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RESEARCH ARTICLE

PREVALENCE, TYPING, AND ASSOCIATED FACTORS OF ORAL *CANDIDA* COLONIZATION IN DENTURE WEARING AND FIXED ORTHODONTIC APPLIANCE COMPARING TO NORMAL TEETH INDIVIDUALS

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Abstract

Background and objectives: Oral Candida colonization (OCC) is one of the most common opportunistic fungal infections among people who wear dentures and have FOA. The prevalence of OCC and Candida among denture patients and FOA patients may change due to the presence of these dental appliances. This study aims to identify the different Candida species, determine the prevalence of OCC in 3 groups of individuals with removable or fixed dentures and fixed orthodontic appliances (FOA) compared with a control group of people with natural teeth and study the risk factors associated with contracting oral Candida species among these groups.

Subjects and methods: The investigational group was selected from denture and orthodontic patients who were clinically examined at the earliest time to obtain basic information before any effective treatment of dental infection. The group included 104 dental patients; 104 fixed orthodontic appliance (FOA) patients and 102 controls. The oral mucosa of each participant was first swabbed and then placed in a liquid medium. Then, after creating successive dilutions and applying them to Sabouraud dextrose agar, the isolates were diagnosed by culturing them on *Candida* agar with color, observing the colony characteristics of the species, in addition to studying the biochemical reactions to confirm the species.

Results: Of the 108 persons with OCC, 44 (34.8%) had a positive OCC. 148 oral *Candida* spp. were found in 108 OCC patients. *C. albicans* (49.1%) was the most frequently isolated species, followed by *C. glabrata* (35.2%) and *C. dubliniensis* (13%). *C. kefyr, C. krusei, C. famata, C. africana,* and *C. stellatoidea* were initially isolated for the first time in the oral cavities of Yemeni dentistry patients. A higher rate of colonization was observed in the male group (48.5%) versus 25% in the female group, with odds ratio equal to 2.8, *CI*=1.7–4.6, X^2=18, and p<0.001. Also there was association of denture wearing (44.2%, OR=1.8, p =0.01) and older ages, (52.5%, OR= 2.3, p=0.01) with high rate of oral *candida* colonization.

Conclusion: *Candida* colonization remains a major problem in the oral cavity. Although the prevalence of non-*albicans Candida* (NAC) species is increasing, *C. albicans* was still the dominant species. A higher rates and significant risk factors of OCC were observed in the male group, denture patients and the elderly.

Keywords: Denture, fixed orthodontic appliance (FOA), non-*albicans Candida* (NAC), Oral *Candida* colonization (OCC), Yemen.

INTRODUCTION

Thirty to eighty percent of healthy people may harbor *Candida* species (*Candida* spp.), one of the typical components of the physiological oral microbiota[1-5]. Of them, *Candida albicans* appears to be the most common, followed by *Candida glabrata, Candida tropicalis, Candida krusei, Candida parapsilosis,* and *Candida glabrata*[1,6]. Although the presence of *Candida* in the oral cavity does not always signal that a problem has been found, this fungus has all the characteristics of an oportunistic pathogen, which means that it has the potential to cause an infection in the oral cavity under specific circumstances. *Candida* can cause a

range of illnesses, from superficial to systemic, and in severe situations, it can even be fatal[7]. Mucosal resistance and factors changing the local oral environment affect the incidence of infection. These include immunological system alterations in the host, the use of antibiotics, diabetes, smoking, advancing age, pregnancy, hyposalivation, poor dental hygiene, and different kinds of dentures[8-12]. A patient may develop oropharyngeal candidiasis as a result of oral candidiasis[13]. Transplant patients also experience colonization with *Candida* spp. and the associated illnesses more frequently than non-transplant patients[14]. Moreover, there is a strong correlation between *Candida* and both malignant and possibly malignant oral lesions[15]. The information that is now available indicates that oral infections caused by *Candida* species are indicated by candida cell counts in concentrated oral rinse samples of more than 600 CFU/mL[16,17].

