Rev Iberoam Micol. 2010;27(3):119–124

Revista Iberoamericana de Micología

www.elsevier.es/reviberoammicol

Original

Oral *Candida* carriage of patients attending a dental clinic in Braga, Portugal

Margarida Martins [a], Mariana Henriques [a], Ana Paula Ribeiro [a], Ricardo Fernandes [a], Vânia Gonçalves [a], Álvaro Seabra [b], Joana Azeredo [a] and Rosário Oliveira [a,*]

[a] *IBB-Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, Universidade do Minho, Campus de Gualtar, Braga, Portugal*
[b] *Clínica Dentária dos Congregados, Lda, Braga, Portugal*

ARTICLE INFO

Article history:
Received 12 June 2009
Accepted 31 March 2010
Available online 18 de abril de 2010

Keywords:
Candida
Polymicrobial cultures
Oral carriage
Braga—Portugal

ABSTRACT

Background: The ability of the *Candida* species to colonize surfaces can be considered as a risk factor for oral infection.

Aims: To establish oral *Candida* carriage in patients attending a dental clinic in Braga, Portugal.

Methods: A total of 97 patients were analysed. Swab samples were collected and directly cultured onto CHROMagar Candida. Representative yeasts were identified by polymerase chain reaction.

Results: From the samples analysed 54.6% ($n=53$) were *Candida* positive, and *Candida albicans* was the most frequently isolated species, accounting for 79% of all the species identified. Non-*C. albicans Candida* (NCAC) species recovered included *Candida parapsilosis*, *Candida glabrata*, *Candida tropicalis*, and *Candida guilliermondii*. There was a lack of association between the presence of *C. albicans* or NCAC species, and age, gender, or prostheses wearing in this population. In 17% of the cases ($n=9$), polymicrobial cultures, with two different *Candida* species, were identified.

Conclusions: This study shows a high *Candida* carriage rate among this population, thus pointing to the relevance of an accurate diagnostic approach in *Candida* species identification.

Colonización oral por *Candida* en pacientes que asisten a una clínica dental en Braga, Portugal

Palabras clave:
Candida
Braga-Portugal
Colonización oral
Colonización mixta

RESUMEN

Antecedentes: La capacidad que poseen las diferentes especies de *Candida* de colonizar las superficies, puede ser considerada como un factor de riesgo para la infección oral.

Objetivos: Establecer la colonización oral por *Candida* en pacientes que asisten a una clínica dental en Braga, Portugal.

Métodos: Un total de 97 pacientes fueron estudiados. Se recogieron muestras bucales con hisopo y fueron cultivadas directamente en CHROMagar Candida. Las levaduras seleccionadas se identificaron mediante reacción en cadena de la polimerasa.

Resultados: De las muestras analizadas 54,6% ($n=53$) fueron positivas para *Candida*. *Candida albicans* fue la especie más frecuentemente aislada, representado el 79% de todas las especies identificadas. Las especies de *Candida* no-*Candida albicans* (CNCA) aisladas fueron *Candida parapsilosis*, *Candida glabrata*, *Candida tropicalis* y *Candida guilliermondii*. En la población estudiada no se observó asociación entre la presencia de *C. albicans* o CNCA con la edad, el sexo o el uso de prótesis. En el 17% de los casos ($n=9$) se encontró una colonización mixta con dos especies de *Candida*.

Conclusiones: Este estudio muestra una alta incidencia de colonización por *Candida* en esta población; por lo tanto, se sugiere la necesidad de un diagnóstico preciso para la identificación de las especies de *Candida*.

Colonization of the oral cavity by *Candida* species was defined as the acquisition and maintenance of yeast cells without clinical signs. This process entails *Candida* species acquisition, growth, and removal.[3]

Within the yeast oral community, *Candida albicans* is the most frequently found (47–75% of the yeasts isolated).[4] However, other yeast species have been increasingly identified, such as non-*C. albicans Candida* (NCAC) species (*Candida glabrata*, *Candida parapsilosis*, *Candida krusei*, *Candida tropicalis*, *Candida dubliniensis*, and *Candida guilliermondii*), *Saccharomyces cerevisiae*, *Trichosporon* species, and *Yarrowia lipolytica*.[2,15,18,29]

* Corresponding author.
E-mail address: roliveira@deb.uminho.pt (R. Oliveira).

