

Oral Colonization by Different *Candida* Species: First Comparative Study between Denture and Nondenture Wearers in Tunisia

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Abstract

Objective This study aimed to compare different *Candida* species present in patients with and without removable dentures to identify alterations in biofilm composition following denture wear within a Tunisian population.

Materials and Methods A cross-sectional study was conducted, comprising a group of patients wearing removable dentures (test group) and a control group without dentures. In the test group, two mycological samples were obtained: one from the prosthetic intaglio and another from the osteomucosal area bearing the denture. For the control group, mycological samples were collected from the oral mucosa. The collected swabs were cultured on CHROMagar *Candida* medium, and yeast counts were quantified as colony forming units (CFUs). *Candida* species were identified through chromogenic analysis.

Statistical Analysis The normality of quantitative variables was evaluated using the Kolmogorov–Smirnov’s test. To compare means and ranks between the test and control groups, the independent samples *t*-test and the Mann–Whitney’s *U* test were employed, respectively. Qualitative variables were compared using Fisher’s exact test. Statistical significance was determined at a critical uncertainty value of $p < 0.05$.

Results A total of 150 participants were involved in this study, with 75 patients in each group. Wearing an acrylic removable denture was found to increase the number of detected *Candida* species ($p < 0.001$) and significantly increases the overall growth of *Candida* spp. ($p = 0.001$). Specifically, the numbers of CFUs of *Candida tropicalis* and *Candida glabrata* were elevated in denture wearers ($p < 0.001$).

Keywords

- ▶ *Candida albicans*
- ▶ *Candida tropicalis*
- ▶ *Candida glabrata*
- ▶ *Candida krusei*
- ▶ removable dentures

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Conclusion Findings stemming from this study indicate that removable dentures promote the growth of *Candida* species. This can be a predisposing factor for *Candida*-associated denture stomatitis in cases of poor oral hygiene or compromised immunity. Therefore, it is imperative to emphasize the fabrication of high-quality dentures and the implementation of rigorous postdenture maintenance protocols to prevent or limit *Candida* infection.

Introduction

Candida species commonly inhabit the oral cavity as commensal microorganisms in healthy individuals. Among them, *Candida albicans* is the most common species and typically remains nonpathogenic.^{1,2} However, wearing removable dentures can alter the oral environment, leading to changes in the physical and biological properties of saliva and other oral structures.³ These alterations often cause an imbalance in the local microbiota, fostering the formation of biofilm with complex structure and composition.⁴ Denture wear can therefore constitute vectors of microbial virulence and act as predisposing factors to the onset of pathologies related to *Candida* spp., such as *Candida*-associated denture stomatitis (CADS).^{2,5-9} *Candida* species contribute to localized diseases on mucosal surfaces and are associated with invasive opportunistic mycoses, constituting a major cause of nosocomial bloodstream infections.¹⁰ Although *C. albicans* has been reported to be the principal *Candida* strain responsible for CADS, recent evidence suggests the rise of non-*albicans* *Candida* species as predominant pathogens in this condition.^{8,11,12}

The emergence of oral *Candida* species other than *C. albicans*, such as *C. krusei*, *C. glabrata*, *C. tropicalis*, and *C. parapsilosis* has garnered significant attention from research groups around the world.^{3,13-16} The increase in oral *Candida* species counts following the insertion of dentures predicts the pathogenicity of these microorganisms.¹² Importantly, some of these species display resistance toward antifungal agents.^{17,18}

Most studies related to the identification and quantification of *Candida* species in patients wearing removable dental prosthesis, or dentures, have focused on those exhibiting symptoms of CADS, with few mycological studies exploring typical denture flora.^{10,19-22}

To our knowledge, this study marks the first investigation conducted in Tunisia to discern changes in mycological profiles related to *Candida* colonization among healthy wearers of removable dentures. Our primary objective was to investigate and compare the prevalence and the abundance of different *Candida* species in cohorts of Tunisian patients with and without dentures.²³

Materials and Methods

Ethical Approval

The study was approved by the Ethics Committee of the Faculty of Medicine of Monastir (certificate number IORG 0009738N°129/OMB 0990-0279). Before participation, all

patients were informed of the study's objectives, and written consent was obtained. This study adhered to principles outlined in the Declaration of Helsinki guidelines.