The preservation of saliva is crucial in the fight against oral candidiasis. *Candida* reproduction is inhibited by lubrication, cleaning, hydration, and antimicrobial action based on elements such lactoferrin, lysozyme, sialoperoxidase, and histidine-rich polypeptides[18,19]. As a result, factors that cause changes in salivary quantity and quality also foster the growth of infection through salivary reproduction. This may lower the salivary flow rate and result in the formation of lesions in the salivary glands[18]. Furthermore, it has been discovered that patients who wear dentures are more likely than those who do not to have *Candida* spp. invade their mucosa[4,7,20,21]. The narrow space between the denture and mucosa between removable dentures creates an ideal environment for unrestricted *Candida* reproduction because they replace a portion of the resorbed alveolar ridge next to the missing teeth and have a wide contact area with oral mucosa[3,12,20].

Cobalt-chromium alloys (Co-Cr) and polymethyl methacrylate (PMMA) are materials utilized in the denture base's manufacture. Because of their inherent characteristics, the materials used to make prosthetic appliances have the potential to directly impact the amount of *Candida* present in the oral cavity (mainly through variations in surface structure, such as the degree of porosity, roughness, and surface-free energy). Similar to PMMA-based materials[22], appliances with a cast metal base made from Co-Cr alloys have a more stable and smooth surface and are not susceptible to the development of surface porosity, with the exception of cases of non-intimate adhesion of the compound Co–Cr and PMMA on the basis of dentures, which may act as a predilection site for fungal colonization. Thus, the use of less durable materials usually has a higher risk of *Candida* growth and dissemination, which might result in an oral infection that presents clinically.

Understanding the elements that lead to the development of diseases makes infection prevention easier and lowers its incidence[7]. Evidence currently available indicates a relationship between the occurrence of clinical oral signs and symptoms and the presence of *Candida* spp.[23]. Patients frequently describe red sores on the tongue and oral mucosa, dry mouth, and changed taste[16].

With the use of innovative techniques and supplies that benefit both patients and professionals, orthodontics is a field that is always evolving[24]. Controlling bacterial and yeast biofilms around the arcs has been emphasized as a means of preventing the development of carious sores or oral infections in orthodontic patients[4,5,20]. Orthodontists are also in charge of guarding against dental caries and other oral infections while a patient is receiving treatment[4,5,20,26]. The orthodontic appliance functions as an alternative location where biofilms can grow[27]. It is hypothesized that stainless steel feet above critical surface tension will have a better plaque retention capacity according to a study by Eliades *et al.*,[28]. Additionally, it has been discovered that metal orthodontic brackets promote particular alterations in the oral environment, such as low pH levels and the microbial inclination towards metal surfaces as a result of electrostatic responses[29], as well as greater plaque accumulation and microbial colonization. Other research, however, was unable to draw any firm conclusions on possible variations in the initial convergence and adherence of microorganisms on metal, ceramic, and plastic brackets over time[5,20,30,31].

This study aims to identify the different Candida species, determine the prevalence of OCC in 3 groups of individuals with removable or fixed dentures and fixed orthodontic appliances (FOA) compared with a control group of people with natural teeth, and study the risk factors associated with contracting oral Candida species among these groups.

MATERIALS AND METHODS

Over the course of two years, beginning in January 2022 and ending in June 2024 (time allowed for doctoral work), *Candida* tests were conducted on 310 people (104 with dentures, 104 with orthodontic abaratus, and 102 controls without dental prostheses) in the dental clinics of the Faculty of Dentistry, Sana'a University, Yemen, and the Department of Medical Microbiology Laboratory, Faculty of Medicine and Health Sciences. Healthy people without systemic illness or clinical indications of a *Candida* infection met the inclusion requirements for the selection of subjects. Furthermore, those who had smoked or were taking immunosuppressive, steroid, antibiotic, or antifungal medications.

