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Oral candidiasis identification and profile of *Candida* in Brazilian patients undergoing radiotherapy in the head and neck region and in patients with removable complete dentures

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ABSTRACT

Introduction: Candidiasis is the most common oral fungal infection in humans affecting mainly people who are debilitated or immunocompromised patients, such as those irradiated for head and neck cancer. Pourpose: Identifing Candida species in head and neck irradiated patients and in those with total removable dental prosthesis by three microbiological methods. Material and Methods: Samples were collected with disposable sterile swabs of twenty-seven patients through exfoliative cytology. The Sabouraud media containing the inoculum were left in an aerobic environment at 36oC during 24 hours. Thus, yeasts were taken to the Laboratory of Taxonomy, Biodiversity and Biotechnology of Fungi to have the respective test for Candida identification with Candifast®, CHROMagar® and PCR EI1®. Results: The pseudomembranous clinical candidosis was more prevalent in Group 1, while the denture stomatitis was the most frequent clinical form in Group 2. There were differences in identification when comparing the three methods. On the other hand, PCR was faster and more effective when compared with CHROMagar, followed by Candifast®. PCR (EI1 and NLI/NL4) identified in Group 1 as C. albicans (37.1%), C. tropicalis (55%), (7.4%) C. krusei. In Group 2 PCR allowed the identification of 68% C. albicans, 20% as C. tropicalis, 8% as C. glabrata and 4% C. parapsilosis. Conclusion: The Candifast® Kit was found to be defective in the identification of several species of Candida, as compared to the molecular identification method used (PCR EI1 and PCR NL1 / NL4). CHROMagar® Candida medium was a good method for the presumptive identification of Candida species.

Keywords: Radiotherapy, Oral candidiasis, Denture stomatitis, Microbiology methods, Candida identification.

1 INTRODUCTION

Candidiasis is the most common oral fungal disease in humans, and can present several oral clinical manifestations, many times making it a difficult diagnosis [1,2].

In the past, candidiasis was considered as an opportunistic infection only, which affected patients debilitated by other diseases. However, today it is known that oral candidiasis can occur in relatively healthy people as a result of a complex interaction between host and microorganism [3,4].

Among the known species of *Candida*, *Candida albicans* is the most common in the mouth and the most cited in scientific papers [5]. However, other species are being identified in patients with candidiasis, which currently justifies a greater interest in researching other microorganisms of this genus. Advances in medicine have led to prolonged survival of immunocompromised patients and the increasing importance of opportunistic fungal infections [6,7]. *Candida* is the most common opportunistic yeast and causes severe infections characterized by high mortality rates. Antifungal agents are particularly toxic to human cells and the use of such drugs has to obey strict criteria. However, one early and aggressive antifungal therapy that takes the etiologic agent involved into account may improve the prognosis of infected patients [8].

Oral candidiasis is a common comorbidity associated with oral mucositis in patients with cancer and irradiated head and neck region. Complications such as oral mucositis, dysgeusia, interruption of feeding and speech, and of oncological treatment are comorbidities observed in these patients [9]. Candidiasis is usually associated with oral mucositis in these patients, which makes treatment even more difficult. Hyposalivation caused by radiotherapy facilitates the colonization by *Candida* species [10,11].

Due to the fact that the treatment instituted for these patients is not always specific, there is a significant increase in the resistance of the species of *Candida* to the antifungals present on the market. This may be due to the institution of a long treatment and the incorrect selection of active principles in relation to the agent etiology of the infection, or even by the selection of species or strains with resistance intrinsic to the drugs used. This whole process generates side effects such as nephrotoxicity and hepatotoxicity. Therefore, it is of extreme importance to accurately identify the species of infecting *Candida*, as well as the establishment of specific protocols for treatment, for by knowing the microorganism and its susceptibility to antifungals methods can the patient be treated more effectively and less aggressively [12, 13, 14].

Candidiasis epidemiological studies related patients with prostheses are of great importance to assess this fungal prevalence [15]. The correct identification of isolated species, the virulence factors verification and the profile analysis of antifungal susceptibility agents to these microorganisms may contribute to the development of other research controls and determine a new therapeutic proposal for the candidiasis treatment [16, 17]. Conventional laboratory techniques for yeast identification (culture, biochemical, physiological and morphological characteristics) have a long time of release ("turnaroundtime"). Under these conditions, faster and more sensitive, molecular-based techniques are of great value.

In recent years, some chromogenic culture media capable of differentiating the species *C. albicans* and other yeasts of clinical interest have emerged. These means of culture for fungi are based on the color change developed by the colonies through pH indicators and fermentation of specific compounds or chromogenic substrates. The CHROMagar® *Candida* medium produced by Difco has been used to isolate and identify presumably some species of *Candida* present in the oral cavity, including *C. albicans*, *C. krusei*, *C. dubliniensis* and *C. tropicalis*. This method is based on the use of β-glucosaminidase substrate and differentiates yeasts according to the morphology and color of the colonies. Its use facilitates the detection and identification of these yeasts and also provides results in lesser time than those obtained by traditional methods [18, 19].