Patient Selection

This study was conducted over 3 months, from January 2023 to March 2023, at the Department of Removable Prosthodontics at the Dental Clinic of Monastir and the Mycology Laboratory at Fattouma Bourguiba University Hospital. The study included a total of 150 patients, divided into two groups: the test group and the control group. Inclusion and exclusion criteria are detailed in ►Table 1.

The test group comprised 75 patients wearing dentures who were treated at the department of prosthodontics between 2018 and 2022. Eligible participants were identified through a review of archived files, following which they were contacted by telephone and invited to participate in the test group. The control group consisted of 75 patients who consulted the department of prosthodontics for the first time for prosthetic rehabilitation.

Data and Sample Collection

Clinical information regarding patients' current clinical condition and medical history was collected using a specifically designed clinical information sheet.

Mycological samples were taken in the morning while patients were fasting, without performing any oral or denture hygiene procedures beforehand. Sterile swabs, premoistened with a saline solution, were utilized for sample collection and then transferred to sterile tubes.

For the test group, two mycological samples were taken for each patient: one from the intaglio surfaces of the denture and another from its osteomucosal supporting area. Notably, in cases where participants were wearing bimaxillary removable dentures, the sample was taken from the upper denture.

In the control group, the mycological samples were exclusively collected from the oral mucosa surrounding the retroincisal papilla zone. This zone, covering an area of approximately 1 cm² around the retroincisive papilla, was chosen due to its accessibility and standardized anatomic alignment across all patients.

The collected swabs were transported for culture in CHROMagar *Candida* medium. After 20 to 48 hours of aerobic incubation at 35 to 37°C, yeast counts were determined and expressed as colony forming units (CFUs). Identification of *Candida* species was conducted through chromogenic analysis followed by additional biochemical testing with ID 32C

Table 1 Inclusion and exclusion criteria for control and test groups

	Test group	Control group
Inclusion criteria	<ul style="list-style-type: none"> • Good general health as indicated by clinical record data • Wearing a uni- or bimaxillary acrylic removable dentures for at least 6 mo • Positively responding to the phone call to take part in the study 	<ul style="list-style-type: none"> • Good general health
Exclusion criteria	<ul style="list-style-type: none"> • Incomplete medical history • Lack of contact number in the medical record • Being unreachable when called • Reporting during phone call that they lost or did not wear their dentures • Discovery of changes in patient's health status during the interview (e.g., diabetes, xerostomia, autoimmune diseases, HIV-positive status, undergoing chemotherapy, chronic illnesses involving immunosuppressive treatments, etc.) • Use of antifungal medications, corticosteroids, antibiotics, or antiseptic mouthwash in the previous 2 mo • Use of adhesive creams • Presence of tissue conditioners on the intaglio surface • Symptoms of CADS during clinical examination 	<ul style="list-style-type: none"> • History of wearing removable dentures • Clinical signs of oral candidiasis or xerostomia • Use of antifungal medications, corticosteroids, antibiotics, immunosuppressants, or oral antiseptics in the previous 2 mo

Abbreviations: CADS, *Candida*-associated denture stomatitis; HIV, human immunodeficiency virus.

biochemical identification strips (BioMérieux, France). Visual readings were conducted after 48 to 72 hours of incubation at 27°C. Species identification was finalized by analyzing sugar assimilation profiles using the APIWEB website.²⁴

Operational Definitions

The obtained mycological cultures were interpreted following specified operational definitions.