Sample Size and power: The sample size was determined by comparing the rates of variance between cases, or patients wearing dental prostheses, and controls using computing software. If the change in OCC ratio for the control group is 2% and for the cases is 20%, then we need 102 participants in each group, with a power of 80% and a confidence level of 99.9%. A total of 310 individuals were selected: 104 were denture wearers, 104 were receiving orthodontic treatment, and 102 were acting as controls without dentures or FOA. The remaining five cases did not have any issues.

Patient sample collection for *Candida* Count (CFU): Two sterile cotton swabs were taken from each patient and control group in order to gather samples. Three groups of swab samples were sent to the microbiological lab after being put in Stuart transport media. At two separate points in the mouth of the patient, buccal mucosa oral swabs were taken[5,25]. Before culture, the collected swab was dissolved in 1 ml of phosphate-buffered saline and stored at -20°C. The sample was then used for CFU calculation, culture, and *Candida* species determination.

Culturing *Candida*: On Sabouraudus dextrose agar, 100 µl of the concentrated oral sample dissolved in phosphate buffer was added, and the mixture was incubated for 48 h at 370°C. At -20°C, samples dissolved in phosphate buffer were preserved long-term. If *Candida* colonies appeared on Sabouraud dextrose agar, 100 µl of oral sample swab fluid was used to inoculate the chromogenic *Candida* agar, which was subsequently incubated for 48 h to examine the colonies. Using the manufacturer's color reference guide, colony colors were used to identify *Candida* species. A fermentation test involving sucrose, maltose, glucose, lactose, and galactose was performed when color identification was ambiguous. The ability of *Candida* species to generate chlamydospores on glutinous rice agar was another method used for their identification[33].

Statistical Analysis: Epi-Info Statistics version 7 was used to screen the information. The results were expressed as percentages to describe *Candida* isolates by species. Data on associated risk factors were statistically analyzed by determining the odds ratio and 95% CI, and significance testing of association was performed using the chi-square test. A p value of <0.05 was considered significant.

Ethical Consideration: On January 1, 2022, the Medical Ethics and Research Committee of Sana'a University's Faculty of Dentistry granted ethical permission for Contract No. 317 project. The ethical principles set forth by the review committee were consistently followed. The chosen participants provided their informed, signed consent.

RESULTS

The study included 310 individuals, 104 with dentures, 104 with orthodontic abaratus, and 102 controls without dental prostheses, 41.9% males and 58.1 females, ranging in age from 9 to 90 years, with a mean±SD of age equal to 37.01±20.9 years old. Most of the participants were in the age group 21–30 years (25.8%), followed by ≥51 years (23.9%) and 31–40 years (22.3%). The rate of candida colonization was 34.8% (108/310) (Table 1). A total of 310 eligible study participants were included in the final statistical analysis. Of the 108 persons with OCC, 34.8% had a prevalence rate. 148 oral *Candida* spp. were found in 108 OCC patients.

Table 1: General characteristics of participate in the study.

Characters	N (%)	Characters	N (%)
Sex		**Ages (years)**	
Male	130 (41.9)	<21 years	50 (16.1)
Female	180 (58.1)	21-30	80 (25.8)
Type of patients		31-40	69 (22.3)
Denture	104 (33.5)	41-50	40 (12.9)
orthodentic	104 (33.5)	≥51	74 (23.9)
Normal	102 (32.9)	Mean age	37.01Years
Total	310 (100)	SD	20.9 Years
		Mode	23 Years
		Median	26 Years
		Min-Max	9- 90 Years

According to species distribution (Table 2), *C. albicans* (49.1%) was the most frequently isolated species throughout current study, followed by *C. glabrata* (35.2%) and *C. dubliniensis* (13%). *C. kefyr*, *C. krusei*, *C. famata*, *C. africana*, and *C. stellatoidea* were initially isolated for the first time in the oral cavities of Yemeni dentistry patients. Furthermore, out of 310 individuals, mixed cultures of two to three species of *Candida* were discovered in 44 cases (14.2%). Co-infection with *C. albicans* and/or *C. glabrata* was most frequently detected when non-*albicans* species were present. Further details on co-infection of *Candida* species are included in Table 3. Considering the frequency and association of oral *Candida* species colonizing buccal mucosa with denture cases, orthodontic cases, and normal controls, in addition to sex and age groups. The total colonization rate of oral *Candida* species colonizing buccal mucosa was 34.8%.