Biochemical tests for the detection of *Candida* species and for testing the resistance of microorganisms to antifungal agents, such as Candifast®, have been shown to be useful, practical and effective, as well as molecular techniques, such as the Polymerase Chain Reaction (PCR). The secret of PCR's success lies in its ability to amplify a precise DNA sequence allied to its simplicity, accuracy, high sensitivity and specificity [20].

The objective of the present study is to identify *Candida* species in Brazilian head and neck irradiated patients with malignant neoplasms and in the ones who make use of removable dental prostheses, using CHROMagar® culture medium *Candida*, biochemical tests as Candifast® and molecular tests such as PCR (Polymerase Chain Reaction).

2 MATERIAL AND METHODS

2.1 PATIENTS AND SAMPLE UNIVERSE

The project was approved by the Research Ethics Committee of the Federal University of Minas Gerais (COEP/UFMG), with Project number CAAE- 0561.0.203.438-11. The volunteers signed the Free and Informed Consent Term to authorize the collection of samples.

Patients irradiated in the head and neck region were referred by public and private hospitals in Belo Horizonte and were selected at the Dental Care Clinic for Patients with Cancer and Irradiated in the Head and Neck region, of the Faculty of Dentistry of the Federal University of Minas Gerais (FOUFMG). Patients with Denture Stomatitis were selected at the Removable Prosthesis Clinic and at the Dental Pathology and Semiology Clinic at FOUFMG. After clinical diagnosis suggestive of candidiasis, the patients underwent an initial clinical examination (baseline) where the study's own forms were filled out based on medical records from the Semiology and Pathology clinics of the UFMG School of Dentistry. Identification and anamnesis data were collected, followed by general objective and intraoral examinations. All study information was confidential, and the names of the participants, as well as photos of their faces were not disclosed.

2.2 INCLUSION AND EXCLUSION CRITERIA

Patients who presented clinical manifestations of candidiasis, such as the presence of removable white plaques, erythematous areas associated with prostheses or not were included in the study. The head and neck irradiated patients included in the sample were undergoing radiotherapy treatment or had it completed within a maximum of 6 months (Inclusion criteria). Patients who 1) did not have candidosis; 2) had clinical manifestations of candidiasis and did not accept the intraoral collection or did not comply with the consent form; 3) were not physically able to undergo material collection (mucous scraping); 4) had prostheses and were head and neck irradiated took part in our exclusion criteria.

2.3 COLLECTION OF MICROORGANISMS

The sample collection period was from November 2011 to April 2012. Samples were collected from all patients who accepted to participate in the survey and presented with candidiasis, whatever the clinical aspect of the disease. The samples were of the "Convenience" type and there was no predilection for gender, age or race. The collection of the material was done by scraping the mucous membranes/exfoliative cytology using a sterile and disposable swab, which was placed directly in the areas where there was presence of candidiasis.

2.4 YEAST DNA EXTRACTION

After presumptive identification of yeasts using CHROMagar® *Candida*, yeasts of different morphotypes were streaked on Sabouraud agar (each morphotype was placed in a separate Petri dish) so that pure colonies were obtained. Thus, these pure yeasts were reassessed in terms of morphotype and were taken to the Laboratory of Taxonomy, Biodiversity and Biotechnology of Fungi at the Department of Microbiology - ICB/UFMG to have the respective DNA extracted. After DNA extraction from the yeasts, the samples were taken to the Nano Drop ® equipment to assess the quality of the extractions.

2.5 MOLECULAR IDENTIFICATION OF YEAST: PCR-EI1

For the molecular differentiation of yeasts, the PCR method known as "fingerprint" was used using the primer - "primer" EI1 (5'-CTGGCTTGTGTATGT-'), which has complementarity to the intron processing sites. Strains of the same species show similar amplification profiles. Thus, after grouping the species that were similar to PCR EI1, a representative of each group was sent for DNA sequencing through PCR EI1. When the PCR procedure was performed for several yeasts, a mix was made with reagents from numbers 1 to 8 and after filling each eppendorf with the solution, the DNA was added lastly and placed in the PCR machine for the beginning of the cycles. PCR products were analyzed by

electrophoresis on a 1.5% agarose gel in 0.5X TBE buffer, diluted in 6X running buffer and Gel Red for approximately 1 hour at 120 V. The bands present on the gel were visualized under ultraviolet light and photographed using a gel photo-documentation system (Vilber Lourmat, France). The amplification results were compared with the reference samples (standard 1 KB) that were placed in the initial channels of each experiment gel [21, 22].

