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Original Article

Characterization of oral yeasts isolated from healthy individuals attended in different Colombian dental clinics

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Abstract

The aim of this study was to identify the most frequent yeasts in the oral cavity of adult individuals without immune disorders and to associate the presence of these oral yeasts with different characteristics of each individual. Oral rinse samples were obtained from 96 healthy adults and cultured in Sabouraud dextrose agar media and CHROMagar. Yeasts were identified by sequencing the D1/D2 region of the 28S *rRNA* gene. Probable association among the socio-demographic characteristics, body mass index, family and personal medical history, oral hygiene, tobacco and/or alcohol consumption habits and presence of oral fungi was analyzed. Contingency tables and logistic regression were employed to evaluate possible relationships between the presence of oral fungi and mixed colonization with these variables. 57.3% of the healthy individuals had oral yeasts and 21.8% had mixed colonization. The most prevalent yeasts were *Candida albicans* (52%), *C. parapsilosis* (17.9%), and *C. dubliniensis* (7.57%). Yeasts with most frequently mixed colonization were *C. albicans* and *C. parapsilosis*. No relationships were found among the variables analyzed. However, the presence of mixed colonization was related to the presence of dental prostheses (P<0.006), dental apparatuses (P=0.016) and O'Leary index (P=0.012). This is the first study that characterized oral yeasts in Colombian healthy individuals, determined the most prevalent oral yeasts *C. albicans*, *C. parapsilosis* and *C. dublinensis* and an association of mixed colonization with the use of dental prostheses and aparatology and poor hygiene.

Keywords: oral yeast, *Candida* species, microbial epidemiology

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Introduction

At present, great progress has been achieved in our understanding of the prevalence and diversity of the microbial communities associated with nearly all of our mucosal surfaces and as a result, studies on the microbiota have influenced processes ranging from digestion to behavior, thus increasing their importance as a fundamental component of physiology. The term microbiota has been used in recent studies in order to indicate that other microorganisms, specifically fungi, are also an important with the term 'mycobiota' being used in reference to this fungal component[1]. The human oral microbiome is the microflora that has received most attention. It is easily sampled and appears strongly associated with important oral colonization diseases such as tooth decay (dental caries) and gum disease (periodontitis) with identification of approximately 600 prevalent bacterial species in the human oral cavity[2–3]. Despite the fact that the human oral cavity is inhabited by many different microorganisms including viruses, bacteria and fungi, studies on oral microbiota have focused largely on the bacterial components; rather recently, however, the oral mycobiota of the healthy mouth has been evaluated and shown to contain 74 culturable and non-culturable fungal genera[4]. Additionally, it has also been found that the number of bacterial and fungal genera among uninfected and HIV-infected individuals ranged between 8–14 and 1–9, respectively, with species in the genus *Candida* predominating in both groups[5].

In Colombia, works on oral fungi remain unexplored in immunocompetent persons. However, there is one study available in type 2 diabetic patients, centered on the identification of oral yeasts in which the results showed that species in the genus *Candida* were the most prevalent microorganisms (71%) with *C. albicans* (48.7%) predominating. In addition, other genera represented by *Rhodotorula*, *Trichosporon*, *Saccharomyces*, *Cryptococcus* and *Kloeckera* (16.8%) were also identified[6]. Additionally, some studies in HIV patients have established that oral candidiasis is the second most prevalent lesion, after gingivitis, exhibited by this population (7.27%[7], 12.8%[8] and 35.5%[9]). Nonetheless, in these studies yeast species associated with this pathology were not identified. Furthermore, in 2014 during the Ⅳ National Study on Oral Health, ENSAB Ⅳ (Ⅳ Estudio Nacional de Salud Bucal) headed by the Colombian Ministry of Health and Social Protection with the purpose of determining the oral health status of nearly 20 000 Colombians residing in the 32 of Departments (Sates) in the country, who were surveyed and evaluated clinically. Despite this important coverage, the corresponding oral fungal colonization data remained unreported. However, the results of this study revealed an increase in periodontal disease, mild fluorosis, edentulism, prosthetic lesions and stomatitis, the latter being strongly associated with oral candidiasis and fungal colonization[10].

Some of these studies have provided approximate figures on the prevalence of oral fungal colonization by *Candida* spp. in Colombia despite the fact that available research on the different fungal species associated with this colonization or with data concerning the biological, clinical and epidemiological aspects of the problem were missing. Therefore, considering the lack of information on the fungi that inhabit the oral cavity, a multicenter study was proposed with the aim of identifying the most frequent yeasts found in the oral cavity of adult individuals free of disorders related to the immune system and associate the presence of these oral yeasts with different characteristics of the individuals who attended 9 different dental clinics ascribed to the Antonio Nariño University and located in various Colombian cities.

