Acta Odontologica Scandinavica

ISSN: 0001-6357 (Print) 1502-3850 (Online) Journal homepage: https://www.tandfonline.com/loi/iode20

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To cite this article: Carla Paola Lozano Moraga, Gonzalo Andrés Rodríguez Martínez, Claudia Andrea Lefimil Puente, Irene Cecilia Morales Bozo & Blanca Regina Urzúa Orellana (2017) Prevalence of *Candida albicans* and carriage of *Candida* non-*albicans* in the saliva of preschool children, according to their caries status, Acta Odontologica Scandinavica, 75:1, 30-35, DOI: 10.1080/00016357.2016.1244560

To link to this article: https://doi.org/10.1080/00016357.2016.1244560

ACTA ODONTOLOGICA SCANDINAVICA, 2017
VOL. 75, NO. 1, 30–35
http://dx.doi.org/10.1080/00016357.2016.1244560

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ORIGINAL ARTICLE

Prevalence of *Candida albicans* and carriage of *Candida* non-*albicans* in the saliva of preschool children, according to their caries status

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ABSTRACT

Objective: This study was conducted to establish associations among the *Candida* carriage rate, the diversity of *Candida* species carried and the different caries status of preschool children.

Materials and methods: Sixty-one children between 2 and 5 years of age were examined by a single expert examiner and were divided into three groups, the caries-free, moderate caries and severe caries groups, according to the criteria of the International Caries Detection and Assessment System II (ICDAS). Saliva samples were obtained from the members of each group and were plated on Sabouraud agar plates to assess the *Candida* carriage rates. CHROMagar *Candida* medium was used for the preliminary screening. Biochemical testing or PCR/sequencing was conducted to identify the different *Candida* species in the samples. The differences observed were considered significant if the p value was <0.05.

Results: The *Candida* carriage rate and the number of species of this fungus carried were higher in the group with the highest level of caries severity ($p < 0.05$). Whereas *Candida albicans* was the most predominant *Candida* species in the saliva of all of the children, *C. dubliniensis* was identified only in the most caries-affected group in addition to other rare species of *Candida* non-*albicans*.

Conclusions: A high salivary *Candida* carriage rate and the presence of specific species of this fungus (such as *C. albicans* and *C. dubliniensis*) appear to be related to the severity of caries experienced by preschool children.

ARTICLE HISTORY
Received 30 April 2016
Revised 27 September 2016
Accepted 30 September 2016

KEYWORDS
Candida species; carriage; dental caries; ICDAS II

Introduction

Hundreds of different microbial communities that compose biofilms can be found in the human oral cavity.[1,2] Only 54% of the species in these microbial communities have been officially identified in the Human Oral Microbiome Database (HOMD; www.homd.org). While much remains to be understood, the species within the biofilms live as commensal microorganisms or as competitors involved in highly complex interactions (reviewed in [3]). In addition to bacteria, *Candida* species are also common commensal colonizers of the oral cavity of healthy people, with an ~50% *Candida* carriage rate observed in this population.[4] Under certain conditions such as the disturbance of the local microenvironment, which can result in an imbalance called dysbiosis,[5] this commensal fungus becomes an opportunistic pathogen [6] called a pathobiont, similar to other microorganisms associated with oral diseases.[7] In recent years, *Candida* has been associated with dental caries in children, and pathogenic *Candida* spp. have been isolated at a high frequency from either saliva or supragingival dental plaque samples collected from children with caries lesions compared to the frequency in caries-free subjects. However, the role of *Candida* spp. in the initiation or progression of a caries lesion remains uncertain. *Candida* species, such as *Candida albicans* (the most frequently isolated species) and *Candida* non-*albicans* have been isolated from the oral cavities of subjects with and without caries. [8–10] To date, studies have focused on the presence of this fungus in individuals with oral diseases (such as periodontal disease [11] and denture stomatitis,[12] in addition to dental caries), but less attention has been paid to analyzing the diversity of *Candida* species in children with different dental caries experiences. The complement of emerging and poorly known members of the oral fungal microbiota, including *Candida*, has restricted investigations of the interrelations between oral bacteria and fungi and the roles that they play in health and disease.[13]