2.6 SEQUENCING OF THE D1D2 REGION OF THE LARGE SUBUNIT OF RIBOSOMAL DNA

The strains that presented PCR profiles different from the most common species were sequenced in order to confirm the molecular identification. Sequencing was performed in the D1/D2 region of the ribosomal DNA major subunit using primers-“primer” NL1 (5'-GCATATCAATAAGCGGAGGAAAAG-') and NL4 (5'-GGTCCGTGTTTCAAGACGG-'). a MegaBacetm 1000 automatic sequencer (Amersham Biosciences, USA) at the Biodiversity and Molecular Evolution Laboratory (LBEM-UFMG). Sequences were edited using the DNAMAN program (lynnon BioSoft, Vaudreuil, Québec). The sequences obtained were compared with those already deposited in GenBank and analyzed using the BLASTn program (Basic Local Alignment Search Tool - version 2.215 of BLAST 2.0) available on the NCBI portal (http://www.ncbi.nlm.nih.gov/blast /) developed by the National Center for Biotechnology. The isolates that presented the analyzed sequences with ≥98% similarity in relation to the sequences already deposited in GenBank were considered as belonging to the same species or genus; those that presented sequences with similarity ≤9% were considered as belonging to the same genus [23].

3 RESULTS

3.1 DEMOGRAPHIC PATIENTS’ INFORMATION

Table 1 shows the demographic informations and individuals’ distribution. In Group 1, 13 male patients (86.7%) and 2 female patients (13.3%) were found. Regarding Group 2, 9 patients were female (75%) and 3 were male (25%).

Table 1- Demografic Patients’ information.

Patients Group	Group Description	Number	Gender	Age (Mean/Years)	Race
Group 1	Irradiated in Head and Neck Patients	15	M (13) 75% F (2) 25%	55-83 (63,2)	L: 30,7% F: 30,7% M: 38,5%
Group 2	Dentures Removable Prosthesis	12	M (3) 33,3% F (9) 66,7%	54-73 (61,4)	L:66,6% F: 22,2% M: 11,1%

Legend: Gender M= male, Gender F= female; Race: L= leucoderm/White, F= feoderma, M= melanoderm/Black.

In Group 2 prosthesis patients, the clinical diagnosis followed different patterns from those of Group 1. In this group only two clinical forms of candidiasis were observed: Denture Stomatitis was

present in 10 out of the 12 patients analyzed (83.3%) while Angular Cheilitis was identified in 4 patients (33.3%). In two patients (16.6%), the two forms were found simultaneously. Of all the patients evaluated in this group, eleven (91.6%) used a removable total prosthesis and only one patient (8.4%) used a removable partial denture. These data are described in Figure 1 and Figure 2.

Considering all sites, the tongue was the most affected site (7 patients, 46.6%), followed by the palate (40%), alveolar ridge (26.6%), buccal commissure 26.6%), jugal mucosa (20%), buccal floor and oropharynx (both with 13.3%). However, in 4 patients (26.6%), there was a simultaneous affection in several sites (≥ 3), that is, the candidiasis occurred in a generalized way. In relation to Group 2, the only sites containing lesion involvement were the palate (83.3%) and buccal commissure (33.3%).

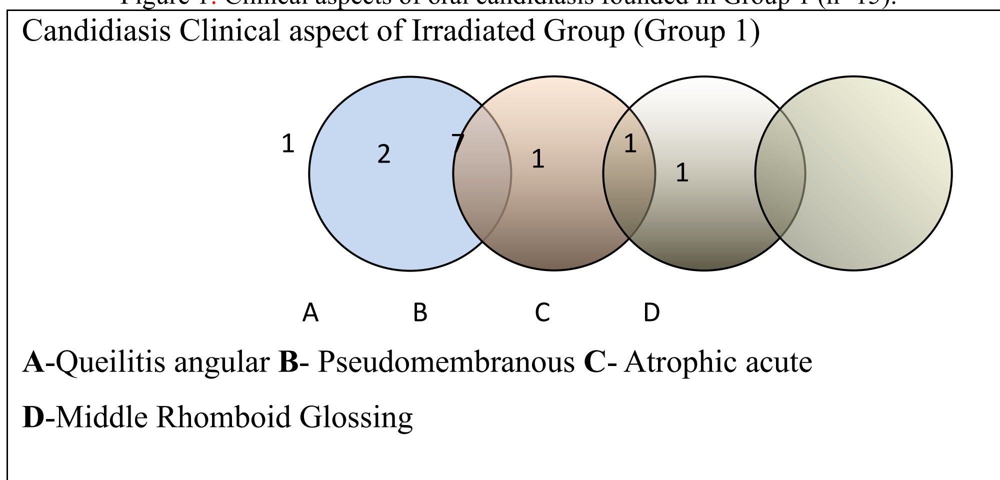


Figure 1: Clinical aspects of oral candidiasis founded in Group 1 (n=15).