Materials and methods

Ethics statements

Informed consent form, according to the regulation 008430 of 1993 of the Ministry of Health of Colombia, was obtained from all the people who accepted to participate in the study. The Ethics Committees from the University of Antonio Nariño approved this study.

Study participants

Oral rinse samples were obtained from 96 adult healthy patients that assisted to the dental clinics from the University of Antonio Nariño in nine Colombian cities (Armenia, Bogotá DC., Cúcuta, Ibagué, Neiva, Palmira, Villavicencio, Popayán and Bucaramanga). We included only patients who had no systemic diseases, were not receiving or had not received pharmacological treatment with antibiotics, antifungal or corticosteroids for the last 6 months. Initially, oral examination was done by a clinician who evaluated presence of dental prostheses and of mucosal lesions and then the following demographic information was obtained from each patient: age, gender, socioeconomic status, occupation, body mass index, familiar and personal medical history, oral hygiene habits, tobacco and alcohol consumption habits, as well as the

existence of immunosuppressive disease.

Sample collection

Individuals attending the clinic were received in the morning before breakfast or brushing their teeth, and were told to do a mouthwash with 10 mL saline solution for approximately 30 seconds, and throwing out the contents of the mouth into a 50 mL Falcon tube. The collected samples were centrifuged at 3 500 r/min for 30 minutes at 4 °C to separate the cells (pellet) from extracellular soluble components (supernatant).

Culture medium and storage isolates

The cell pellets were seeded directly on petri plates with Sabouraud dextrose agar media with™ (Becton Dickinson and Co. BD™, reference 221988, USA) and CHROMagar Candida® (CHOMagar Microbiology, Paris, France). The plates were incubated at 25 °C for 20 days with weekly readings to evaluate the positive cultures. Isolated yeast colonies growing in the plates were collected with a loop and suspended into sterile tubes with distilled water at 4 °C and frozen in milk medium (Skim milk, BD™, reference 232100) at −20 °C. Storage was performed by both methods to reduce the risk of losing the microorganisms during the process.

Molecular identification

Genomic DNA was extracted from the isolated colonies grown on the culture media described above by means of a QIAamp DNA mini kit (QIAGEN, Germantown, MD, USA), following the manufacturer's recommendations. The molecular marker used was the D1/D2 region of the 28S *rRNA* gene that was amplified following international guidelines for the molecular identification of fungi as applied for identification of *Candida* species[11–12]. The amplified products from the D1/D2 region (~600 bp) were sent to Macrogen (Maryland, USA) for Sanger bidirectional sequencing and the amplification productxs size was assessed by agarose gel electrophoresis. Sequencher 5.0 software (Gene Code Corporation) for editing and aligning the sequences was used. A search was then made for each sequence to establish similarity with known strains according to the following databases: the NCBI (BLAST) (National Center for Biotechnology Information), CBS-KNAW (Fungal Biodiversity Centre) and Mycobank (International Mycological Association). The assays were done in triplicate. To confirm the species name assigned to each yeast, we aligned the DNA sequences obtained with DNA sequences reported for each species in Genbank using Clustal W (https://embnet.vital-it.ch/software/ClustalW.html). The phylogenetic trees were generated using the UPGMA method[13] in Molecular Evolutionary Genetics Analysis (MEGA) software, Version 7 (available at: http://www.megasoftware.net/). The bootstrap consensus tree was inferred from 500 replicates[14] and the evolutionary distances were computed using the Maximum Composite Likelihood method[15].

Statistical analysis

Correspondence analysis and contingency tables by means of the Infostat software 2010 version[16] were used to assess the association among the variables age, gender, socio-economic status, occupation, body mass index, family and personal medical history, oral hygiene habits, tobacco or alcohol consumption habits and presence of oral fungi. Subsequently, logistic regression analysis was performed to evaluate possible relationships between the presence of oral fungi, mixed colonization or type of yeast with the variables age, gender, socioeconomic status, occupation, body mass index, family medical history and personal oral hygiene habits, as well as tobacco and/or alcohol consumption habits (significance of $P<0.05$) in STATGRAPHICS Centurion XVI Statistics software version 16.1.15.