1130-1406/$ - see front matter © 2010 Revista Iberoamericana de Micología. Published by Elsevier España, S.L. All rights reserved.
doi:10.1016/j.riam.2010.03.007

Although the presence of *Candida* species is not an indication of disease, the ability of the yeasts to overcome host clearance mechanisms and to colonize surfaces can be considered as a risk factor for oral infection. The balance between *Candida* colonization and candidiasis relies on the balance between pathogen characteristics (e.g. production of adhesins, secreted aspartyl proteinases), and host factors.[12] Host local predisposing conditions comprise: (i) reduced saliva secretion, (ii) epithelial changes and local mucosal diseases, (iii) changes in commensal flora, (iv) high carbohydrate diet, and (v) denture wearing. Additionally, host systemic factors have also been associated with *Candida* oral colonization, and include: (i) age, (ii) tobacco smoking, (iii) endocrine disorders, including diabetes, hypothyroidism, and hyperparathyroidism, (iv) rheumatic diseases, (v) nutritional deficiencies (iron or folate deficiencies), (vi) immunosuppressive conditions, such as chemotherapy, deficiencies of humoral or cell-mediated immunity, human immunodeficiency virus infection, and acquired immunodeficiency syndrome, and (vii) drugs such as broad-spectrum antibiotics, and corticosteroids.[1,4,10,18]

To the authors' knowledge, oral *Candida* carriage prevalence and aetiology studies have not yet been performed in the Portuguese population. Thus, the main objective of this study was to evaluate oral *Candida* carriage in patients attending a dental clinic in Braga, Portugal.

Patients and Methods

Candida type strains

For quality control purposes, the following *Candida* type strains were used in the identification procedures of *Candida* isolates: *C. albicans* CECT 1472, *C. dubliniensis*, strain provided by Biognostica from United Kingdom National External Quality Assessment Service, *C. glabrata* ATCC 2001, *C. guilliermondii* ATCC 6260, *Candida kefyr* ATCC 204093, *C. krusei* ATCC 6258, *C. parapsilosis* ATCC 22019, and *C. tropicalis* ATCC 750. These strains were maintained on Sabouraud dextrose agar.

Patients

Samples were collected from a total of 97 asymptomatic individuals (77 females and 20 males) attending a dental clinic in Braga, Portugal, over a 12-month period (May 2005–2006). Each of the potential subjects was informed of the aims and methods of the study, and anticipated benefits and potential risks, according to the World Medical Association Declaration of Helsinki. Data on patient age, oral hygiene habits, health status, medications, and prosthesis wearing were collected.

Sample collection

Samples were collected by passing a sterile swab (UNI-TER, MEUS, Padua, Italy) across the oral mucosa, tongue, hard palate, and gums, and replacing it in its sterile container tube. Samples were kept at 4 °C and analysed within 24 h.

Candida species identification

Medium for the primary isolation

CHROMagar Candida medium (CHROMagar, Paris, France) was prepared according to the manufacturer's instructions. The swab was inoculated into CHROMagar Candida medium rotating the swab head on the surface of the medium. The plates were incubated at 37 °C for 48 h. Colony morphology and colour description were assigned in a standard manner by a single investigator. Presumptive species identification was performed according to Odds and Bernaerts.[20] At least one colony exhibiting each colour was streaked into a new CHROMagar Candida plate and then cryopreserved.

Molecular identification

Yeast DNA was extracted using the QIAamp® DNA Mini Kit (QIAGEN, Lisbon, Portugal) according to the manufacturer's instructions. Genomic DNA content was determined by spectrophotometry readings at 260 nm. Aliquots of 10 µl were analysed by electrophoresis in a 0.8% agarose (Bio-Rad, Lisbon, Portugal) gel in 1 × TBE buffer (Bio-Rad), and visualized with a UV transilluminator, after ethidium bromide (Bio-Rad) staining (0.5 mg/ml).

To assess *Candida* speciation, a polymerase chain reaction method (PCR) previously described[14] was followed. This method uses primer mixtures of the *Candida* DNA topoisomerase II genes (Table 1). In a multiplex PCR strategy it allows identification of *C. albicans*, *C. guilliermondii,* and *C. parapsilosis* using primer set A (Table 1) and *C. dubliniensis*, *C. krusei*, *C. kefyr*, and *C. glabrata* using primer set B (Table 1). *C. tropicalis* is identified in a third PCR reaction using a single pair of primers (Table 1).