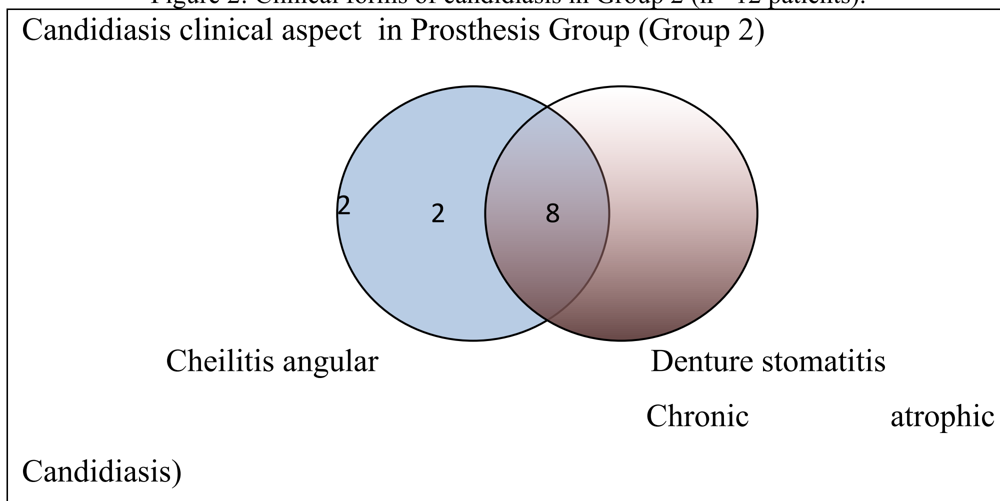


Figure 2: Clinical forms of candidiasis in Group 2 (n= 12 patients).

The identification of the samples was done by using three methods: Biochemical test (Candifast®), physiological test with CHROMagar® Candida medium and PCR EI, to confirm their identification through molecular biology.

3.2 CANDIFAST® IDENTIFICATION TEST

The biochemical test, of the 15 patients of Group 1 (irradiates in the head and neck region) 18 samples were obtained that were inoculated in Sabouraud agar. Two samples were obtained in three patients (20%). Samples I7 (tongue) and I7.1 (palate) presented different clinical forms of candidiasis (Pseudomembranous Candidosis and Acute Atrophic Candidosis, respectively). Samples I9 and I9A presented the same clinical form (Pseudomembranous Candidosis), however the first one affected the tongue, and the second had an oropharynx. In another evaluated patient (samples I14 A and I14B), the colonies presented different morphotypes in the Sabouraud agar, and were thus identified separately. Therefore, out of the 18 samples identified, 7 (38.8%) were identified as *C. albicans* and 7 (38.8%) as *C. tropicalis*. The other four samples were identified as distinct species: one as *C. krusei* (5.5%), one as *C. lusitaniae* (5.5%), one as *C. parapsilosis* (5.5%) and the last one as *C. glabrata*.

For Group 2 (prosthesis carriers), the biochemical test allowed the identification of a total of 14 samples from the 12 patients evaluated. In two patients (16.6%) it was possible to obtain two samples, as they presented two clinical types of candidosis simultaneously: Denture stomatitis on the hard palate and Angular cheilitis in the buccal commissure. In these four described isolates (P2, P2.1, P3 and P3A), the Candifast® kit identified *C. albicans*. The other samples were also identified as *C. albicans* (100%).

3.3 CHROMAGAR® IDENTIFICATION TEST

The CHROMagar® *Candida* medium allowed the presumptive identification of three species of *Candida*: *C. albicans*, which shows green coloration; *C. tropicalis* showing blue coloration and *C. krusei* with pink coloration (Figures 3A, 3B and 3C). Through the CHROMagar® *Candida* medium, 27 samples from Group 1 (irradiated) and 25 samples from Group 2 (Prosthesis holders) were isolated. Out of the samples tested (n = 52), 25 (48%) were identified as *C. albicans*, 18 (34.6%) were identified as *C. tropicalis* and 4 (7.7%) as *C. krusei*. However, 5 samples (9.7%) could not be identified by using this method because the colonies did not present the colour stains described by the manufacturer - green, blue or pink. These colonies showed white, beige and purplish rose colorations, which could not be presumptively identified by the CHROMagar® *Candida* medium.

Figure 3: Presumptive identification of *C. albicans* (A), *C. tropicalis* (B), and *C. krusei* (C). CHROMagar® *Candida* method.