- Negative culture: The culture was interpreted as negative if no yeast colonies were identified.
- Positive culture: The culture was interpreted as positive if at least one yeast colony was identified.
- Positive patient (test group): A patient in the test group was classified as positive when the oral and/or denture mycological results were positive.
- Negative patient (test group): A patient in the test group was classified as negative when both the oral and denture mycological results were negative.

Statistical Analysis

Statistical analysis of data was performed with SPSS (Statistical Package for the Social Sciences) version 26. The normality of quantitative variables was evaluated using the Kolmogorov–Smirnov's test. Differences in means and ranks between test and control groups were compared using the independent samples *t*-test and the Mann–Whitney's *U* test, respectively. Qualitative variables were compared using Fisher's exact test. The differences observed were considered statistically significant when the critical uncertainty value was $p < 0.05$.

Results

The study comprised a total of 150 participants. The test group consisted of 75 subjects, including 51 men and 24 women, resulting in a sex ratio of 2.12. Participants' ages ranged from 35 to 92 years, with a mean age of 60.29 ± 10.74

years. The control group consisted of 75 subjects, including 41 men and 34 women, with a sex ratio of 1.2, an age range of 35 to 81 years, and a mean age of 57.28 ± 8.74 years. Statistical analysis revealed no significant differences between the two groups regarding age and gender ($p > 0.05$), indicating their independence for these variables.

Cultures were performed for both groups, revealing important differences in positivity rates. In the test group, 90.6% of the patients (68 out of 75) were positive, including 55 (73.33%) patients who were positive for *Candida* in both oral and denture samples, 4 (5.33%) positive only in oral samples, and 9 (12%) positive exclusively in denture samples. In the control group, 81.3% (65 out of 75) of the patient samples were positive for *Candida*.

When distinguishing the species of *Candida* detected in positive mycological samples, 72.6% of positive oral samples from the test group contained more than one *Candida* species (►Table 2). Further, in this group, all four *Candida* species (*C. albicans*, *C. glabrata*, *C. krusei*, and *C. tropicalis*) were identified in two oral samples and one denture sample (►Fig. 1).

A significantly greater diversity of *Candida* species was observed in the mycological samples derived from the test group as compared with the controls ($p < 0.001$) (►Table 3). The detection frequencies of each *Candida* species exhibited notable differences between samples from the test and control groups (►Fig. 2). *Candida albicans* was the most frequently identified species in positive cultures from both the test and control groups, with detection rates of 68 and 60% for the oral and denture samples, respectively, in samples from the test group and 76% in the control samples. Importantly, non-*albicans* species including *C. tropicalis*, *C. glabrata*, and *C. krusei* displayed higher detection frequencies in the oral and denture samples from the test group as compared with the control samples (►Fig. 2).

The number of CFUs in samples derived from test and control groups were compared, revealing a higher total

Table 2 Distribution of *Candida* species in the positive cultures from denture-wearing and control patient samples

Group		Test		Control
Sample site		Oral n (%)	Denture n (%)	Oral n (%)
Number of <i>Candida</i> species detected	1 species	16 (27.1)	18 (28.1)	44 (72.13)
	2 species	20 (33.8)	20 (31.2)	13 (21.31)
	3 species	21 (35.5)	25 (39)	04 (6.56)
	4 species	2 (03.3)	1 (1.5)	0 (0)

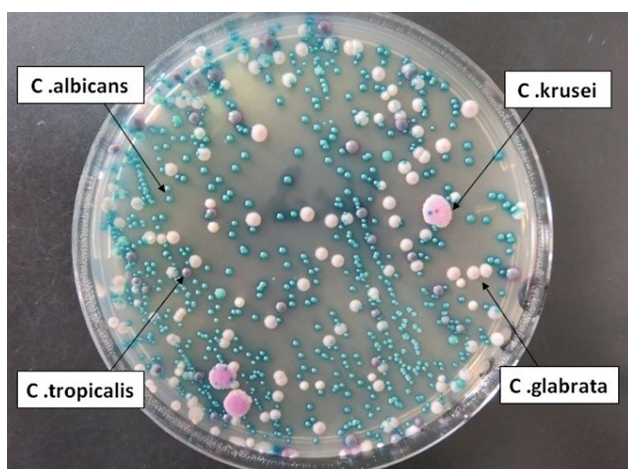


Fig. 1 Representative mycological culture from a denture-wearing patient, demonstrating the presence of four *Candida* species.