Candida spp. have several virulence traits that affect caries development, such as the abilities to adhere to tooth surfaces and degrade proteins and the extracellular matrix,[14] to colonize the oral mucosa, ferment carbohydrates [15] (contributing to oral acidity) and produce extracellular enzymes, all of which contribute to their pathogenicity.[14,16]

It would be interesting to analyze the conditions of the oral cavity microenvironment that affect the dental caries status. These conditions might increase the carriage rate and counts of *Candida* in individuals with caries, as well as lead to a change in the contributions of other species, including

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Candida spp., to the oral microbiome. These results obtained in such a study might provide relevant information for the development of a novel caries therapy, based not only on bacterial targets but also on the fungi present in oral biofilms, which might create conditions allowing the transition from health to disease. It is a relevant question with very limited answers to date.[13]

The aim of this study was to evaluate the *Candida* carriage rate and the diversity of *Candida* species, other than *C. albicans*, present in the saliva and their associations with the different caries status of preschool children.

Materials and methods

Subjects

Our non-probabilistic sample included 61 children (girls and boys) between 2 and 5 years of age who were selected based on the presence/absence of carious lesions. Of these participants, 30 children had caries and 31 children were caries-free. All of the participants (Chileans) were recruited from a single kindergarten located in the northern area of the Santiago metropolitan region in Chile. The oral examinations were performed by a single trained and expert dentist using a dental mirror, a probe and head-mounted LED light. The status of the visually evident dental caries was determined following the ICDAS II criteria.[17] The children in the caries-free group did not exhibit caries lesions (ICDAS II Code 0). The children in the group with caries were divided between those who had moderate caries (ICDAS II Codes 2–3) and those who had severe caries (ICDAS II Codes 4–6). The study was performed in accordance with the principles of the Declaration of Helsinki. The protocol of the study and the informed consent form were approved by the Ethics Committee of the Faculty of Dentistry at the University of Chile (certificate no. 2011/03). The inclusion criteria were an informed consent form signed by the parents of each child after they had heard a description of the study. The exclusion criteria were receiving antibiotic or antifungal treatment within the previous 6 months, the presence of chronic diseases, such as allergies and diabetes, and the existence of any condition that affected salivary flow.

Saliva samples

A total of 2 mL of unstimulated whole saliva was collected from each child in the morning between 10–12 AM at least 2 h after breakfast by the child spitting or through direct sampling using with a soft plastic Pasteur pipette. The children were allowed to consume food until 8 AM, that is, up to 2 h before sampling. The samples were placed in sterile plastic vials maintained at 4 °C and were immediately transported to the laboratory for microbiological analysis.

Isolation of Candida

One milliliter of unstimulated whole saliva was homogenized for 30 s using a vortex mixer (Labnet International, Edison, New Jersey) and was then diluted using phosphate-buffered saline (PBS; 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4 and 2 mM KH_2PO_4, pH 7.4). A 100-μL aliquot of the undiluted unstimulated whole saliva and an equal volume of the 1:10 (v/v) diluted sample were plated in duplicate on Sabouraud dextrose agar (SDA; BD, BBL, Franklin Lakes, NJ) medium supplemented with tetracycline (50 μg/mL) to allow the development of *Candida* colonies. The plates were incubated at 37 °C for 48–72 h under aerobic conditions. After the incubation period ended, phenotypically different isolates that resembled *Candida* genus colonies were randomly selected, sub-cultivated, and then grown in 5 mL of Sabouraud dextrose broth (Oxoid, Basingstoke, New Hampshire, UK) under the growth conditions described above. Aliquots of the cultures were stored in 20% glycerol at −80 °C until further analysis. SDA was used as the primary isolation medium to allow recognition of the morphological characteristics of *Candida* colonies (convex and cream-colored) and to determine the number of colony-forming units per milliliter of saliva, although we finally dichotomized the children based on the presence/absence of salivary *Candida*.