Table 2: Distribution of *Candida* strains (n=108 patient) 148 isolated from denture, FOA and normal teeth individuals (n=310).

Species	Number (%)
C. albicans	53 (49.1)
C. glabrata	38 (35.2)
C. dubliniensis	14 (13)
C. tropicalis	15 (13.9)
C. famata	8 (7.4)
C. kefyr	8 (7.4)
C. krusei	4 (3.7)
C. parapsilosis	3 (2.8)
C. africana	3 (2.8)
C. stellatoidea	2 (1.9)
Single growth candida isolates	68/148 (45.9)
Mixed growth candida isolates	80 /148 (54.1)
Total candida isolates	148
Mono-infection cases	64/310 (20.6)
Co-infection cases	44/310 (14.2)
Positive candidaiasis cases	108/310 (34.8)

Table 3: Distribution of co-infection with different *Candida* species in 108 patients with OC.

Co-infection species	Number (%)
C. albicans, C. glabrata	15 (34.1)
C. albicans, C. dubliniensis	4 (9.1)
C. albicans, C. africana	1 (2.3)
C. albicans, C. famata	3 (6.8)
C. albicans, C. stellatoidea	1 (2.3)
C. albicans, C. tropicalis	3 (6.8)
C. albicans, C. kefyr	1 (2.3)
C. albicans, C. kefyr	1 (2.3)
C. albicans, C. krusei	1 (2.3)
C. dubliniensis, C. glabrata	2 (4.6)
C. dubliniensis, C. famata	1 (2.3)
C. dubliniensis, C. tropicalis	1 (2.3)
C. glabrata, C. tropicalis	3 (6.8)
C. glabrata, C. kefyr	1 (2.3)
C. tropicalis, C. parapsilosis	1 (2.3)
C. famata, C. kefyr	1 (2.3)
C. famata, Candida krusei	1 (2.3)
C. albicans, C. famata, C. krusei	1 (2.3)
C. albicans, C. glabrata, C. dubliniensis	1 (2.3)
C. albicans, C. glabrata, C. kefyr	1 (2.3)
Total co-infections out of positive cases	44/108 (40.7)

A higher rate of colonization was observed in the male group (48.5%) versus 25% in the female group, and the variance was statistically significant (p<0.001), with the associated odds ratio for male patients equal to 2.8, *CI*=1.7–4.6, X^2=18, and p<0.001. According to age group, there was a high rate of *Candida* colonization in the 41–50 year old age group, equal to 52.5%, with an associated odds ratio of 2.3, *CI*=1.2–4.5, X^2=6.3, and p=0.01. However, the mean±SD of the positive *Candida* colonization cases was 39.5±20.9 years higher than 37.01±20.9 years for the total participants. Comparing oral *Candida* species colonizing buccal mucosa rates between denture/orthodendic cases and normal control, the highest rate was with the denture group (44.2%), with an associated odds ratio equal to 1.8, *CI*=1.1–3.0, X^2=6.1, and p=0.01. However, a lower rate of *candida* colonization was observed in orthodentic appliance patients, equal to 36.5%, but the results were not statistically significant (p=0.65) (Table 4).

Table 4: Frequency and association of oral Candida species colonizing buccal mucosa with denture cases, orthodontic cases and normal controls, in addation to sex, and age groups.