Considering only Group 1, 9 samples (33.3%) were identified as *C. albicans*, 14 (51.9%) as *C. tropicalis*, and 2 (7.4%) as *C. krusei*. 2 samples (7.4%) were not identified because they had other colorations (white and beige) not described by the manufacturer. In relation to Group 2, out of the 25 samples isolated by CHROMagar® *Candida*, 15 (60%) were identified as *C. albicans*, 4 (16%) as *C. tropicalis*, 2 (8%) as *C. krusei* and 3 (12%) were not identified.

Figure 4: Presumptive identification (Group 1=27 samples) using the CHROMagar® medium.

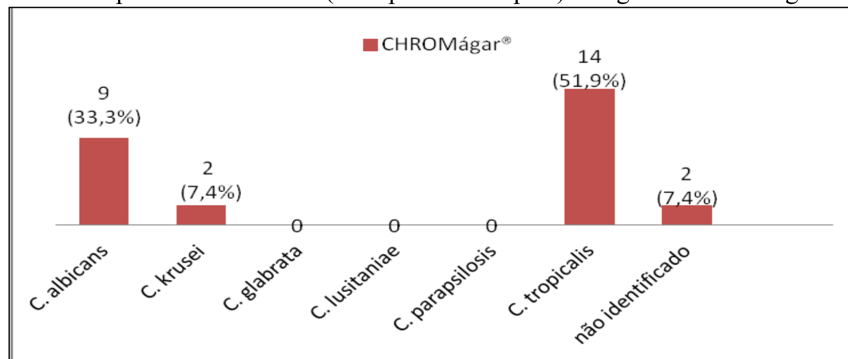
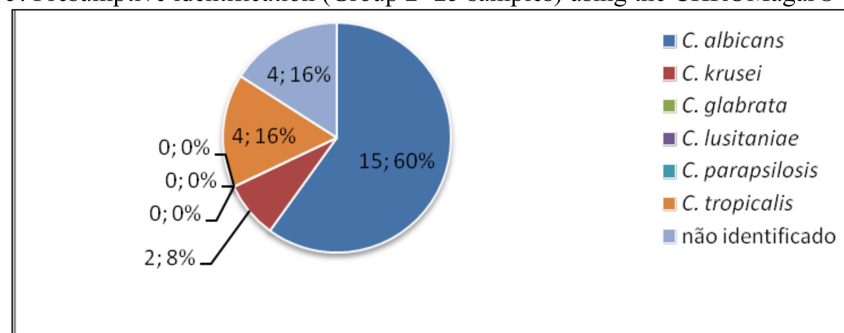


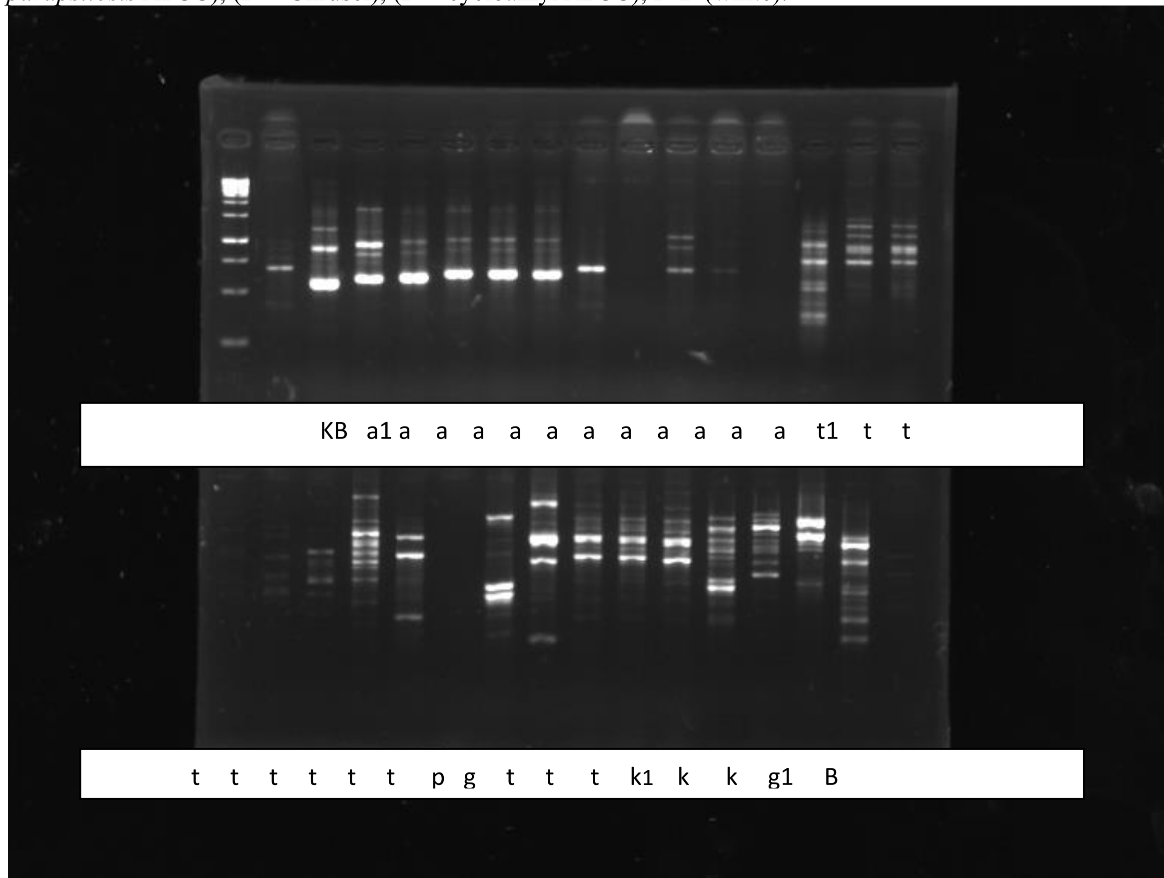
Figure 5: Presumptive identification (Group 2=25 samples) using the CHROMagar® medium.