number of *Candida* spp. CFUs in test samples ($p=0.001$) (► **Table 4**). Quantitative analysis of distinct species indicated that *C. tropicalis* and *C. glabrata* were notably higher in denture wearers than in nonwearers ($p < 0.001$), while no significant differences in the abundance of *C. krusei* and *C. albicans* were noted between the test and the control groups.

Discussion

Removable dentures are intended to replace missing teeth, fulfilling an esthetic and functional role while prioritizing biocompatibility and safety.³ Our study shows that wearing an acrylic dentures increases the number of detected *Candida* species and significantly promotes the growth of *Candida* spp. Specifically, the number of CFUs of *C. tropicalis* and *C. glabrata* are significantly higher in denture wearers than nonwearers.

According to some reports, patients with removable dentures are at a higher risk of developing CADS due to the development of pathogenicity in oral commensal *Candida* spp. as a result of alterations to local conditions associated with denture wear.^{6,20} While most of these reports predominantly focused on *C. albicans*,^{25,26} few groups have evaluated the role of non-*albicans* species.^{13,20,27}

To the best of our knowledge, this study represents the first qualitative and quantitative mycological investigation of the different *Candida* species in denture wearers and nonwearers in Tunisia. All patients included in the study were in good general health, enabling the elimination of confounding factors that might influence the mycological microflora. Specifically, patients with weakened immune systems have a disrupted oral microflora, increasing susceptibility to oral infection. Similarly, immunosuppressive treatments and xerostomia favor elevated yeast counts and increase the risk of CADS. Consequently, patients with xerostomia or those undergoing immunosuppressive treatments were excluded from the study.^{3,28-30}

Previous studies exploring this topic have been carried out only 1 month after patients initiate denture wear.^{31,32} Our study required patients in the test group to wear removable dentures for at least 6 months. This duration, as determined by Dar-Odeh and Shehabi,²⁷ is sufficient to detect the true effect of wearing dentures on the oral mycological microflora.

The sampling technique chosen in this study involved oral and denture swabs, while other authors utilized oral rinse samples or unstimulated saliva.^{3,14} *Candida* species, particularly *C. albicans*, are known for their strong adhesive capacity and propensity to form biofilms.³³ Swab sampling of the palatal mucosa or prosthetic intaglio is therefore the optimal approach for examination as it can mechanically obtain biofilm samples. This technique also offers additional advantages in terms of simplicity, rapidity, reproducibility, and ease of transport and storage.³⁴

Table 3 Comparison of the number of *Candida* species detected between denture-wearing and control patient samples

Group	N (%)	Median (Q1; Q3)	Minimum	Maximum	p-Value
^a Test group	75 (50)	2 (1; 2)	0	4	
Control group	75 (50)	1 (1; 1)	0	3	<0.001 ^b
Total	150 (100)	1 (1; 2)	0	4	

^aThe maximum number of *Candida* species detected in the denture or oral cultures was used for statistical analysis.

^bSignificant value as determined by the Mann-Whitney's *U* test ($p < 0.05$).