Results

Participants' demographics and frequency of oral yeasts

A total of 96 individuals (64 females and 32 males) from nine Colombian cities were enrolled in the study with their origin being as follows: 21 Armenia (21.9%), 10 Bogotá DC. (10.4%), 10 Cúcuta (10.4%), 10 Ibagué (10.4%), 10 Neiva (10.4%), 10 Palmira (10.4%), 10 Villavicencio (10.4%), 8 Popayán (8.3%) and 7 Bucaramanga (7.3%) (***Table 1***).

Yeasts colonization of the oral cavity according to gender was 59.4% in female and 53.1% in males, among whom 26.3% and 11.8% had mixed colonization, respectively. The age ranges with the highest frequency of oral yeast were 72.7% (>60 years), 66.7% (25–35 years) and 63.3% (48–59 years). According to the body mass index, 84.6% of individuals were categorized with normal weight (54.2%) and overweight (34.4%), and they presented oral yeasts in 61.5% and 54.5%. On the other hand, a higher yeasts count was found in individuals in the middle to low socio-economic status while the only two individuals in the high middle status had no oral yeast isolated. Although most of the study population reported being

Table 1 **Frequency of oral yeasts and mixed colonization found in 96 healthy individuals attended in consultation at dental clinics from nine different Colombia cities in 2013**

Presence of oral yeasts	Armenia	Bogotá	Bucaramanga	Cúcuta	Ibagué	Neiva	Palmira	Popayán	Villavicencio	Total [*n* (%)]
No	5	6	4	7	5	5	4	2	3	41 (42.7)
Yes	16	4	3	3	5	5	6	6	7	55 (57.3)
Mixed colonization (more than one identified species)	4	1	1	0	1	0	0	3	2	12 (21.8)
Total	21	10	7	10	10	10	10	8	10	96

non-smokers (91.7%) or alcohol consumers (78.1%), frequencies of oral fungi were similar in smokers (50%) and non-smokers (58%), as well as in those who consumed alcohol (52.4%) and those that did not (58.7%). These findings were also evident for other variables such as oral hygiene habits, presence of prostheses or apparatuses, state, color and texture of oral mucosa and O'Leary index (***Table 2***).

DNA sequencing and molecular identification of oral yeast

The UPGMA method was used to construct a dendrogram of sequences based on sequence similarity in order to find out how the sequences are grouped and how they are related. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) was shown next to the branches. The evolutionary distances were computed using the Maximum Composite Likelihood method and occurred in the units of the number of base substitutions per site. The analysis involved 112 nucleotide sequences. Codon positions included were 1_{st}+2_{nd}+3_{rd}+noncoding. All positions containing gaps and missing data were eliminated. There were 409 positions in the final dataset. The cluster obtained from all yeast species sequences found in this study with the yeast species sequences from Genbank allowed us to confirm a correct identification of yeast species found in oral cavity of healthy patients (***Fig. 1***).

In this study, among collected 67 isolated of oral yeast, 35 were *C. albicans*, 29 non-*C. albicans* and one isolated of other yeasts, including *Pichia kluyvery*, *Geotrichum candidum* and *Rhodotorula mucilaginosa*. The most prevalent oral yeast was *C. albicans* (52%), within non-*C. albicans* isolates were *C. parapsilosis* (18%), *C. dubliniensis* (7%), *C. glabrata* (6%), *C. tropicalis* (3%), *C. intermedia* (3%) and other less common yeasts found were *C. lipolytica, C. lusitaniae, C. haemulonni, C. fermentati, Pichia kluyvery, Rhodotorula mucilaginosa, and Geotrichum candidum* with a frequency of 1.5% each one (***Fig. 2***).

For individuals who had mixed colonization, two species of yeasts were identified and co-colonization between *C. albicans* and *C. parapsilosis* was the most common (5 subjects). We also observed co-colonization among *C. albicans*, *C. intermedia*, *C. tropicalis*, *C. glabrata* and *C. fermentati*, as well as other co-colonization between *C. dublinensis* and *C. parapsilosis* with *C. glabrata*, *C. lusitaniae* and *Geotrichum candidum* (***Table 3***).

Data analysis

The correspondence analysis was performed on the socio-demographic characteristics, body mass index, family and personal medical history, oral hygiene, tobacco and/or alcohol consumption, habits and presence of oral fungi. It showed a possible association between age, family medical history, use of dental prosthesis or dental apparatuses, presence of oral yeast, mixed colonization, species of yeast from individuals with only a microorganism (Ⅰ) and of those with two (Ⅱ) enclosed by the red circle in ***Fig. 3***.

Similarly, the result of contingency tables evidenced these associations and, additionally, included an association between O'Leary index with mixed colonization and yeast species from individuals who had a single fungus in their oral cavity and those with two (***Table 4***).

