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Isolation and identification of *Candida* spp. from dental samples in patients with orthodontic appliances and without

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Abstract
Background: Long-term orthodontic device use fosters an environment that is conducive to the growth of normal oral microbiota, especially Candida species, which can raise the risk of periodontal diseases. The study's objective was to determine how common Candida spp. were in individuals who had and did not have orthodontic equipment.
Materials and methods: Two hundred and fifty samples were collected from individuals who visited the Specialized Dental Center in Kirkuk Governorate, as well as some outpatient clinics. Each sample consisted of two swabs: one from the oral cavity and the other from areas of caries and root canal inflammation. Participants were divided into two groups: those wearing braces and those without. It was confirmed that all participants had not taken any antibiotics for at least two days prior to the swabs being taken. Participants ranged in age from 3 to 70 years and included both men and women. Samples were collected between October 1, 2024, and January 7, 2025, and were then immediately sent to the laboratory for microbial culture.
Result: The results of the current study indicate that out of 202 dental swabs taken from teeth with braces, 173 (79.7%) were positive for fungal isolation. For teeth without braces, 44 (20.3%) were positive. According to age group the results showed that the age group (11–18 year) with braces recorded the highest percentage of positive samples, with 52 samples (26.1%), followed by the 7–10 year age group with braces at 21.6%, while the lowest percentage of positive samples was in the males aged 18 years and older with braces at 10.6%. According to diet, the "other treatments" category recorded the highest percentage of positive samples, at 47 (90.4%), followed by smokers at 87.1%, and then pregnant women at 85.7%. The results showed that *Candida albicans* was the predominant yeast species isolated from both oral and dental samples, accounting for 49.2% of oral isolates and 52.3% of dental isolates, followed by *C. krusei* and *C. glabrata*. Lower isolation rates were recorded for *C. tropicalis* and *C. parapsilosis*.
Conclusion: Oral Candida colonization is more common when removable orthodontic equipment are worn for extended periods of time.

Keywords: Orthodontic Appliances, dental caries, Candida Spp.

Introduction

The oral cavity hosts various microorganisms, including bacteria, viruses, and fungi [1]. Some fungi are part of natural microflora and typically do not harm the host, suggesting a mutualistic relationship. However, some biologists view isolated fungal colonies as a form of parasitism, indicating potential harm [2]. Numerous local oral variables, including mouth dryness, high-sugar diets, constant and detachable orthodontic equipment, and replaceable full dentures, and poor oral hygiene can increase the oral *Candida* carriage changed to pathogenic form and caused *Candida* -associated buccal lesions [3].

The presence of orthodontics fixed device can increase plaque accumulation, affecting the balance of bacteria and fungi in the mouth [4]. One of the biggest challenges in orthodontics is to maintain proper oral hygiene during treatment, bands and other accessories further preserve the dental plaque, that can worsen these illnesses by generating gingivitis and enamel demineralization, which can result in white spots, caries, and Candida stomatitis. Microbiological research has demonstrated that the number of bacteria, particularly Streptococci, Lactobacilli, and Candida, rises significantly following the installation of a permanent orthodontic device, subjecting the oral cavity to an imbalance and facilitating the development of illnesses [5-8]. About 50–60% of people are infected with the opportunistic pathogen Candida. Candida infections, also known as candidiasis or candidosis, continue to be a significant clinical issue, particularly in patients with impaired immune systems [9].

Candida albicans is virulent due to a variety of mechanisms, including acid phosphatases, phospholipases, and proteases. Nonetheless, the majority of researchers concur that the hyphal form of Candida albicans attaches to tissue and tissues more readily than the yeast phase; adherence to host cell surfaces is thought to be the initial and crucial stage of colonization and infection [10]. Determining the oral microbial change in patients receiving orthodontic treatment is crucial since the doctors are dedicated to maintaining the patients' oral health in some circumstances that include lengthy treatment durations.

Materials and methods

Specimens Collecting

Two hundred and fifty samples were collected from individuals who visited the Specialized Dental Center in Kirkuk Governorate, as well as some outpatient clinics. Each sample consisted of two swabs: one from the oral cavity and the other from areas of caries and root canal inflammation. Participants were divided into two groups: those wearing braces and those without. It was confirmed that all participants had not taken any antibiotics for at least

two days prior to the swabs being taken. Participants ranged in age from 3 to 70 years and included both men and women. Samples were collected between October 1, 2024, and January 7, 2025, and were then immediately sent to the laboratory for microbial culture.

Isolation and identification of *candida spp*.