Characters	N (%)	Positive *Candida* species n=108 N (%)	*OR (95% CI)*	X^2	*p*
Sex					
Male	130 (41.9)	63 (48.5)	2.8 (1.7-4.6)	18	<0.001
Female	180 (58.1)	45 (25)	0.3 (0.2-0.5)	18	<0.001
Ages (years)					
<21 years	50 (16.1)	13 (26)	0.68 (0.3-1.3)	1.2	0.27
21-30	80 (25.8)	24 (30)	0.74 (0.4-1.2)	1.1	0.29
31-40	69 (22.3)	18 (26.1)	0.3 (0.21-0.69)	10.6	0.001
41-50	40 (12.9)	21 (52.5)	2.3 (1.2-4.5)	6.3	0.01
≥51	74 (23.9)	32 (43.2)	1.6 (0.9-2.7)	3.0	0.08
Mean age	37.01Years	39.5 years			
SD	20.9 Years	20.9 years			
Type of patients					
Denture	104 (33.5)	46 (44.2)	1.8 (1.1-3)	6.1	0.01
Orthodentic appliance	104 (33.5)	38 (36.5)	1.2 (0.67-1.8)	0.19	0.65
Normal	102 (32.9)	24 (23.5)	0.45 (0.2 -0.77)	8.5	0.003
Total	310 (100)	108 (34.8)			

DISCUSSION

Although several species of *Candida* may be present in the oral cavity, *C. albicans* is unquestionably the most common[16,19,34]. About 80% of all recovered *Candida* spp. are thought to be *C. albicans*, and these proportions are thought to be constant in patients with and without clinical symptoms[19,35]. Since different species can cause infections in different ways and can definitely affect how a patient responds to medication, it is important to identify the exact species[16]. In addition to *C. albicans*, other often isolated species are *C. tropicalis*, *C. dubliensis*, and *C. glabrata*. However, it is not uncommon for two or more distinct species to be recovered at the same time in certain[34,36,37]. Current analysis revealed that *C. albicans* was the most frequently isolated species across all three groups of participants in this study. This finding is consistent

with the findings of other studies that are currently accessible, which also indicated that *C. albicans* predominated[19,36,37]. Additionally, metal framework denture wearers and FOA are the primary populations from which *C. albicans* is isolated; in comparison to the control group, there was a notable rise in *C. albicans* colonization in these groups. The only other statistically significant difference, besides *C. albicans*, was discovered for colonization with *C. glabrata*. This result is comparable to that of Nayak *et al.*[40], who isolated much greater quantities of *C. glabrata* together with *C. albicans*. In recent years, there has been an upsurge in the prevalence of non-*albicans Candida* species, particularly *C. glabrata* and *C. krusei*. Since these two species are recognized for responding inadequately to normal treatment regimens, it is imperative to determine the precise species[41,42]. Furthermore, the present results differed with those of Neha *et al*., which showed that *C. tropicalis* was the most frequent NCAC, followed by *C. glabrata*, and that *C. parapsilosis* was infrequently isolated from healthy oral colonization or from patient diseases[41]. *C. kefyr*, *C. krusei*, *C. famata*, *C. africana*, and *C. stellatoidea* were first isolated in Yemeni dentistry patients' oral cavities in the current investigation. These species have not been isolated in the oral cavity[36,37], or even in other locations such the vaginal mucus membren[43,44].

The male group in the current study had a greater colonization rate (48.5%) than the female group did, and the variance was statistically significant ($p<0.001$). For male patients, the related odds ratio was equivalent to 2.8, CI=1.7–4.6, $X^2=18$, and $p<0.001$. The current result was consistent with earlier reports from Yemen, where it was noted that male patients had greater rates of OCC than female respondents[36,37]. The present findings, however, differed with those published by Thein[45], who noted a comparable colonization rate in both adult sexes.

About 52% of the 41–50 year old age group in the current study had a high rate of *Candida* colonization, with an associated odds ratio of 2.3, CI=1.2–4.5, $X^2=6.3$, and $p=0.01$. The pattern of increased OCC in cases and controls with advancing age. The results of the current study were consistent with those of studies by Chopde *et al.*[46], and Alhasani *et al*. [37], which found that OCC is more common in older adults. The elderly's propensity for systemic ailments, dietary alterations, and altered salivary features can all be used to explain this conclusion[39,45,47]. Advancing age was also a risk factor for denture stomatitis in the elderly, because cell-mediated immunity, which provides protection against *Candidal* infection, declines with age. Certain oral environmental characteristics, such the age of the individuals and their unstimulated salivary flow rate, were linked to greater levels of microorganisms in the saliva of people who wear complete dentures, according to Chopde *et al.*[46]. These authors proposed that an increase in salivary microbe concentration was caused by an aging-related decrease in salivary flow rate[36,48,49].