To confirm these associations, logistic regression analysis was performed with presence of oral yeast and mixed colonization as the independent variables by the stepwise method (cut off value, 0.05). Association with presence of oral yeast with any variable studied was not found. Nevertheless, the mixed colonization was associated with the use of dental apparatuses (P=0.016), use of dental prosthesis (P=0.006), O'Leary Index (P=0.012) and the adjusted odds ratio (95% CI), for use of dental apparatuses was 8.88 (1.33–59.35), used dental prosthesis 7.19 (0.52–3.43), O'Leary Index-questionable 0.04 (0.00–0.65) and O'Leary Index-deficient 0.02 (0.00–0.38) (***Table 5***).

Discussion

It has been demonstrated that at least 15 different

Table 2 **Frequency of oral yeasts and mixed colonization according to the groups analyzed in 96 healthy individuals studied in dental clinics from nine cities in Colombia in 2013**

Variables	Category	*n* (%)	Presence of oral yeasts		
			No [*n* (%)]	Yes [*n* (%)]	Mixed colonization [*n* (%)]
Gender	Female	64 (66.7)	26 (40.6)	38 (59.4)	10 (26.3)
	Male	32 (33.3)	15 (46.9)	17 (53.1)	2 (11.8)
Age	13−24	16 (16.7)	9 (56.3)	7 (43.8)	0 (0.0)
	25−35	18 (18.8)	6 (33.3)	12 (66.7)	2 (16.7)
	36−47	21 (21.9)	12 (57.1)	9 (42.9)	3 (33.3)
	48−59	30 (31.3)	11 (36.7)	19 (63.3)	5 (26.3)
	>60	11 (11.5)	3 (27.3)	8 (72.7)	2 (25.0)
Body mass index	Underweight: thinness severe	1 (1.0)	0 (0.0)	1 (100.0)	1 (100.0)
	Normal weight	52 (54.2)	20 (38.5)	32 (61.5)	7 (21.9)
	Overweight	33 (34.4)	15 (45.5)	18 (54.5)	4 (22.2)
	Obese: Type Ⅰ	6 (6.3)	3 (50.0)	3 (50.0)	1 (33.3)
	Obese: Type Ⅱ	2 (2.1)	1 (50.0)	1 (50.0)	0 (0.0)
	Obese: Type Ⅲ	2 (2.1)	2 (100.0)	0 (0.0)	0 (0.0)
Socioeconomic status	Low-low	23 (24.0)	12 (52.2)	11 (47.8)	2 (18.2)
	Low	28 (29.2)	13 (46.4)	15 (53.6)	4 (26.7)
	Medium-low	29 (30.2)	9 (31.0)	20 (69.0)	4 (20.0)
	Medium	14 (14.6)	5 (35.7)	9 (64.3)	2 (22.2)
	Medium-high	2 (2.1)	2 (100.0)	0 (0.0)	0 (0.0)
Smoke	Yes	8 (8.3)	4 (50.0)	4 (50.0)	0 (0.0)
	No	88 (91.7)	37 (42.0)	51 (58.0)	12 (23.5)
Drink alcohol	Yes	21 (21.9)	10 (47.6)	11 (52.4)	1 (9.1)
	No	75 (78.1)	31 (41.3)	44 (58.7)	11 (25.0)
Tooth brushing (Once a day)	Yes	88 (91.7)	38 (43.2)	50 (56.8)	10 (20.0)
	No	8 (8.3)	3 (37.5)	5 (62.4)	2 (40.0)
Dental yield	Yes	48 (50.0)	18 (38.5)	30 (62.5)	5 (16.7)
	No	48 (50.0)	23 (47.9)	25 (52.1)	7 (28.0)
Dental prosthesis	Yes	32 (33.3)	12 (37.5)	20 (62.5)	8 (40.0)
	No	64 (56.3)	29 (45.3)	35 (54.7)	4 (11.4)
Dental aparatology	Yes	37 (38.5)	13 (35.1)	24 (64.9)	9 (37.5)
	No	59 (61.5)	28 (47.5)	31 (52.5)	3 (9.7)
Status of oral mucosa	Normal	64 (56.3)	26 (40.6)	38 (59.4)	9 (23.7)
	Abnormal	32 (33.3)	15 (46.9)	17 (53.1)	3 (17.6)
Color mucosa	Normal	84 (87.5)	35 (41.7)	49 (58.3)	12 (24.5)
	Abnormal	12 (12.5)	6 (50.0)	6 (50.0)	0 (0.0)
Texture mucosa	Normal	89 (92.7)	37 (41.6)	52 (58.4)	11 (21.2)
	Abnormal	7 (7.3)	4 (57.1)	3 (42.9)	1 (33.3)
O'Leary index	Acceptable	5 (5.6)	2 (40.0)	3 (60.0)	0 (0.0)
	Questionable	26 (29.2)	12 (46.2)	14 (53.8)	4 (28.6)
	Deficient	58 (65.2)	25 (43.1)	33 (56.9)	5 (15.2)