Identification of Candida *species*

For the preliminary identification of the *Candida* species in the samples, all of the *Candida* isolates were plated on CHROMagar *Candida* medium (CHROMagar™, Paris, France). To determine the levels of *C. albicans* and *C. dubliniensis* in the samples, isolated colonies were selected according to their coloration on the selective medium (light-green and dark-green colonies for *C. albicans* and *C. dubliniensis*, respectively).[18] To confirm the presence of both species, the *HWP1* gene was amplified using PCR.[19] Other *Candida* species distinct from *C. albicans*/*C. dubliniensis* (colonies of other colors) were identified using the Api ID 32C (bioMérieux, Marcy-l'Etoile, Lyon, France) biochemical test according to the manufacturer's protocol. When the results of this biochemical *Candida* spp. identification test were ambiguous, the ITS1-5.8S-ITS2 rDNA region of each clinical isolate was sequenced using the universal fungal ITS1 and ITS4 primers.[20] The DNA amplicons obtained from the isolates were sequenced by Macrogen Inc. (Seoul, Republic of Korea). The sequences obtained and their levels of homology based on sequence alignment were analyzed using the web-based program BLASTn (Basic Local Alignment Search Tool) (http://blast.ncbi.nlm.nih.gov/) provided by the NCBI (National Center for Biotechnology Information).

Statistical analysis

The Shapiro–Wilk test was used to evaluate the normality of the distribution of the data. The chi-squared and Kruskal–Wallis tests were used to compare the groups. The data were analyzed using Stata SE version 14.0 software (StataCorp LP, College Station, Texas). Differences were considered significant when the p value was < 0.05.

Results

The average age of the 61 children enrolled in the study was 3.4 ± 0.12 years, of which 27 were males (44.3%) and 34 were

females (55.7%). The average age of the individuals with caries was 3.6 years, whereas that of the caries-free individuals was 3.2 years. There were 12 males (40%) and 18 females (60%) in the caries groups, and there were 15 males (48.4%) and 16 females (51.6%) in the caries-free group. A total of 31 individuals were caries-free (ICDAS II Code 0), whereas 30 individuals had caries. Of these, 12 individuals (40%) had ICDAS II Code 2–3 caries and 18 individuals (60%) had ICDAS II Code 4–6 caries.

Thirty of the 61 participants carried *Candida* in their saliva (49.2%). In the caries-free group, there were 11 individuals (of 31 total) that carried this fungus (35.5%); in the moderate caries group, there were six individuals (of 12 total) that carried this fungus (50%); and in the group with severe caries, there were 13 individuals (of 18 total) that carried this fungus (72.2%). Thus, analysis of the *Candida* carriage rates showed that a higher percentage of individuals in the severe caries group carried this fungus compared with that of the moderate caries group or the caries-free group ($p < 0.05$). Interestingly, the *Candida* carriage rate was higher in the group of individuals who had a greater severity of carious lesions. In contrast, the *Candida* carriage rate was the lowest in the group of individuals who were caries-free.

The *Candida* species that were isolated from the saliva samples and were identified are listed in Table 1. *Candida albicans* was found in all of the groups but was more prevalent in the group with severe caries ($p < 0.05$). Of the 135 colonies isolated from the 13 individuals in this group who carried *Candida*, 102 (75.6%) were identified as *C. albicans* colonies. Additionally, a larger variety of *Candida* species were isolated from this group, including *C. dubliniensis*, *C. pulcherrima*, *C. lusitaniae*, *C. parapsilosis*, *C. tropicalis* and *C. rugosa*, in addition to *C. albicans* (for the number of colonies and the frequency of the species identified, see Table 1). All of the *Candida* colonies isolated from the individuals in the moderate caries group ($n = 33$, corresponding to 100%) were identified as *C. albicans* colonies and no *Candida* non-*albicans* colonies were detected. One-hundred *Candida* colonies were isolated from the 11 individuals in the caries-free group that carried *Candida*, and 46 of these (46%) were *C. albicans* isolates, 28 were *C. lusitaniae* isolates (28%) and 26 were *C. parapsilosis* isolates (26%). Therefore, the number of *Candida* species identified in the caries-free group (three species) was lower than that of the severe caries group (seven species), and one *Candida* species was identified in the moderate caries group.