3.4 FINGERPRINT PCR METHOD

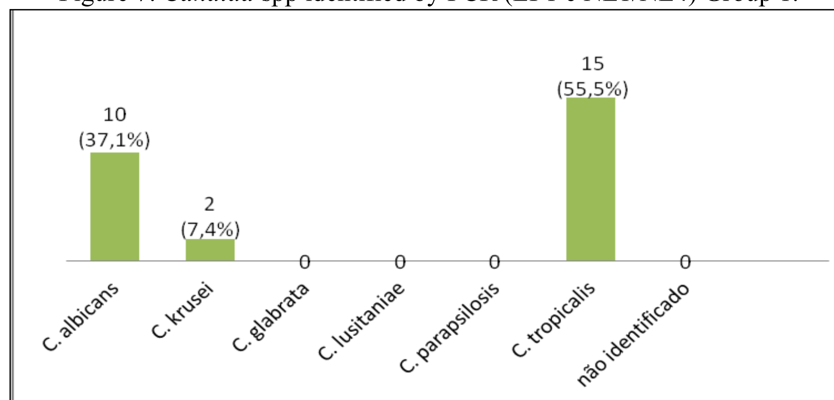
After the identification of the yeasts by the biochemical method and the presumptive identification of the CHROMagar® medium, representatives of each morphotype were selected for the identification confirmation, using the DNA fingerprint technique with the first EI1. When the PCR cycles were completed, the products of each sample were placed on 1.5% agarose gel and analyzed by electrophoresis. Samples that resembled CHROMagar® were placed side by side on the gels and the bands were stained and photographed (Figure 6). Samples that were identical in electrophoresis were considered to be from the same species. Each representative of each group was subsequently sequenced (D1 / D2 region of the major subunit of rDNA) to confirm this identity by comparing the nucleotide sequences obtained in this work with other sequences previously deposited in the bank gene. Two software programs were used: Electropherogram Quality Analysis (Embrapa) and Blast (Basic Local Alignment Search Tool) that found regions of similarity between the biological sequences.

Figure 6: Similar samples in CHROMagar® *Candida* were placed side by side for run on 1.5% agarose gel. (Standard), (a- *C. albicans*), (a1- *C. albicans* ATCC), (t-*C. tropicalis*), (t1- *C. tropicalis* ATCC) (g- *C. glabrata*), (g1 -*C. glabrata* ATCC), (p *C. parapsilosis* ATCC), (Î ± -Ckrusei), (Î ± -cycloalkyl ATCC), Î²-B (white).



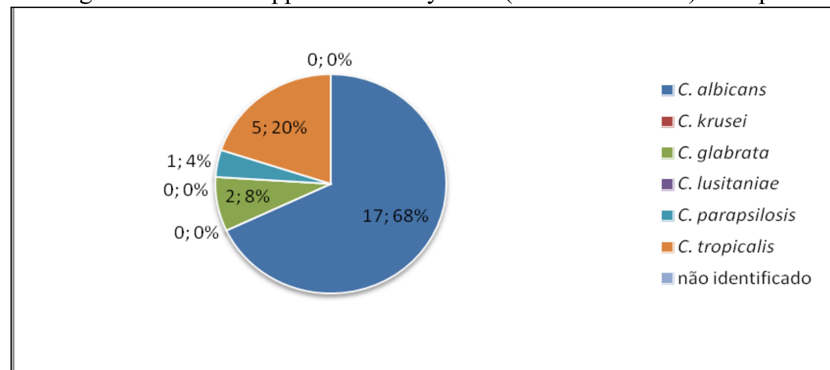
After identification by PCR and confirmation by sequencing, the number of yeasts for Group 1 (Irradiated in head and neck region) was: from 27 isolates, 10 (37.1%) were identified as *C. albicans*, 15 (55, 5%) were identified as *C. tropicalis and* 2 (7.4%) as *C. krusei* (Figure 7). PCR EI 1 did not confirm the identification of *C. lusitaniae*, *C. parapsilosis* and *C. glabrata* as determined by the Candifast® Kit and the 2 samples that the CHROMagar® *Candida* medium had not identified were actually *C. albicans* and *C. tropicalis*.