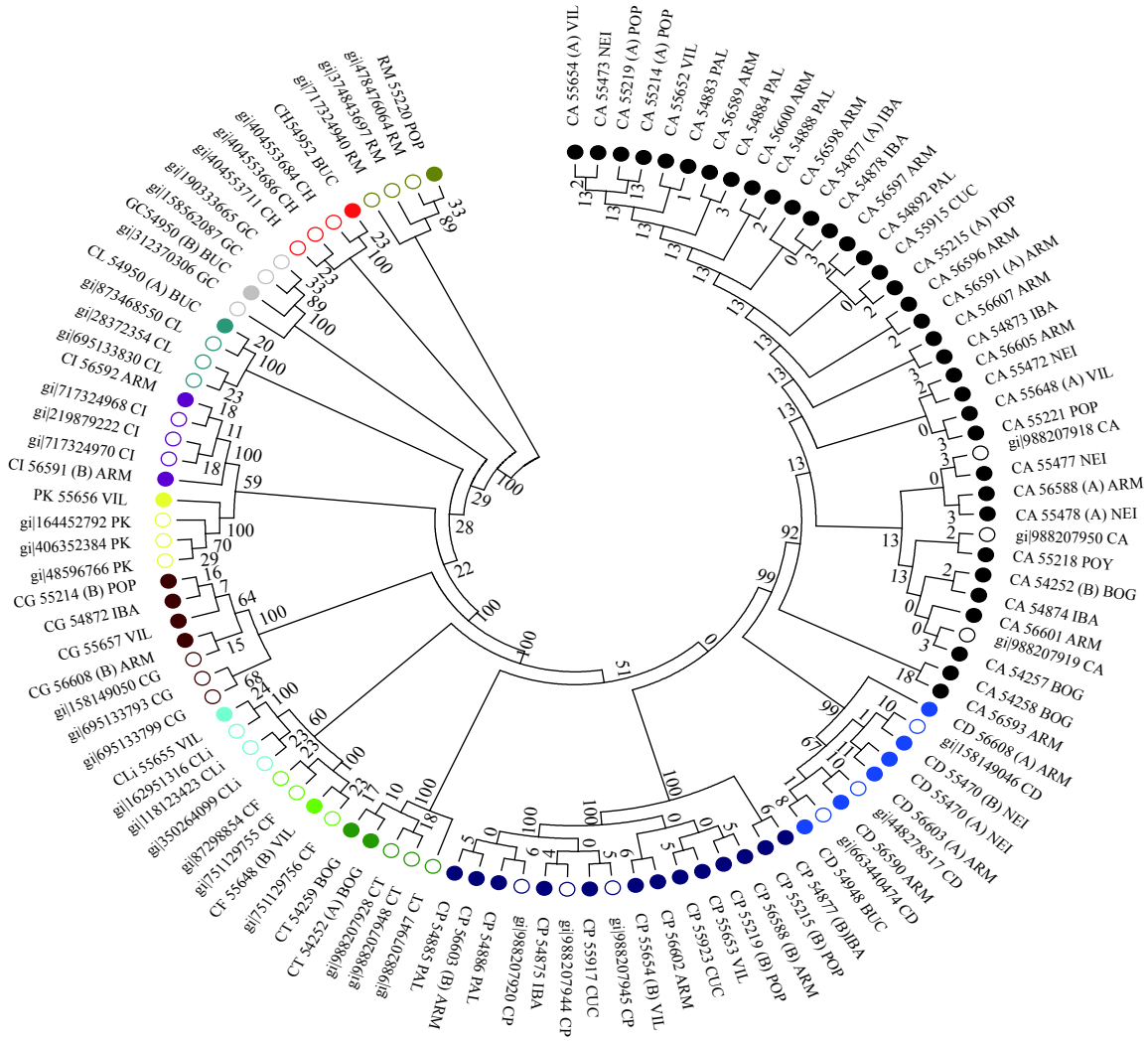

Fig. 1 **Alignment DNA sequences of yeast species found in the oral cavity of healthy patients with yeast species sequences reported in the Genebank collection (NCBI) using the UPGMA method in Mega 7 software.** Each yeast identified is represented by a color circle filled, the name of the species CA: *C. albicans*, CD: *C. dubliniensis*, CP: *C. parapsilosis*, CT: *C. tropicalis*, CLI: *C. lipolytica*, CF: *C. fermentati*, CG: *C. glabrata*, PK: *Pichia kluyvery*, CI: *C. intermedia*, CL: *C. lusitaniae*, GC: *Geotrichum candidum*, CH: *C. haemulonni*, RM: *Rhodotorula mucilaginous*; the sample code and the place of origin of the individual from which the yeast was isolated: Armenia (ARM), Bogotá DC. (BOG), Cúcuta (CUC), Ibagué (IBA), Neiva (NEI), Palmira (PAL), Villavicencio (VIL), Popayán (POP) and Bucaramanga (BUC). Color circle indicates the control sequences obtained from the Genbank for each yeast species.

Candida species can cause human diseases and more than 90% can produce invasive infections, namely, *C. albicans, C. glabrata, C. tropicalis, C. parapsilosis* and *C. Kruse*: however, *Candida* spp. mucosal infections such as oropharynx, esophagus and vagina are not classically considered invasive[17]. Invasive fungal infections in the oral cavity are less frequent and include aspergillosis (*Aspergillus* spp.), Cryptococcosis (*Cryptococcus neoformans*), histoplasmosis (*Histoplasma capsulatum*), paracoccidioidomycosis (*Paracoccidioides