PCR amplification was performed in 25 µl volume consisting of 1 × PCR buffer (160 mM $(NH_4)_2SO_4$, 670 mM Tris–HCl pH 8.8, 0.1% Tween 20, and 25 mM $MgCl_2$; Bioron, Porto, Portugal); dNTP mixture (200 µM each; Bioron); primer mixture (300 nM each); 1.25 U Taq DNA polymerase (Bioron); and 10–100 ng genomic DNA template; the remaining volume consisted of sterilized ultrapure water. PCR was carried out in a MyCycler thermal cycler (Bio-Rad) under the following cycling conditions: 35 cycles of 30 s at 94 °C, 15 s at 57 °C, and 45 s at 65 °C, after a 10-min initial period of DNA denaturation and enzyme activation at 94 °C. One blank reaction was performed simultaneously per every 10 tests run by replacing the template DNA by sterilized ultrapure water. DNA from type strains was also included in each reaction as positive and negative controls.

An aliquot of 15 µl of each PCR product was analysed by electrophoresis in a 1.2% agarose gel in 1 × TBE buffer. Fragments were visualized by ethidium bromide staining (0.5 mg/ml) with a UV transilluminator. The size of the amplified DNA fragments was determined by comparison with a 100-bp DNA marker (Bioron).

All isolates whose presumptive identification in CHROMagar Candida did not correspond to the molecular identification were re-tested by two independent researchers in a blind assay.

Statistical analysis

CHROMagar Candida medium sensitivity was calculated as: [No. of true positives × 100/(No. of true positives + No. of false negatives)], and specificity as: [No. of true negatives × 100/(No. of true negatives + No. of false positives)]. Statistical analysis was performed using GraphPad Prism, version 5.00 software for Windows. Data were analysed using two-tailed chi-square test or Fischer test to measure association between *Candida* species distribution within groups. A statistical confidence interval of 95% was established.

Results

Candida *species identification*

Presumptive identification of yeasts clinical isolates was based on their colour on CHROMagar Candida medium. Samples were

Table 1
Primers sets and species-specific primers used in this study (as described by Kanbe et al.[14])

Primers sets	Target species	Forward primer sequence (name)	Reverse primer sequence (name)	Expected PCR product size (bp)
A[a]	*C. albicans*	5′-TTGAACATCTCCAGTTTCAAAGGT-3′ (CABF59)	5′-AGCTAAATTCATAGCAGAAAGC-3′ (CADBR125)	665
	C. guilliermondii	5′-CCCAAAATCACAAAGCTCAAGT-3′ (CGLF41)	5′-TACGACTTGAAGTTGCGAATTG-3′ (CGLR61)	205
	C. parapsilosis	5′-GGACAACATGACAAAAGTCGGCA-3′ (CPPIIF41)	5′-TTGTGGTGTAATTCTTGGGAG-3′ (CPPIIR69)	310
B[b]	*C. dubliniensis*	5′-AAATGGGTTTGGTGCCAAATTA-3′ (CDBF28)	5′-GTTGGCATTGGCAATAGCTCTA-3′ (CDBR110)	816
	C. krusei	5′-GAGCCACGGTAAAGAATACACA-3′ (CKSF35)	5′-TTTAAAGTGACCCGGATACC-3′ (CKSR57)	227
	C. kefyr	5′-CTTCCAAAGGTCAGAAGTATGTCC-3′ (CKFF35)	5′-CTTCAAACGGTCTGAAACCT-3′ (CKFR85)	532
	C. glabrata	5′-CCCAAAAATGGCCGTAAGTATG-3′ (CGBF35)	5′-ATAGTCGCTACTAATATCACACC-3′ (CGBR103)	674
C[c]	*C. tropicalis*	5′-CTGGGAAATTATATAAGCAAGTT-3′ (CTPIIF36)	5′-TCAATGTACAATTATGACCGAGTT-3′ (CTPIIR121)	860

[a] In the original report,[14] this primer set had the designation PsI.
[b] In the original report,[14] this primer set had the designation PsII.
[c] In the original report,[14] this primer set had the designation PsIII.

processed in parallel with *C. albicans*, *C. krusei*, and *C. tropicalis* type strains that presented the expected colours on CHROMagar Candida medium: green, pink, and blue, respectively.