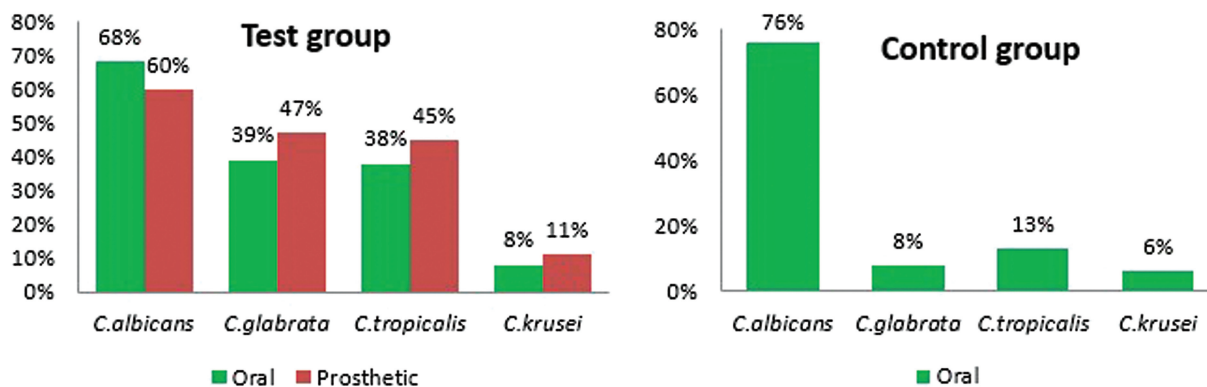


Fig. 2 Detection frequencies of *Candida* species in positive cultures from denture-wearing and control patient samples.

The CHROMagar *Candida* culture medium was chosen due to its selective nature and direct speciation based on color production, surpassing the performance of the commonly used Sabouraud dextrose agar.^{14,35}

Mycological examination revealed that 90.6% of the patients in the test group exhibited oral cultures that were positive for *Candida* spp., compared with 81.33% in the control group. In a study by Prakash et al,¹³ 100% of the patients wearing dentures were found to be positive for *Candida* spp., compared with 52% of patients without dentures. *Candida* spp. are commonly found in patients not wearing dentures, as they are saprophytes of the oral cavity. In the human oral microbiota, *Candida* spp. are present subclinically as commensals, and their presence is regulated by other residents of microbiota and by host immunity.^{13,36,37} However, when comparing the mycological profile of the two groups, the number of *Candida* species was significantly higher in the test group than in the control samples. The oral cavity of denture wearers was found to be colonized by a mixture of *Candida* species, a trend observed in most patients in the test group.

Sant'Ana et al reported that the presence of two or more *Candida* species in the same patient could predispose the patient to recurrent stomatitis.³⁸ Apart from *C. albicans*, the detection frequency of the various *Candida* species was

higher in denture wearers compared with patients without dentures. In our study, although *C. albicans* was the most frequently detected species in both groups, it was more frequently isolated in patients without dentures than in denture wearers. Prakash et al also reported that *C. albicans* is the predominant species (96.2%) in patients without dentures, whereas its prevalence is 58% in denture wearers.¹³ This suggests a probable competition among the different *Candida* species in denture wearers that could explain the lower detection frequency of *C. albicans* in favor of other *Candida* species.

Contrary to the results of this study, Gleiznys et al concluded in their review that the prevalence of *C. albicans* is 45 to 65% in healthy individuals and that this prevalence increases to 60 to 100% in denture wearers.⁶

The detection frequency of non-*albicans* *Candida* species was also found to be higher in the test group compared with the control group. Analysis of non-*albicans* species indicated that *C. glabrata* was most frequently isolated in denture wearers, followed by *C. tropicalis* and *C. krusei*. The frequency at which these *Candida* species are observed can have significant implications for human infections.³¹

In line with our findings, Vanden et al observed a higher prevalence of *C. glabrata* (44.1%) than *C. tropicalis* (19.1%) in a

Table 4 Quantitative comparison of *Candida* species identified denture-wearing and control patient samples