Discussion

This study demonstrated a correlation between the presence of *Candida* spp., principally *C. albicans*, and a high prevalence of *Candida* carriage in children with dental caries, specifically children with the highest ICDAS II Code (4–6) caries. It can be speculated that the prevalence of this fungus would be increased under this pathological condition.

A diet high in carbohydrates is a rich source of nutrients for *Candida*, which generates acidic products through fermentation. Thus, these microorganisms contribute to the maintenance of a lower than neutral salivary pH,[15] which is associated with dental caries. Furthermore, this low pH is optimal for the activity of extracellular enzymes (such as aspartyl proteinases), which are considered one of the most important virulence factors of this fungus.[16] Some of these enzymes are expressed under the following conditions: (i) when *Candida* is in biofilms containing streptococci,[21] (ii) in the oral cavities of healthy individuals carrying *Candida* and (iii) in individuals with oral candidiasis.[16]

Our results show that most of the salivary isolates identified as *Candida* species were *C. albicans*, as previously reported. *C. albicans* is considered the most pathogenic species of its genus and it was highly prevalent in the three groups analyzed in this study. In this study, other species of this genus were also isolated, with *C. dubliniensis* only from the group with severe caries in addition to *C. pulcherrima* and *C. rugosa*. Therefore, this group had the highest level of carriage of *Candida* non-*albicans* species (six species) (Table 1), which agrees with the results of De la Torre et al.,[22] who identified 11 species of *Candida* non-*albicans* in subjects with caries. Only *C. albicans* was present in the moderate caries group, and this species was also found in the caries-free group, in addition to two *Candida* non-*albicans* species.

Our study demonstrated *Candida* carriage in the oral cavities of approximately half of the subjects, as it has been found in other studies of similar subjects.[8,10] We can state that under healthy oral conditions (caries-free), a lower level of *Candida* carriage exists and that few species of this genus

Table 1. Identification of *Candida* species isolated from the saliva of children in the ICDAS II Code 2–3 and 4–6 caries groups and the caries-free group.

	Isolated colonies *n* (%)			
	Caries lesions (ICDAS II Codes)			
Candida species	2–3 ($N = 6/12$)	4–6 ($N = 13/18$)	Caries-free ($N = 11/31$)	Total identified isolates
Candida albicans	33 (100)	102 (75.6)*	46 (46)	181
Candida lusitaniae	0	3 (2.2)	28 (28)	31
Candida parapsilosis	0	2 (1.5)	26 (26)	28
Candida dubliniensis	0	15 (11.1)	0	15
Candida tropicalis	0	1 (0.74)	0	1
Candida pulcherrima	0	11 (8.1)	0	11
Candida rugosa	0	1 (0.74)	0	1
Total	33	135	100	268

The composition of *Candida* species identified is expressed as the number of isolated colonies and their percentage (%). the microbiological analysis was performed using 1-mL samples of whole unstimulated saliva.
*$p < 0.05$, as determined using the non-parametric Kruskal–Wallis test.
N: number of individuals in each group carrying particular *Candida* species..

are present in the oral cavity. However, we isolated only 10% of the colonies of the fungus from each individual that carried *Candida* for identification at species level. Therefore, although this strategy was sufficient for the proposed objective, we believed that more *Candida* species could have been detected in all groups, particularly in the moderate caries group.

Candida dubliniensis is not generally found in the oral microbiota, being present in approximately 3.5% of healthy individuals.[23] Surprisingly, this species has been isolated from the plaque and caries dentine of the primary teeth of children with early childhood caries [24] and from the saliva of children with active caries.[25] Thus, this study is most likely the first in which this *Candida* species was isolated from the saliva of children with severe caries lesions. *Candida* non-*albicans* species are known to cause candidiasis of reduced virulence, which is attributed to their lack of all or part of the virulence factors that *C. albicans* possesses. Therefore, the participation of these species is not known in dental caries.