For each culture, representative isolates were identified by PCR using *Candida* specific primers pairs for the genomic sequences of DNA topoisomerase II gene (Table 1).[14] Reference DNA strain was included in each assay as control. DNA of *C. albicans* (Fig. 1A, lane 1), *C. guilliermondii* (Fig. 1A, lane 3), and *C. parapsilosis* (Fig. 1A, lane 4) were amplified using primer set A (Table 1). Primer set B (Table 1) allowed identification of *C. glabrata* (Fig. 1B, lane 2), *C. dubliniensis*, *C. krusei*, and *C. kefyr* reference strains (data not shown). Finally, the set of primers C (Table 1) allowed identification of *C. tropicalis* reference strain (Fig. 1C, lane 5). For all *Candida* species the amplicon size obtained (Fig. 1) was as expected (Table 1). Clinical isolate identification was based on the comparison of size of the amplified DNA products (assessed by the DNA ladder) with the respective type strain PCR product. Fig. 1 shows an example of the identification of an isolate of *C. albicans* (lane 6), *C. glabrata* (lane 7), *C. guilliermondii* (lane 8), *C. parapsilosis* (lane 9), and *C. tropicalis* (lane 10).

CHROMagar Candida phenotypic characteristics of the *Candida* species identified by PCR are specified in Table 2. As expected, *C. glabrata*, and *C. parapsilosis* did not present a distinguishable colour in this medium. Using PCR as a standard method, and considering the CHROMagar Candida identifying colours claimed by the manufacturer, CHROMagar Candida sensitivity and specificity for *C. albicans* were found to be 97.9% and 83.3%, respectively. For *C. tropicalis* CHROMagar Candida sensitivity was 66.7%, and specificity 100%.

Candida *species carriage*

From the 97 patients evaluated, 53 were identified as oral *Candida* carriers: 81.1% were females ($n=43$), and 18.9% males ($n=10$), with ages ranging from 28 to 91 years old (mean=61 and median=62 years old). Denture wearers accounted for 84.9% of the individuals ($n=45$).

C. albicans was identified in 79% of the samples, being the predominant *Candida* species. Additionally, *C. parapsilosis* comprised 6.5% of the isolates, followed by *C. glabrata* (4.8%), *C. tropicalis* (3.2%), and *C. guilliermondii* (1.6%). Five percent of the CHROMagar Candida positive samples (Table 2) were not identified. The distribution of *Candida* isolates within gender, and age groups is presented in Table 3. There was no association between *C. albicans* or NCAC species carriage within (i) gender ($P=0.7$), (ii) the age groups defined ($P=0.83$), or (iii) denture wearing ($P=1$).

Table 2
Colour of colonies on CHROMagar Candida medium for species identified by PCR

Species	Colony colour		
	Green	Blue	Pink
C. albicans	48	–	1
C. parapsilosis	–	–	4
C. glabrata	–	–	3
C. tropicalis	1	2	–
C. guilliermondii	–	–	1
Unidentified	1	–	2

Seventeen percent of the individuals ($n=9$) presented more than one *Candida* species per sample (Table 4). These individuals (seven females, two males) had a mean age of 58 years old (range=38–88 years old). The percentage of patients wearing denture in this sub-population (88.9%) was similar to that observed in the population studied.

Discussion

Microbiological testing in the field of oral medicine facilitates diagnosis, choice of therapy, treatment assessment, and risk evaluation.[6] This study focused on diagnostics and risk evaluation.

The diagnostic approach used herein included a primary isolation on a chromogenic medium (CHROMagar Candida) followed by *Candida* species identification using a previously described PCR method.[14] The CHROMagar Candida phenotypic characteristics of the *Candida* species identified by PCR are specified in Table 2. In comparison with PCR, there was lower sensitivity and specificity of CHROMagar Candida in the identification of *C. tropicalis*, and of sensitivity regarding *C. albicans*, also described by other authors.[27] In fact, the low sensitivity shown by *C. tropicalis* to CHROMagar Candida (66.7%) is due to the fact that one isolate further identified as *C. tropicalis* exhibited green colour (Table 2), instead of the characteristic blue colour. *C. tropicalis* isolates developing dark pink,[28] lavender,[19,27] and white colours.[27] on CHROMagar Candida have also been reported. Regarding *C. albicans*, two isolates that developed green colour on CHROMagar Candida were not identified as *C. albicans* (Table 2). In addition, one of the isolates, further identified as *C. albicans*, developed pink colour on CHROMagar Candida. These colour variations are in accordance with literature reports showing that *C. albicans* isolates can develop atypical colours in CHROMagar Candida, which includes pink,[25] white,[27] blue, or lavender.[19] It