<i>Candida</i> species	Group	N (%)	Median (Q1; Q3)	Minimum	Maximum	p-Value
<i>Candida</i> spp. (all species)	Test	75 (50)	1,070 (5; 5,000,507)	0	505,050,005	0.001 ^a
	Control	75 (50)	45 (1; 1,000)	0	20,000,000	
<i>Candida albicans</i>	Test	75 (50)	22.5 (0; 50,004)	0	505,000,000	0.729
	Control	75 (50)	45 (1; 1,000)	0	10,000,000	
<i>Candida tropicalis</i>	Test	75 (50)	0.5 (0; 50,001)	0	5,050,000	<0.001 ^a
	Control	75 (50)	15 (0; 63)	0	100	
<i>Candida glabrata</i>	Test	75 (50)	0.5 (0; 57)	0	500,050,000	<0.001 ^a
	Control	75 (50)	0 (0; 0)	0	10,000,000	
<i>Candida krusei</i>	Test	75 (50)	2 (3; 42)	0	5,000,000	0.935
	Control	75 (50)	0 (0; 0)	0	100,000	

^aSignificant value as determined by the Mann-Whitney's *U* test ($p < 0.05$).

group of elderly subjects with dentures.³⁹ Calcaterra et al also reported that the most frequently isolated non-*albicans* *Candida* species in the oral mucosa and dentures is *C. glabrata*, followed by *C. dubliniensis* and *C. tropicalis*.⁴⁰

While *C. glabrata* is frequently coisolated with other *Candida* species from the oral lesions, its role in the pathogenesis of CADS is still poorly understood.⁴¹ Studies suggest that *C. glabrata* and *C. albicans* coinfection may lead to more severe symptoms and treatment complexity.⁴² *Candida glabrata* can produce proteases, phospholipases, and hemolysins, and can promote biofilm formation, enabling evasion of the host immune response and generation of fungal resistance.⁴¹ Overexpression of drug transporters and genetic mutations that modify thermotolerance are mechanisms which contribute to resistance to antifungals, such as azoles, polyenes, and echinocandins, ultimately enhancing virulence.⁴¹

Semiquantitative analysis revealed a significant increase in the total number of *Candida* CFUs in the test group compared with the control group. Removable dentures are not inert elements; their insertion leads to changes in the physiology of the oral microbiota by inducing plaque formation that favors an abundance of potentially pathogenic bacteria and *Candida* spp.^{43,44} The abundance of *Candida* in individuals wearing dentures is crucial for infection development,²⁰ where a higher yeast count, often exceeding 400 CFU/mL of saliva, is indicative of CADS.⁴⁵ Ghani et al reported that wearing dentures significantly enhances the pathogenic activity of oral *Candida*, leading to a mean pH decrease of 1.9 compared with 0.8 in nondenture wearers.⁴⁴ Additionally, the presence of exogenous material, particularly acrylic resin, provides new adhesion surfaces for oral biofilm.³⁶ Wearing dentures reduces the exposure of the osteomucosal surface to the mechanical action of saliva and the tongue, thus facilitating colonization by microbes, particularly acidogenic bacteria and *Candida* spp.⁴⁰

As shown by Du et al,⁴⁶ *Candida* spp. interact with the coexisting oral bacteria through physical attachment, extracellular signaling, and metabolic cross-feeding. These interactions enhance the cellular and biochemical composition of the biofilm, thereby increasing its pathogenic potential.

Salmanian et al demonstrated that yeast, present in the oral cavity and the digestive tract, could serve as a reservoir for *Helicobacter pylori* and play an important role in bacterial reinoculation of more distal locations in the gastrointestinal tract.⁴⁷ Consequently, diagnosing patients wearing dentures, even in the absence of symptoms, is paramount due to the importance of the oral mucosa as a primary source of bacterial reinfection.

Quantitative analysis of *Candida* species showed a significant increase in the number of CFUs of *C. tropicalis* and *C. glabrata* in the test group. Building upon this finding, the quantitative increase of these two non-*albicans* *Candida* species could be a predictive indicator of the development of CADS.