Figure 7: *Candida* spp identified by PCR (EI 1 e NL1/NL4) Group 1.



In Group 2 (Prosthesis Carriers), 25 yeasts were isolated and 17 (68%) of the samples were identified as *C. albicans*, 5 (20%) as *C. tropicalis*, 2 (8%) as *C. glabrata* and 1 (4%) as *C. parapsilosis*. Figure 15 shows the results described (Figure 8). Thus, the Candifast® Kit allowed the identification of 18 yeast isolates in Group 1 and 14 yeasts in Group 2. By the other two methods of identification, it was possible to isolate a larger number of microorganisms because the CHROMagar® *Candida* medium allows a better visual separation of morphotypes, differently from the Sabouraud agar medium, where the yeast distinction is very difficult.

Figure 8- *Candida* spp identified by PCR (EI 1 e NL1/NL4) Group 2.



Therefore, when comparing the three identification methods used in this present study, it was observed that the results obtained with the CHROMagar® *Candida* medium coincided in almost 100% with those presented with PCR EI1. However, when the PCR was compared with the Candifast® kit, there were divergences in the results in the 2 groups. The kit erroneously identified some yeasts such as *C. lusitaniae*, *C. parapsilosis* and *C.glabrata* in Group 1, which were not confirmed by PCR and in relation to Group 2, Candifast® identified only *C.albicans*, which was not confirmed by PCR EI1, which identified three other yeast species: *C.tropicalis* (5 samples), *C. glabrata* (2) and *C. parapsilosis* (1). Thus, in Group 1, 11 species (4 *C. albicans*, 6 *C.tropicalis* and 1 *C. krusei*) were correctly identified with Candifast® (61.8% of agreement with PCR) whereas in the Group 2 evaluation, 7 isolates of *C. albicans* were found (50% of agreement with PCR).

4 DISCUSSION

This study allowed the identification of the main *Candida* species that colonize the mouth by three methods: the biochemical methods Candifast® and CHROMagar® *Candida* medium and the molecular identification by PCR EI 1 - NLI / NL4 PCR.

Several studies have shown that during radiotherapy, the number of non-albicans species increases significantly, but *C. albicans* is still the most prevalent species [24,25,26]. The present study did not confirm these findings, demonstrating a greater prevalence by *C. tropicalis* (55.5%) of the

samples identified, followed by *C. albicans* (37.1%) and *C. krusei* (7.4%). This increased incidence can be attributed to the intense radiotherapy to which patients undergo, to the association with chemotherapy or to the sometimes variate methods used in the identification of these microorganisms by others authors.

In relation to Group 2, 68% of *C. albicans* were identified, followed by 20% *C. tropicalis,* 8% *C. glabrata* and 4% *C. parapsilosis*. The results of this research are in agreement with Oliveira et al. [27] and Qiu et al. [28], who found a higher prevalence of *C. albicans* in patients with total prosthesis, and the palate was the most affected site by the lesions. In another study [28, 29], which aimed to analyze the genetic variability of *Candida* yeasts in the oral cavity of users of total prosthesis and control individuals, the author also concluded that *C. albicans* was the most often found. However, he cites that *C. glabrata* was the second most prevalent species, followed by *C. tropicalis*, *C. parapsilosis* and *C. krusei*. These results differ from those found in this study. Table 2 shows the comparative results of species identification by the three methods.

Table 2- Comparison of three presunctive identification methods for oral clinical *Candida* species in Group 1 and Group 2 patients.

Microorganisms	Group 1			Group 2		
	Candifast (n=18)	Chromagar (n=27)	PcrEll (n=27)	Candifast (n=14)	Chromagar (n=25)	PcrEll (n=25
	Diagnosed cases number			Diagnosed cases number		
C. albicans	7 (38.8%)	9 (33.3%)	10 (37.1%)	14 (100%)	15 (60%)	17 (68%)
C. tropicalis	7 (38.8%)	14 (51.9%)	15 (55.5%)		4 (16%)	5 (20%)
C. krusei	1 (5.5%)	2 (7.4%)	2 (7.4%)		2 (8%)	-
C. lusitaniae	1 (5.5%)	-	-		-	-
C. parapsilosis	1 (5.5%)	-	-		-	1 (4%)
C. glabrata	1 (5.5%)	-	-		-	1 (4%)
Not identified		2 (7.4%)	-		3 (12%)	-

Identification using the CHROMagar® *Candida* medium coincided in almost 100% with the PCR method. This shows that the first can be used to identify three *Candida* species: *C. albicans* (green), *C. tropicalis* (blue) and *C. krusei* (pink). It is a fast method and easy to implement, however the cost should be considered on choice. Some colonies showed small color changes, which may have occurred due to metabolic changes among the isolates. Most authors agree that other species could not be identified with this method, as they may present several morphotypes and stains not compatible with those described by the manufacturer [30, 31, 32].

