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\textsuperscript{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 C. albicans 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 similar 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)**

<table>
<thead>
<tr>
<th>Isolated pairs</th>
<th>Similarity coefficient</th>
<th>Percentage similarity</th>
</tr>
</thead>
<tbody>
<tr>
<td>04 – 04FR</td>
<td>0.45</td>
<td>45%</td>
</tr>
<tr>
<td>08 – 08FR</td>
<td>0.95</td>
<td>95%</td>
</tr>
<tr>
<td>14 – 14FR</td>
<td>0.45</td>
<td>45%</td>
</tr>
<tr>
<td>16 – 16FR</td>
<td>1.0</td>
<td>100%</td>
</tr>
<tr>
<td>19 – 19FR</td>
<td>0.45</td>
<td>45%</td>
</tr>
<tr>
<td>22 – 22FR</td>
<td>0.95</td>
<td>95%</td>
</tr>
<tr>
<td>27 – 27FR</td>
<td>0.45</td>
<td>45%</td>
</tr>
<tr>
<td>33 – 33FR</td>
<td>0.45</td>
<td>45%</td>
</tr>
<tr>
<td>37 – 37FR</td>
<td>1.0</td>
<td>100%</td>
</tr>
<tr>
<td>Mean</td>
<td>0.68</td>
<td>68%</td>
</tr>
</tbody>
</table>

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)
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
Additionally, some isolates showed high genetic variability indicating intrafamilial transmission of 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 23.

The difficulty in finding primers for intrafamilial 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 dendrograms suggest the intrafamilial transmission of this yeast (Figure 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 laboratory 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 1,6,9,11,12,16.

A higher degree of genomic similarity of Candida sp. has been found in interspecific analysis of species of yeast samples. Pinto 22 (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 interspecific genetic similarity among C. albicans samples from several nosocomial specimens has been found to range from 49 to 91% among isolates analyzed 19. 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 (O4RF, 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. 23 (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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