Candida parapsilosis and *C. tropicalis*, have been isolated from the oral samples [10,22,26] of individuals with or without oral lesions but always with less frequency compared with that of *C. albicans*. *Candida lusitaniae* is an emerging opportunistic nosocomial pathogen, whose presence has been detected in samples of root-caries lesions [27] and in individuals with oral candidiasis.[28] This species is of interest because it is resistant to fluconazole and can develop resistance to amphotericin B and caspofungin during clinical treatment (reviewed in [29]). The occurrence of *C. pulcherrima* and *C. rugosa* in the participants in this study merits attention because these species are rarely detected in the oral cavity.[26,27] The uncommon presence of these species could have been due to the participants having received long-term antibiotic treatment.

There is sufficient scientific evidence for the presence of *Candida* in the oral cavities of subjects with or without dental caries,[8–10] but these studies did not determine which species were present under each condition or which of the species predominated. To date, the role of *Candida* in the development and progression dental caries is not entirely clear, although some recent analyses have helped to elucidate this issue. For example, experiments of ÓSullivan et al. [30] demonstrated the presence of basic proline-rich proteins (bPRP) in the saliva, which acted as receptors for *C. albicans*, allowing it to adhere to the enamel pellicle and oral streptococci. Moreover, it was reported that *Candida* spp. can dissolve dentine and consequently cause the release of calcium into the microenvironment at different rates.[31]

Taken together, the above evidence supports the colonization and persistence of *C. albicans* in the oral cavity, but it has not been determined which factor(s) is (are) the functional contributors of *Candida* and which factor(s) is (are) directly involved in the pathogenesis of dental caries based on the prevalent metabolic activities performed by the dominant bacteria associated with this disease. This situation is complex, and a full understanding of it is far from being achieved. How such different microorganisms interact and organize in oral biofilms during health or in caries are topics that deserve further investigation.[13] Research efforts should be directed toward clarifying whether the increase in the number of oral *C. albicans* is a result of factors directly involved in the onset of the carious process or whether this fungus plays a direct role in creating the conditions favoring this disease.

It is important to note that the average numbers of *Candida* CFUs observed in the samples from the three test groups did not accurately represent the counts because there was a high level of intra-group variability. The samples from individuals with caries and those carrying *Candida* has $<$100 CFU/mL, with some exceptions in which $>$500 CFU/mL were found. In contrast, in samples from the caries-free group, an average of 26 CFU/mL was found, with some exceptions in which values between 200 and 600 CFU/mL were observed. Thus, we cannot state that *Candida* counts $>$400 CFU/mL are characteristic of individuals with caries.[32] In such individuals, the effects of other factors on oral *Candida* colonization cannot be ignored, such as the salivary pH, salivary flow rate, buffering capacity of the saliva, and the characteristics of the microenvironment that this fungus colonizes. These factors could affect the *Candida* carriage of individuals with and without caries. Due to the high variability in the intra-group *Candida* colony counts, we decided to dichotomize the participants into groups according to the presence and absence of *Candida*.

Importantly, to obtain representative and similar samples containing the species present in the oral cavity, we decided upon the strategy of collecting unstimulated saliva using a non-invasive, simple method,[33] which is an important consideration for studying preschool children.

In spite of the limitations, the results obtained in this study demonstrated the rate of *Candida* carriage, mainly *C. albicans*, in the saliva of preschool children with different severities of caries lesion development. Because *C. albicans* was the *Candida* species most frequently isolated from the saliva of children with caries lesions, it is important to analyze the genetic variability of the clinical isolates of this species and to evaluate the possible associations of genotypes of this fungus with the development and promotion of caries. Additionally, more research on the interaction between *Candida* non-*albicans* spp. and bacteria is necessary because some of the former are emerging species that have been isolated from individuals with caries, although at low frequencies. More *in situ* and *in vivo* caries models must be developed to determine how and when the virulence and competition factors of *Candida* are expressed during the onset and progression of caries. The use of such models will facilitate understanding which biotic (such as the prevalence of cariogenic bacteria) or abiotic (such as a low pH) signals elicit a change in the composition and abundance of *Candida* in the oral microbiota of individuals with a healthy oral cavity and those with dental caries. Finally, it is also important to characterize the oral mycobiome in health and disease (such as dental caries) to gain knowledge about the role of fungi in the oral cavity.