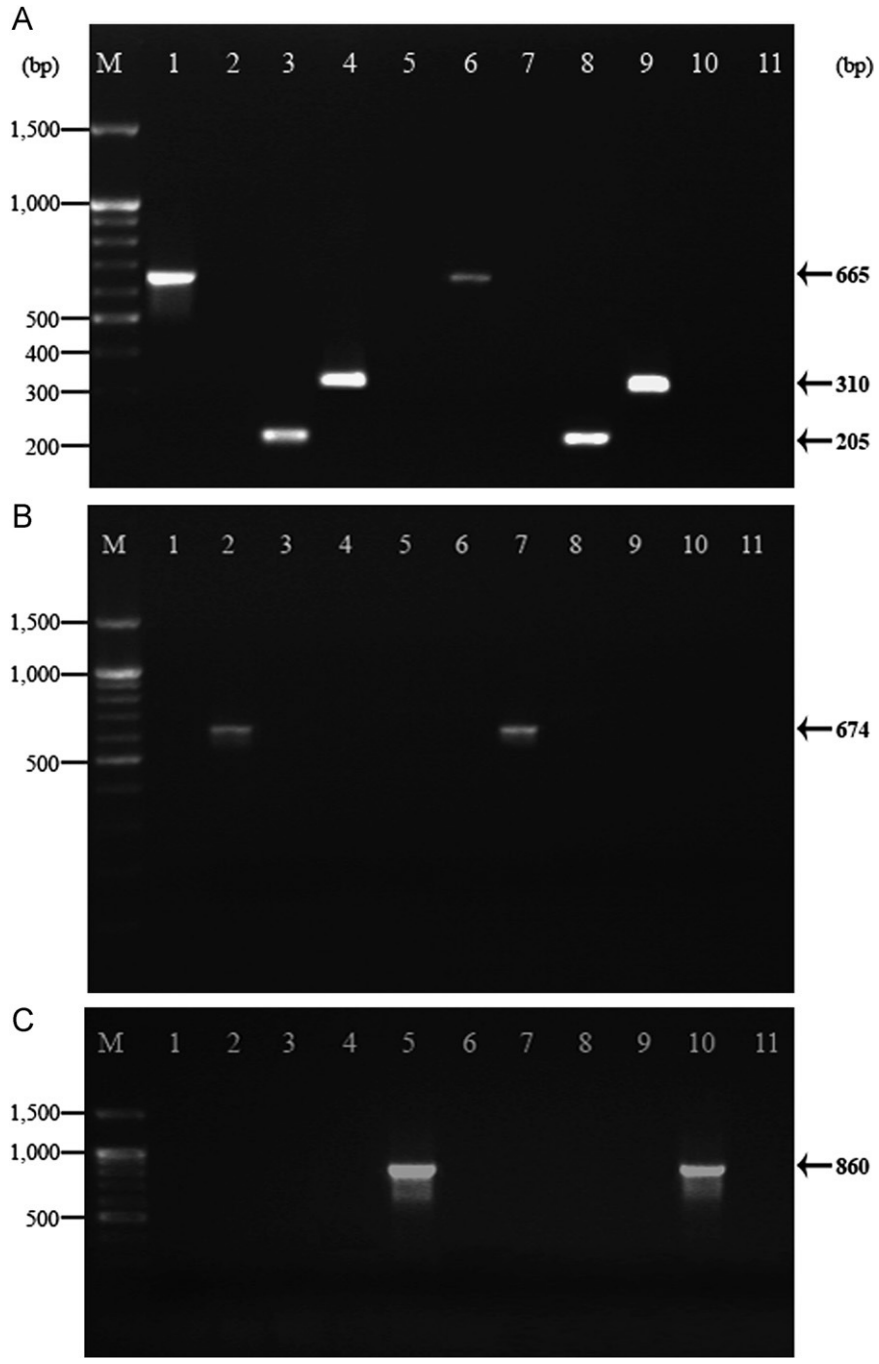

Fig. 1. *Candida* species-specific amplification of DNA topoisomerase II fragments. Genomic DNA was amplified using different sets of species-specific primers (from A to C), in accordance with Table 1. Lanes: M, 100-bp DNA marker and their molecular size in bp is indicated in the left margin; 1. *C. albicans* CECT 1472; 2. *C. glabrata* ATCC 2001; 3. *C. guilliermondii* ATCC 6260; 4. *C. parapsilosis* ATCC 22019; 5. *C. tropicalis* ATCC 750; 6–10. Example of clinical isolates of each species; 11. Blank. *C. dubliniensis*, *C. krusei* ATCC6258, and *C. kefyr* ATCC 204093 identification was omitted for simplicity. Arrows on the right indicate molecular weight of the amplified products.

should be noted that in the same plates containing these atypical strains, isolates of *C. albicans* and *C. tropicalis* with the expected colour phenotypes were also present.