In comparing the rates of oral *Candida* species colonizing buccal mucosa between the denture group and the normal control, the denture group had the greatest rate (44.2%), with an odds ratio of 1.8, CI=1.1–3.0, $X^2=6.1$, and $p=0.01$. The microenvironment of the oral cavity is altered just by the presence of prosthetic equipment, as this encourages *Candida* colonization and growth. Additionally, the appliance itself sticks to the mucosal surface, inhibiting natural saliva flow and physiological cleansing[11,50,51]. It was found in this study that, in contrast to 23.5% of people who did not use any appliances, 44.2% of subjects with prosthetic appliances tested positive for *Candida* spp. In comparison to data on *Candida* colonization in denture wearers, which indicated a percentage of positive findings in 80–100% of patients compared to 45–65% in subjects without dentures, our result is lower[38]. Numerous studies have established that a large majority of individuals with prosthetic appliances have *candida*[8,35,40]. Only 52% of individuals without dentures had the same results as Prakash *et al.*[35], who demonstrated that *Candida* was present in nearly all people wearing prosthetic prostheses. A removable prosthetic appliance in the mouth speeds up colonization and biofilm production, but *Candida's* capacity to stick to surfaces and create complex biofilms serves as the first step in the onset and course of the disease. The physical characteristics of the material used in its construction also affect the denture's initial adherence to its surface. Adherence is influenced by a number of factors, including porosity, free surface energy, hydrophobicity, and surface roughness. The process of polymerization, surface changes, and denture manufacture techniques also have a major impact[39]. It has been shown that the previously listed material properties could influence how biofilm forms in *in vitro* settings.

On the other hand, patients wearing orthodontic appliances had a higher rate of *candida* colonization—36.5% compared to 23.5% for the control group, with a 13% increase in FOA patients (Table 4). The current study, which examined the OCC rate after fixed orthodontic treatment, finds that using these appliances increases the carriage and significantly alters the population of oral microorganisms. This is likely because the appliance causes ecological changes in the oral cavity. The OCC primary absence of the baseline patient cluster was not unexpected, as applicants were requested to ascertain good oral hygiene prior to the trial. Conversely, the test group's OCC rate increased by 13.8% following the implementation of FOA. This discovery is most likely due to the presence of orthodontic attachments on the labial and lingual surfaces of these teeth, which make it difficult to thoroughly brush the gingival tissue. Several publications have documented similar changes in the OCC rate with fixed and removable appliances during orthodontic therapy[5,20,36]. Additionally, the bonding material in FOA has a rough surface, which acts as a *Candida* trap and irritates the gingiva[5,12,21,52].

Limitation of the study

Overall, our study offers intriguing new information on the first species of *candida* to be isolated from the oral mucous of healthy adults in Yemen; concerning *C. albicans*, however, it remains the most frequently

colonized species in oral mucus. However, we recognize that there are certain limitations with respect to the number of participants in the research and that additional data concerning the genotyping of the *candida* isolates is required.

CONCLUSION

Candida colonization remains a major problem in the oral cavity. Although the prevalence of non-*albicans Candida* (NAC) species is increasing, *C. albicans* was still the dominant species. A higher rates and significant risk factors of OCC were observed in the male group, denture patients and the elderly.

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CONFLICT OF INTEREST

Regarding this project, there is no conflict of interest.

AUTHOR'S CONTRIBUTIONS

Madar EM: conducted the fieldwork for this study as part of her doctoral dissertation, writing the original draft, methodology, and investigation**. Al-Khamesy KSA:** formal analysis, data curation, conceptualization. **Al-Shamahy HA:** writing, review and editing, methodology. **Abbas AMA:** formal analysis, data curation, conceptualization. All authors read and approved the final manuscript for publication.

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