Conclusions

A high *Candida* carriage rate and the presence of diverse *Candida* species in the saliva of preschool children,

particularly *C. albicans* and *C. dubliniensis*, appear to be related to their caries severity. The results of our study strongly suggests an ecological contribution of *Candida* to the process of caries formation, although this relationship was not examined in this investigation.

Acknowledgements

The authors thank Dr. R. Giacaman (Department of Oral Rehabilitation, Cariology Unit, University of Talca, Talca, Chile) for helpful comments and are grateful for the support of this study by the students of dentistry at the University of Chile. Thanks to Mr. Juan Fernández, from the Language and Traslation services of the Faculty for kindly proofreading and checking the spelling and grammar of this article.

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

Funding

National Commission for Scientific and Technological Research (Conicyt), The Chilean Government [Postdoctoral Fondecyt 3120164 to C. Lozano].

Notes on contributors

Carla Paola Lozano Moraga coordinated and conducted this investigation and is the PI of the major project that gave rise to it. G.R.M. performed the oral examinations of all of the subjects. C.L.P. performed part of the laboratory work. I.M.B. and B.U.O. contributed to the design of the study. All of the authors evaluated and discussed the results. C.L.M. drafted the article, and all of the authors read and approved the final article.

References

[1] Takahashi N, Nyvad B. The role of bacteria in the caries process: ecological perspectives. J Dent Res. 2011;90:294–303.
[2] Marsh PD. Dental plaque as a biofilm and a microbial community - implications for health and disease. BMC Oral Health. 2006;6(Suppl. 1):S14.
[3] Filoche S, Wong L, Sissons CH. Oral biofilms: emerging concepts in microbial ecology. J Dent Res. 2010;89:8–18.
[4] Samaranayake LP, Fungi of relevance to dentistry. Essential microbial for dentistry. 3rd ed. Edinburgh, UK: Churchill Livingstone; 2006.
[5] Fejerskov O. Changing paradigms in concepts on dental caries: consequences for oral health care. Caries Res. 2004;38:182–191.
[6] Kam A, Xu J. Diversity of commensal yeasts within and among healthy hosts. Diagn Microbiol Infect Dis. 2002;43:19–28.
[7] Simón-Soro A, Mira A. Solving the etiology of dental caries. Trends Microbiol. 2015;23:76–82.
[8] Moalic E, Gestalin A, Quinio D, et al. The extent of oral fungal flora in 353 students and possible relationships with dental caries. Caries Res. 2001;35:149–155.
[9] Signoretto C, Burlacchini G, Faccioni F, et al. Support for the role of *Candida* spp. in extensive caries lesions of children. New Microbiol. 2009;32:101–107.
[10] Raja M, Hannan A, Ali K. Association of oral candidal carriage with dental caries in children. Caries Res. 2010;44:272–276.
[11] Urzúa B, Hermosilla G, Gamonal J, et al. Yeast diversity in the oral microbiota of subjects with periodontitis: *Candida albicans* and *Candida dubliniensis* colonize the periodontal pockets. Med Mycol. 2008;46:783–793.
[12] Chopde N, Jawale B, Pharande A, et al. Microbial colonization and their relation with potential cofactors in patients with denture stomatitis. J Contemp Dent Pract. 2012;13:456–459.
[13] Diaz PI, Strausbaugh LD, Dongari-Bagtzoglou A. Fungal-bacterial interactions and their relevance to oral health: linking the clinic and the bench. Front Cell Infect Microbiol. 2014;4:101–106.
[14] Li W, Yu D, Gao S, et al. Role of *Candida albicans*-secreted aspartyl proteinases (Saps) in severe early childhood caries. Int J Mol Sci. 2014;15:10766–10779.