Although *C. dubliniensis* may also show green-colored stains, MÄHNß et al. [33] conducted a study for phenotypic differentiation between the *C. albicans* and *C. dubliniensis* species and failed to create a precise way to distinguish them using CHOMagar® *Candida*, which is in accordance with our results.

In the present study it was observed that the colonies of *Candida glabrata* presented a pink color, with a bright appearance. As it was a pink colony, it was identified by CHROMagar® *Candida* as *C. krusei*. However, Madhvan et al. [18] studied the sensitivity and specificity of the CHROMagar® *Candida* medium in the identification of oral yeasts and concluded that *C. glabrata* could also be identified by this method and presented a different morphotype of *C. krusei*: its colonies were convex, more punctiform, with regular edges and darker pink coloring. They did not have the "dry" aspect of a *C. krusei*. Species that were not identified by CHROMagar® had white or beige colorations. They were identified by PCR as *C. albicans*, *C. tropicalis* or *C. parapsilosis*. The latter, according to Madhvan et al. [18] and Sampath et al. [34] can present a huge number of colors and different aspects of morphotype.

The Candifast® Kit showed a 50% agreement with the PCR method for Group 2 and 61.8% agreement for Group 1. The manufacturer cited a degree of agreement above 90% with other biochemical methods, however does not compare it with PCR, which is the gold standard in yeast identification. A negative aspect of the test which can be cited is the subjectivity in reading the results of Candifast®. The degree of turbidity of the dilution vial is measured visually, as well as the color reading in the wells. The manufacturer elucidates that colors "yellow-orange", "yellow" and "fuchsina" are "positive growth" and "reddish orange" is "negative growth". What can be appreciated is that these colors often get confused. The Sabouraud agar medium is the recommended way to use the Candifast® Kit. Due to the fact that this medium most often did not distinguish the yeast morphotypes, the number of isolates determined by the Candifast® Kit was much lower in the two groups compared to the CHROMagar® *Candida* medium. Using the Sabouraud agar medium, 18 samples were identified in Group 1 and 14 samples in Group 2. For CHROMagar® *Candida*, 27 and 25 were isolated, respectively. Thus, the biochemical method allowed a sub-identification of the yeasts and was not efficient in the differentiation of other species tested. This suggests that there is a need to reformulate this kit's methodology. This could be done by replacing Sabouraud agar in the first step of identification with the CHROMagar® *Candida* medium. Thus, after identification of different morphotypes, these samples would now be spiked on Sabouraud agar and the identification process would be continued following the steps described by the manufacturer [19, 35].

Several authors cite the co-infection in their works and their possible relation with the degree of virulence of these microorganisms [11, 17, 36]. Some patients tested in this study had co-infection, where it was possible to detect more than one *Candida* species causing the disease. In Group 1, the presence of two species of *Candida* was detected in four patients, and *C. albicans-C.tropicalis* co-infection occurred in three patients and in one *C. albicans-C.krusei* patient. In Group 2, co-infection occurred in five patients, and in three patients the C. albicans and *C.glabrata* species were present simultaneously, in one case *C. albicans-C.parapsilosis* and *C. albicans-C. tropicalis*. Infections by

multiple species of *Candida* (co-infections) can be considered a response to the resistance of microorganisms to the various antifungals used by patients. In conclusion, *C. tropicalis* was the most found species in Group 1 (Irradiated in the head and neck region). The species *C. albicans* was the most common species in Group 2 (with prostheses). The Candifast® Kit was found to be defective in the identification of several species of *Candida*, as compared to the molecular identification method used (PCR EI1 and PCR NL1 / NL4). CHROMagar® *Candida* medium was a good method for the presumptive identification of *Candida* species. It has limitations when the colors found in the colonies differ from green, blue or pink.

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DECLARATION COMPETING

The authors have declared no conflict of interest.

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AUTHOR CONTRIBUTION STATEMENT

All authors contributed both in the experimental performance and in patient care, as well as in reviewing text.

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DATA AVAILABILITY STATEMENT

Information about the names and clinical information were recorded and filed at the Faculty of Dentistry of the UFMG and will not be exposed to maintain the privacy of each patient. All data were included in this article.

AUTHOR DECLARATION

The authors declare their agreement with the publication of this work publishing as a chapter of the book HEALTH and MEDICINE: Science, Care, and Discoveries, by Seven Publicações.

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