Multiple research groups have observed a significant increase in *C. glabrata* CFUs following the insertion of removable dentures, highlighting the species' ability to adapt to different environmental conditions.^{39,48}

Despite higher numbers of *C. albicans* CFUs in the test group compared with the control group, no statistically significant association was found between denture wear and *C. albicans* abundance. However, as a saprophyte of the oral cavity, *C. albicans* may enhance its pathogenicity or become an opportunistic pathogen after denture insertion. Certain strains of *C. albicans* can produce carcinogenic nitrosamines from saliva and promote neoplastic changes.⁴⁹

Wearing dentures was not found to significantly affect the number of *C. krusei* CFUs. Quantitative analyses of *C. krusei* are not frequently performed, limiting our capacity to make direct comparisons. Further, the exclusion of patients with CADS might explain the lack of association between *C. krusei* abundance and denture wear. Indeed, some reports have identified *C. krusei* as the most frequently non-*albicans* *Candida* species isolated from patients with CADS.⁵⁰

In their research, Samaranyake and Samaranyake reported that *C. krusei* is less virulent than *C. albicans* in terms of its capacity to adhere to epithelial and denture surfaces.⁵¹ Notably, *C. krusei* demonstrates distinct structural and metabolic characteristics from other *Candida* species and exhibits different behaviors in response to host defenses. Further understanding of the pathogenic potential of this yeast, together with advancements in molecular biology techniques, could further elucidate the epidemiology and the pathogenesis of *C. krusei* infections.

Denture wearers were found to be asymptomatic carriers of multiple *Candida* species more frequently than non-wearers. This underscores the potential of dentures as vectors of virulence and their role in exposing individuals to the risk of CADS. As there are no established diagnostic tests to reliably distinguish infection from colonization, the susceptibility of patients to infection could not be predicted using quantification of *Candida* CFUs alone.^{52,53} Other local and systemic predisposing factors linked to host conditions, involving changes from commensal to pathogenic forms, must be considered.^{8,54}

Due to variations in sampling methods (swab, oral rinse, etc.) and sampling sites (tongue, oral mucosa, dentures, saliva, etc.), comparing our findings with those of other researchers constitutes a challenge. Differences in laboratory techniques and methods used to identify *Candida* species, including real-time polymerase chain reaction and diverse biochemical tests, further complicate the direct comparison of results between research groups. These discrepancies likely contribute to contradictory findings reported across different studies (► **Supplementary Table S1**, available in the online version).

The proliferation and coexistence of non-*albicans* *Candida* species in individuals wearing removable dentures may also present a challenge in the management of candidal infections. In cases where a resistant oral candidal infection is diagnosed, it is crucial to request species identification from the laboratory, to adjust treatment strategies, including the choice of antifungal agent and dosage, accordingly.^{15,55,56}

Finally, although this study made efforts to quantify CFUs, the method employed was semiquantitative and therefore only provided an approximate assessment. Moreover,

additional parameters that could influence oral colonization by *Candida* spp., such as the patient's age, gender, smoking habits, the duration of denture wear, and oral hygiene, were not studied. Further investigation using quantitative methods, next-generation sequencing, and controlling for confounding population variables may provide additional insight into the colonization of non-*albicans* *Candida* in wearers of removable dentures.

Conclusion

Findings stemming from this study underscore the potential role of removable dentures as reservoirs for *Candida* spp. The increase in both the total number of *Candida* colonies and the variety of non-*albicans* *Candida* species present in samples derived from healthy denture wearers suggests an increased risk for CADS mediated by denture wear. Preventive measures should be implemented to mitigate this risk, including the provision of well-fabricated dentures and rigorous professional postdenture maintenance. Additionally, patients should be instructed on how to undertake daily denture and oral hygiene practices. Further investigation is required to delineate the exact mechanisms by which dentures alter oral *Candida* populations.

Conflict of Interest

None declared.

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