Additionally, three yeast isolates were not identified by the PCR method using specific primers for the most common species (Table 2). A review of the literature reporting yeast species frequently isolated from the oral cavity,[18] and colours developed by *Candida* species in CHROMagar Candida,[20] suggest that the unidentified isolate developing green colour may be a *Trichosporon* species, and that the pink isolates could be *Candida famata*, *Candida inconspicua*, *Candida lusitaniae*, *Candida norvegensis*, *Candida pelliculosa*, or *S. cerevisiae*.

Results presented herein provide evidence that CHROMagar Candida medium fails to identify some yeast isolates, and that species identification should be supported by other methods, such as molecular characterization. Nevertheless, this medium does facilitate the recognition of polymicrobial species in cultures, as exemplified in Table 4.

The analysis of epidemiological literature on recovery of *Candida* species from the oral cavity is not clear concerning

Table 3
Frequency of distribution of *Candida* species isolated and patients' characteristics

Patient characteristics	Species[a] frequency, % (n)					
	C. albicans	NCAC species				
		C. parapsilosis	*C. glabrata*	*C. tropicalis*	*C. guilliermondii*	Unidentified
Gender						
Female	80 (40)	6 (3)	4 (2)	4 (2)	0 (0)	6 (3)
Male	75 (9)	8.3 (1)	8.3 (1)	0 (0)	8.3 (1)	0 (0)
Age (years)						
≤ 54	82.6 (19)	4.3 (1)	4.3 (1)	4.3 (1)	0 (0)	4.3 (1)
55–79	75.9 (22)	6.9 (2)	3.4 (1)	3.4 (1)	3.4 (1)	6.9 (2)
≥ 80	80 (8)	10 (1)	10 (1)	0 (0)	0 (0)	0 (0)

[a] PCR identification

Table 4
Number of patients with more than one *Candida* species

Species[a]	Patients
C. albicans–*C. glabrata*	3
C. albicans–*C. tropicalis*	2
C. albicans–*C. parapsilosis*	1
C. albicans–unidentified	2
C. parapsilosis–*C. guilliermondii*	1

[a] PCR identification.

factors determining colonization. The reasons for such variability may include differing patient selection criteria, collection data periods, geographic region in which the patients live, sampling collection methods, and methodologies used for sample analysis.

In the present study, the prevalence of yeasts isolated was 54.6%, and between 41% and 67% in previous studies.[2,7,9,17,29] However, due to the irregular distribution of *Candida* in the oral cavity,[26] it must be recognized that swab samples can yield false-negative results, and thus a misclassification of true carriers as non–carriers. The *Candida* carriage frequency observed in this study was as follows: *C. albicans* > *C. parapsilosis* > *C. glabrata* > *C. tropicalis* > *C. guilliermondii* (Table 3), with NCAC species accounting for 21% of the total *Candida* species. The increased prevalence of *C. parapsilosis* within NCAC species was also observed in Portuguese patients with fungaemia,[5] suggesting that *C. parapsilosis* might be an important fungal pathogen in Portugal. Various reports have also shown the prevalence of *C. tropicalis*,[9] *C. parapsilosis*,[11] *C. famata*,[15] or *C. glabrata*[17,29] over other NCAC species in the oral cavity, and such variation may be due to patient age or underlying disease.

The distribution of *Candida* isolates within gender and age groups are presented in Table 3. In the current study there was no association between *C. albicans* versus NCAC species carriage within gender or age as similarly observed by other authors.[13] Nevertheless, NCAC species recovered from patients greater than 80 years old were exclusively *C. parapsilosis* and *C. glabrata* (Table 3), suggesting an association between this age group and these *Candida* species. An increase in the sample size would be necessary to establish a conclusive association between these two NCAC species and this age group.