brasiliensis*), geotrichosis (*Geotrichum candidum*), blastomycosis (*Blastomyces dermatitidis*) and mucorrmycosis (*Mucoraceae* spp.), commonly diagnosed in immunosuppressed patients, a situation unlike superficial fungal infections caused mainly by *Candida* spp. with the most frequent being *C. albicans*, species that comprise 70% to 80% of all oral isolates, followed by *C. glabrata* 5% and *C. tropicalis* 8%[18]. Undoubtedly, *Candida albicans* is the most common yeast in the oral cavity. However, the frequency of others yeasts including non-*C.albicans* may vary according to the type of patient and the methods used for isolation and identification. In this case, a molecular identification of the oral yeasts recovered from healthy patients allowed to define a prevalence of 55.3% of oral yeast colonization with *C. albicans* being the most common species (52%) followed by *C. parapsilosis* (17.9%), *C. dubliniensis* (7.5%), *C. glabrata* (6%) and the only yeasts different to *Candida* species, namely *Pichia kluyvery*, *Geotrichum candidum* and *Rhodotorula mucilaginosa* had a very low frequency (1.5%). Of

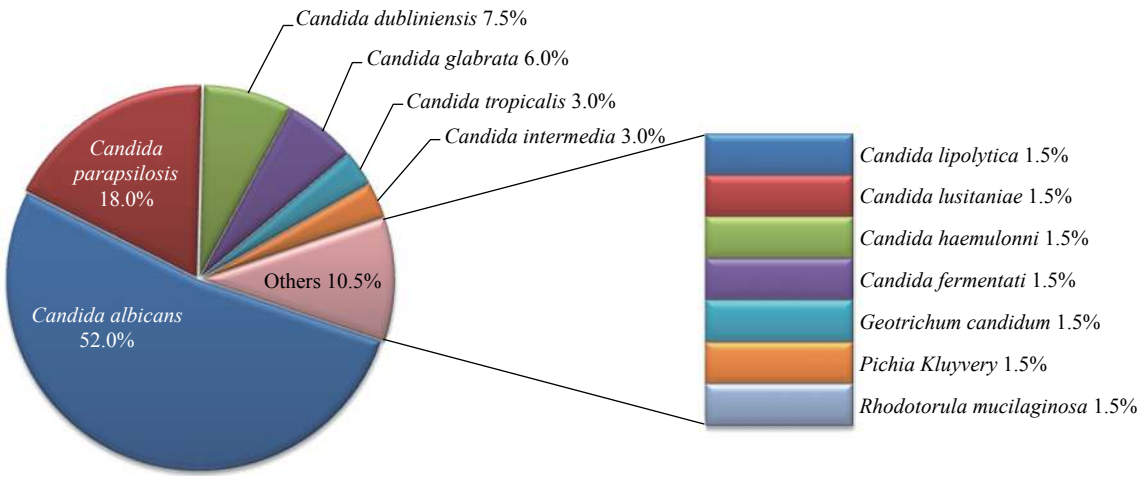

Fig. 2 **Distribution of yeast species identified in the oral cavity of 96 healthy individuals studied in dental clinics from nine Colombia cities in 2013.**

these patients, 21.8% had mixed colonization generated by only two species of yeasts were related to the use of dental prostheses and dental apparatuses and questionable O'Leary or deficient index.