[15] Falsetta ML, Klein MI, Colonne PM, et al. Symbiotic relationship between *Streptococcus mutans* and *Candida albicans* synergizes virulence of plaque biofilms *in vivo*. Infect Immun. 2014;82:1968–1981.
[16] Naglik JR, Nweport G, White TC, et al. *In vivo* analysis of secreted aspartyl proteinase expression in human oral candidiasis. Infect Immun. 1999;67:2482–2490.
[17] Ismail AI, Sohn W, Tellez M, et al. The International Caries Detection and Assessment System (ICDAS): an integrated system for measuring dental caries. Community Dent Oral Epidemiol. 2007;35:170–178.
[18] Odds FC, Bernaerts R. CHROMagar *Candida*, a new differential isolation medium for presumptive identification of clinically important *Candida* species. J Clin Microbiol. 1994;32:1923–1929.
[19] Romeo O, Racco C, Criseo G. Amplification of the hyphal wall protein 1 gene to distinguish *Candida albicans* from *Candida dubliniensis*. J Clin Microbiol. 2006;44:2590–2592.
[20] White TJ, Bruns T, Lee S, Taylor J. PCR protocols: a guide to methods and applications. In: Innis M, Gelfand D, Sninsky J, White T, editors. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Academic Press: Orlando, FL; 1999:315–322.
[21] Cavalcanti YW, Wilson M, Lewis M, et al. Modulation of *Candida albicans* virulence by bacterial biofilms on titanium surfaces. Biofouling. 2016;32:123–134.
[22] De la Torre J, Marichalar-Mendia X, Varona-Barquin A, et al. Caries and *Candida* colonisation in adult patients in Basque Country (Spain). Mycoses. 2016;59:234–240.
[23] Pinjon E, Moran GP, Coleman DC, et al. Azole susceptibility and resistance in *Candida dubliniensis*. Biochem Soc Trans. 2005;33:1210–1214.
[24] Kneist S, Borutta A, Sigusch BW, et al. First-time isolation of *Candida dubliniensis* from plaque and carious dentine of primary teeth. Eur Arch Paediatr Dent. 2015;16:365–370.
[25] Al-Ahmad A, Auschill TM, Dakhel R, et al. Prevalence of *Candida albicans* and *Candida dubliniensis* in caries-free and caries-active children in relation to the oral microbiota-a clinical study. Clin Oral Investig. 2015. doi: 10.1007/s00784-015-1696-9.
[26] Sahand IH, Maza JL, Eraso E, et al. Evaluation of CHROM-Pal medium for the isolation and direct identification of *Candida dubliniensis* in primary cultures from the oral cavity. J Med Microbiol. 2009;58:1437–1442.
[27] Zaremba ML, Stokowska W, Klimiuk A, et al. Microorganisms in root carious lesions in adults. Adv Med Sci. 2006;51 Suppl 1:237–240.
[28] Muadcheingka T, Tantivitayakul P. Distribution of *Candida albicans* and non-*albicans Candida* species in oral candidiasis patients: correlation between cell surface hydrophobicity and biofilm forming activities. Arch Oral Biol. 2015;60:894–901.
[29] Sanchis M, Guarro J, Sutton DA, et al. Voriconazole and posaconazole therapy for experimental *Candida lusitaniae* infection. Diag Microbiol Infect Dis. 2016;84:48–51.
[30] ÓSullivan JM, Jenkinson HF, Cannon RD. Adhesion of *Candida albicans* to oral streptococci is promoted by selective adsorption of salivary proteins to the streptococcal cell surface. Microbiology. 2000;146:41–48.
[31] Szabó B, Majoros L, Papp-Falusi E, et al. Studies on the possible aetiological role of different *Candida* species in pathogenesis of dentine caries by monitoring the calcium release from tooth particles. Acta Microbiol Immunol Hung. 2014;61:11–17.

[32] Moreira D, Spoldorio DMP, Rodrigues JA de O, et al. *Candida* spp. biotypes in the oral cavity of school children from different socio-economic categories in Piracicaba-SP, Brazil. Pesqui Odontol Bras. 2001;15:187–195.

[33] Gomar-Vercher S, Cabrera-Rubio R, Mira A, et al. Relationship of children's salivary microbiota with their caries status: a pyrosequencing study. Clin Oral Investig. 2014;18:2087–2094.