Earlier studies scarcely report identification of mixed *Candida* cultures. However, in recent years, refinement in identification procedures has allowed discrimination of multi-*Candida* species in culture. As observed in other studies,[2,8,16,17,24,29] the most common association found herein was *C. albicans* plus *C. glabrata* (Table 4). Nevertheless, the epidemiological data available report association between other *Candida* species.[2,8,15–17,24,29] It is interesting to note that in the current study *C. parapsilosis* was the only NCAC species identified that was not exclusively co-isolated with other *Candida* species (Tables 3 and 4). In fact, other authors have reported that colonization with NCAC species, as the sole species, is lower when compared with its co-colonization with other species.[17] This suggests that multi-species colonization may support maintenance of the oral NCAC population, contributing to increased interactions with molecules and surfaces in the oral cavity. In fact, it was shown that the intensity of colonization by more than one *Candida* species was higher than that observed with a single species.[17] However, when host natural defences decay, benign colonization can develop into oral candidiasis, and antifungal therapy may support NCAC species emergence as the sole detectable species from oral lesions. In fact, results from the ARTEMIS DISK Global Antifungal Surveillance Program[21–23] show that *Candida* species resistance to fluconazole can be ranked as follows: *C. glabrata*, 16%; *C. guilliermondii*, 13%; *C. tropicalis*, 2.6%; *C. parapsilosis*, 2.4%; and *C. albicans*, 1.2%. These findings suggest that multi-species carriers might be at higher risk than the mono-species carriers, of developing oral candidiasis, and of being resistant to antifungal therapy.

According to the World Medical Association Declaration of Helsinki, the purpose of research involving human individuals is to advance our prophylactic, diagnostic, and therapeutic procedures for a better understanding of the aetiology of the disease. The present study fulfilled some of these aims. The diagnosis of oral *Candida* carriage prior to the presentation of clinical symptoms facilitates dental hygiene education for routine oral care, controlling of the spread of colonization through appropriate methods, and use of therapeutic approaches when indicated. Finally, the main observation that may contribute to a better understanding of *Candida* oral carriage is the high frequency of polymicrobial cultures encountered, which may present an increased risk of infection to patients, and require careful surveillance.

Author's declaration

Authors have nothing to declare.

Acknowledgements

We are indebted to Associate Professor Toshio Kanbe (Division of Molecular Mycology and Medicine, Centre for Neurological Diseases and Cancer, Nagoya Graduate School of Medicine, Japan)

for critical discussion and advices on the PCR protocol. We also acknowledge Dr. Angel Gonzalez (Department of Biology, The University of Texas at San Antonio, USA) for Spanish language revision.

Margarida Martins was financially supported by a fellowship from Fundação para a Ciência e Tecnologia (FCT), Portugal (Contract SFRH/BD/28222/2006). This research was supported by FCT through the Project PPCDT/BIO/61112/2004.