Xu and Mitchellin in 2003 analyzed in 722 healthy persons of whom were 239 Chinese and 483 North Americans, growing saliva in CHROMAgar, using API 20C analysis for yeasts identification and amplification of (D1/D2) of the 28S rRNA to differentiate *C. albicans* from *C. dubliniensis*, a finding that revealed that prevalence of oral yeasts in the Chinese population was 66.9%, a group where the 3 most common species were *C. parapsilosis* (39.4%), *C. guilliermondii* (21.3%), and *C. famata* (11.9%) with a fourth one corresponding to *C. albicans* (9.4%). This was unlike the American individuals in whom the largest prevalence (90.6%) corresponded to *C. albicans,* followed by *C. parapsilosis* (3.1%) and *C. tropicalis* (2.6%). Despite these differences, the microflora of oral yeasts was not determined by ethnicity as observed in our work because no significant differences were found in relation to oral yeasts identified in 9 Colombian cities[19].

Cavaleiro *et al* in 2013 used the tRNA-PCR fingerprinting and sequencing of the 28S rDNA D1/D2 domain to identify all yeasts isolated from CHROMagar™ Candida cultures of oral swabs that had been collected from 178 patients, in whom the most frequent yeasts were *C. albicans* (66%), *C. glabrata* (9%), *C. tropicalis* (4.5%), *C. parapsilosis* (4.5%) and *C. lusitaniae* (4.5%), while the non-*Candida* yeast were *Pichia norvegensis, Torulaspora delbrueckii, Debaryomyces hansenii, Trichosporon cutaneum*, and *Rhodotorula mucilaginous*[20]. We identified in our work *G. candidum, P. kluyvery and R. mucilaginous* in one patient. Several authors categorized these isolates as transient species due to accidental environmental exposures to certain foods, water or soil, among others. Additionally, these results determined that 19.4% of patients had colonization by multiple species of yeasts two, three or even four different yeasts in the same patient with the

Table 3 **Frequency of individuals with oral yeasts mixed colonization (with two different species) in the population in studied in dental clinics from nine cities in Colombia in 2013**

Oral yeast species	Individuals with mixed colonization (*n*)
Candida albicans + *Candida parapsilosis*	5
Candida albicans + *Candida intermedia*	1
Candida albicans + *Candida tropicalis*	1
Candida albicans + *Candida glabrata*	1
Candida albicans + *Candida fermentati*	1
Candida dubliniensis + *Candida parapsilosis*	1
Candida dubliniensis + *Candida glabrata*	1
Candida lusitaniae + *Geotrichum candidum*	1

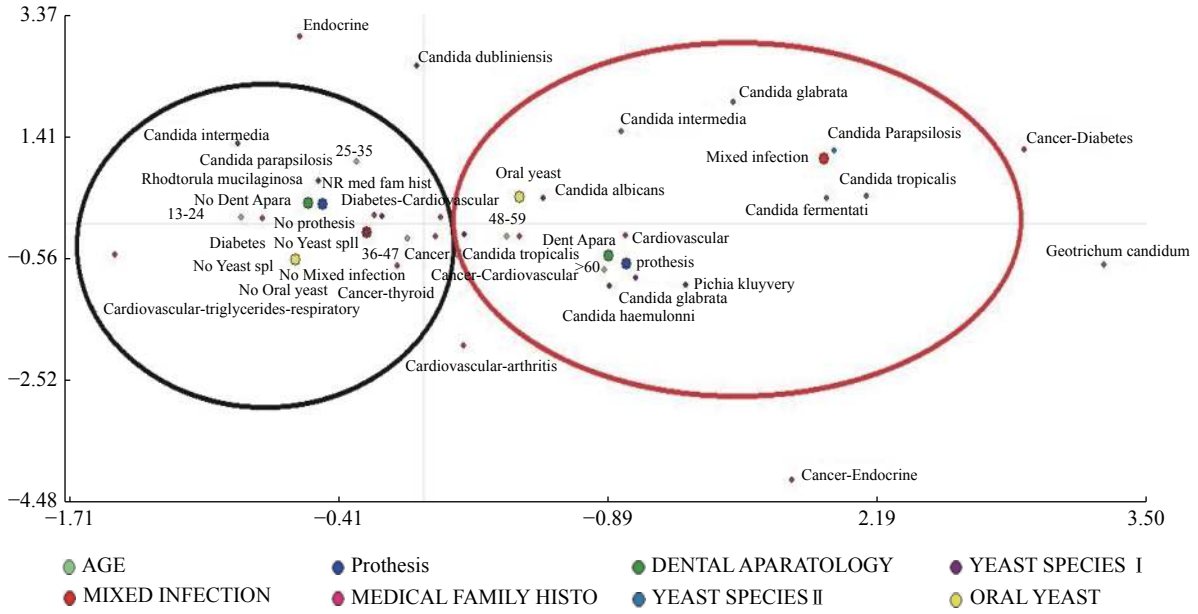

Fig. 3 **Correspondence analysis between age, family medical history, use of prosthesis or dental apparatuses, presence of oral yeasts, mixed colonization and species of yeasts represented by only a microorganism (Ⅰ) and those who had two (Ⅱ) in 96 healthy individuals studied in dental clinics from nine cities in Colombia in 2013.** Black circle represents the variable related to the absence of oral yeast and red circle represents the variable related to the presence of oral yeast.

Table 4 **Statistically significant association between yeast identification results, socio-demographic and oral variables in 96 Colombian healthy individuals studied in dental clinics from nine cities in Colombia in 2013 (significance of *P*<0.05)**

Variables	Pearson chi-square	*P*-value
Age-yeast species Ⅰ	73.25	0.0109
Mixed colonization and dental prosthesis	6.86	0.0088
Dental prosthesis and yeast species (Ⅰ)	21.67	0.0414
Mixed colonization and dental apparatuses	7.7	0.0055
Mixed colonization and O'Leary index	7.61	0.0548
Yeast species (Ⅰ) and O'Leary index	55.04	0.0220

Table 5 **Logistic regression results of the association between mixed colonization of oral yeasts and studied variables in 96 Colombian healthy individuals studied in dental clinics from nine cities in Colombia in 2013 (significance of *P*<0.05)**

Variables	*P*-value	Standard error	Odds ratio	Confidence interval 95 %	
				Lower limit	Upper limit
Used dental aparatology	0.016	0.95	8.88	1.33	59.35
Used dental prosthesis	0.006	0.73	7.19	0.52	3.43
O'Leary index-questionable	0.012	1.45	0.04	0.00	0.65
O'Leary index-deficient	0.012	1.47	0.02	0.00	0.38

commonest co-colonization being that of *C. albicans* and *C. glabrata* found in three patients. Similarly, in our study the frequency of mixed colonization was 21.8%. However, only two yeast species were identified in the same patient, mainly *C. albicans* with *C. parapsilosis* in 5 patients. Previous studies on acrylic substrates and oral epithelium have shown that co-infection of *C. albicans* and *C. glabrata* improve their capacity to form biofilms thus enhancing their invasiveness and increasing tissue damage even though they are phylogenetically, genetically and phenotypically very different[21–23]. Additionally, more

recently it has been reported to occur in patients with periodontal disease[24]. Although newer studies have proposed that, during biofilm formation in experimental candidiasis, a competitive relationship appears to exist among *C. albicans, C. glabrata* and *C. Krusey*[25], no information about the existence of such synergism between *C. albicans* and *C. parapsilosis* has been recorded and could have be seen for the first time in Colombia judging by the results of this study.

Despite the fact that oral colonization is common, several predisposing factors such as age, use of dentures, poor oral hygiene and immune status of the patient do cooperate[26]. In this study conducted in healthy individuals, none of the variables analyzed (alcoholism, smoking, oral prostheses, oral apparatuses, oral hygiene habits or mucosal abnormalities) showed an association with the presence of fungi in the oral cavity, our results only pointed to the fact that a relationship between oral hygiene and the possibility of having a mixed colonization does exist, such as observed in persons who wear dentures or carry dental apparatuses, and who have 7 to 8 times more possibilities of presenting mixed colonization. For Colombia, there is a report on the yeasts found in the oral cavity from diabetic type Ⅱ patients, in whom mixed colonization with two and three different yeasts was found, and as in our results, that the mixed colonization of two most common yeasts was between *C. albicans and C. parapsilosis* (5 individuals). Although the synergy between *C. albicans* and *C. glabrata* has already been studied, there are no reports of synergism between *C. albicans* and *C. parapsilosis,* which could depend on the populations evaluated with the possibility that it is not found in other populations. Finally, this is the first study dealing with yeasts in the oral cavity of Colombian healthy persons that characterizes the species based on molecular techniques and allows from the microbiological context, to better understand the epidemiology and biology of yeast species inhabiting the human oral cavity.

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