References

1. Akpan A, Morgan R. Oral candidiasis. Postgrad Med J. 2002;78:455–9.
2. Belazi M, Velegraki A, Fleva A, Papanaum L, Baka D, Daniilidou N, Karamitsos D. Candidal overgrowth in diabetic patients: potential predisposing factors. Mycoses. 2005;48:192–6.
3. Cannon RD, Chaffin WL. Oral colonization by *Candida albicans*. Crit Rev Oral Biol Med. 1999;10:359–83.
4. Cannon RD, Holmes AR, Mason AB, Monk BC. Oral *Candida*: clearance, colonization, or candidiasis? J Dent Res. 1995;74:1152–61.
5. Costa-de-Oliveira S, Pina-Vaz C, Mendonça D, Gonçalves RA. A first Portuguese epidemiological survey of fungaemia in a university hospital. Eur J Clin Microbiol Infect Dis. 2008;27:365–74.
6. Dahlen G. Microbiological diagnostics in oral diseases. Acta Odontol Scand. 2006;64:164–8.
7. Daniluk T, Tokajuk G, Stokowska W, Fiedoruk K, Sciepuk M, Zaremba ML, et al. Occurrence rate of oral *Candida albicans* in denture wearer patients. Adv Med Sci. 2006;51:77–80.
8. Davies AN, Brailsford SR, Beighton D. Oral candidosis in patients with advanced cancer. Oral Oncol. 2006;42:698–702.
9. de Resende MA, de Sousa LV, de Oliveira RC, Koga-Ito CY, Lyon JP. Prevalence and antifungal susceptibility of yeasts obtained from the oral cavity of elderly individuals. Mycopathologia. 2006;162:39–44.
10. Farah CS, Ashman RB, Challacombe SJ. Oral candidosis. Clin Dermatol. 2000;18:553–62.
11. Gonçalves RH, Miranda ET, Zaia JE, Giannini MJ. Species diversity of yeast in oral colonization of insulin-treated diabetes mellitus patients. Mycopathologia. 2006;162:83–9.
12. Henriques M, Azeredo J, Oliveira R. *Candida* species adhesion to oral epithelium: factors involved and experimental methodology used. Crit Rev Microbiol. 2006;32:217–26.
13. Ikebe K, Morii K, Matsuda K, Hata K, Nokubi T. Association of candidal activity with denture use and salivary flow in symptom-free adults over 60 years. J Oral Rehabil. 2006;33:36–42.
14. Kanbe T, Horii T, Arishima T, Ozeki M, Kikuchi A. PCR-based identification of pathogenic *Candida* species using primer mixes specific to *Candida* DNA topoisomerase II genes. Yeast. 2002;19:973–89.
15. Kleinegger CL, Lockhart SR, Vargas K, Soll DR. Frequency, intensity, species, and strains of oral *Candida* vary as a function of host age. J Clin Microbiol. 1996;34:2246–54.
16. Liguori G, Lucariello A, Colella G, De Luca A, Marinelli P. Rapid identification of *Candida* species in oral rinse solutions by PCR. J Clin Pathol. 2007;60:1035–9.
17. Lockhart SR, Joly S, Vargas K, Swails-Wenger J, Enger L, Soll DR. Natural defenses against *Candida* colonization breakdown in the oral cavities of the elderly. J Dent Res. 1999;78:857–68.
18. Meurman JH, Siikala E, Richardson M, Rautemaa R. Non-*Candida albicans Candida* yeasts of the oral cavity. In: Méndez-Vilas A, editor. Communicating current research and educational topics and trends in applied microbiology. Microbiology book series. Badajoz: Formatex; 2007. p. 719–31.
19. Murray CK, Beckius ML, Green JA, Hospenthal DR. Use of chromogenic medium in the isolation of yeasts from clinical specimens. J Med Microbiol. 2005;54: 981–5.
20. Odds FC, Bernaerts R. CHROMagar Candida, a new differential isolation medium for presumptive identification of clinically important *Candida* species. J Clin Microbiol. 1994;32:1923–9.
21. Pfaller MA, Diekema DJ, Gibbs DL, Newell VA, Meis JF, Gould IM, et al. Results from the ARTEMIS DISK Global Antifungal Surveillance study, 1997 to 2005: an 8.5-year analysis of susceptibilities of *Candida* species and other yeast species to fluconazole and voriconazole determined by CLSI standardized disk diffusion testing. J Clin Microbiol. 2007;45:1735–45.
22. Pfaller MA, Diekema DJ, Gibbs DL, Newell VA, Ng KP, Colombo A, et al., Global Antifungal Surveillance Group. Geographic and temporal trends in the isolation and antifungal susceptibility of *Candida parapsilosis*: a global assessment from the ARTEMIS DISK antifungal surveillance program, 2001–2005. J Clin Microbiol. 2008;46:842–9.
23. Pfaller MA, Diekema DJ, Mendez M, Kibbler C, Erzsebet P, Chang SC, et al., The Global Antifungal Surveillance Group. *Candida guilliermondii*, an opportunistic fungal pathogen with decreased susceptibility to fluconazole: geographic and temporal trends from the ARTEMIS DISK antifungal surveillance Program. J Clin Microbiol. 2006;44:3551–6.
24. Qi QG, Hu T, Zhou XD. Frequency, species and molecular characterization of oral *Candida* in hosts of different age in China. J Oral Pathol Med. 2005;34: 352–6.
25. Saunte DM, Klingspor L, Jalal S, Arnau J, Arendrup MC. Four cases of *Candida albicans* infections with isolates developing pink colonies on CHROMagar Candida plates. Mycoses. 2005;48:378–81.
26. Webb BC, Thomas CJ, Willcox MDP, Harty DWS, Knox KW. *Candida* associated denture stomatitis. Aetiology and management: a review. Aust Dent J. 1998; 43:45–50.
27. Willinger B, Manafi M. Evaluation of CHROMagar Candida for rapid screening of clinical specimens for *Candida* species. Mycoses. 1999;42:61–5.
28. Yücesoy M, Marol S. Performance of CHROMagar Candida and BIGGY agar for identification of yeast species. Ann Clin Microbiol Antimicrob. 2003; 2:8–15.
29. Zaremba ML, Daniluk T, Rozkiewicz D, Cylwik-Rokicka D, Kierklo A, Tokajuk G, et al. Incidence rate of *Candida* species in the oral cavity of middle-aged and elderly subjects. Adv Med Sci. 2006;51:233–6.