A 15-cm-long stick topped with a sterile swab was used to obtain oral swabs (from the mouth and teeth). After a 12-hour fast, all swabs were collected early in the morning. Following collection, the swab stick was promptly put in an AMIES transport medium tube, labeled with each patient's code number, and kept in the container's freezer. The tube was sent to the scientific college's Department of Microbiology right after the swab was collected. Fungi were isolated and cultured using Sabouraud's solid agar medium with gentamicin and chloramphenicol in the shape of circular amber-colored Petri dishes. an inoculation of a main isolation medium, such as Sabouraud's dextrose agar (SDA), using a sterile cotton swab [17]. Chromogenic Candida agar was evaluated whether Candida colonies were visible under a microscope on Sabouraud's dextrose agar. Using the manufacturer's color reference guide, the color of the colonies was used to identify the species of Candida. VITEK 2 was used to further identify each of the Candida isolates [18].

Result

The current study showed an equal isolation rate for oral samples (250 samples, 50%) and dental samples (250 samples, 50%). Dental samples included 202 samples (80.8%) with braces and 48 samples (19.2%) without braces.

The results of the current study, shown in Table (1), indicate that out of 202 dental swabs taken from teeth with braces, 173 (79.7%) were positive and 29 (87.9%) were negative for bacterial and fungal isolation. For teeth without braces, 44 (20.3%) were positive and 4 (12.1%) were negative.

Table 1: Distribution of positive samples according to sources of isolation from teeth

Source	Total number of swabs	Number of positive swabs) % (	Number of negative swabs) % (
teeth with braces	202	)79.7% (173	)87.9% (29
teeth without braces	48	)20.3% (44	)12.1% (4
Total	250	)86.8% (217	)13.2% (33

Table (2) shows the distribution of the studied samples by age group and the presence of braces, indicating the number and percentage of positive and negative samples out of a total of 250 samples. The results showed that 199 samples (79.6%) were positive, while 51 samples (20.4%) were negative.

The age group (11–18 year) with braces recorded the highest percentage of positive samples, with 52 samples (26.1%), followed by the 7–10 year age group with braces at 21.6%, and then the females aged 18 years with braces at 19.6%. while the lowest percentage of positive samples was in the males aged 18 years and older with braces at 10.6%.

Table 2: Distribution of positive samples by age group

Age groups	Total number of samples	Number of positive samples) % (	Number of negative samples) % (
3-6 years without braces	28	26)13.1 % (	2)3.9% (
7-10 yeas with braces	50	43)21.6 % (	7)13.7% (
11-18 years with braces	60	52)26.1 % (	8)15.7% (
18> Males with braces	37	21)10.6% (	16)31.4% (
> 18 years Females with braces	55	39)19.6 % (	16)31.4% (
Older without braces	20	18)9 % (	2)3.9% (
Total	250	199)79.6 % (	51)20.4% (

Table (3) shows the distribution of the studied samples by health status and clinical factors. The total number of samples was 234. The results showed that 181 samples (77.4%) were positive, while 53 samples (22.6%) were negative. The "other treatments" category recorded the highest percentage of positive samples, at 47 (90.4%), followed by smokers at 87.1%, and then pregnant women at 85.7%. Patients with chronic diseases also showed a high percentage of positive samples at 80.7%. In contrast, a clear decrease in the percentage of positive samples was observed among antibiotic users, at only 35.3%, while this category also recorded the highest percentage of negative samples (64.7%). Surgical procedures showed a moderate percentage of positive samples at 64.3%.

Table 3: Distribution of positive samples according to immunocompromised patients and other factors

Groups	Total number of samples	Number of negative samples) % (	Number of positive samples) % (
Pregnant	7	1)14.3% (	6)85.7% (
Chronic disease	57	11)19.3% (	46)80.7% (
Smoker	70	9)12.9% (	61)87.1% (
Antibiotics	34	22)64.7% (	12)35.3% (
Surgical operation	14	5)35.7% (	9)64.3% (
Other medication	52	5)9.6% (	47)90.4% (
Total	234	53)22.6% (	181)77.4% (

The distribution of the sample according to diet, with the sample divided into three main categories. The results showed that the largest percentage of participants (62.8%) followed a diet based on sweets with other added foods, while the percentage of individuals who consumed only sweets was 33.2%. The category that did not consume sweets had the lowest percentage, representing only 4% of the total sample.

The relative distribution of Candida spp. isolates from the mouth and teeth. The results showed that Candida albicans was the most common species in both sources, accounting for 49.2% of oral isolates and 52.3% of dental isolates. C. krusei was the second most common, at 22.6% of oral isolates and 23.6% of dental isolates, followed by C. glabrata at 14.5% and 16.6% in the mouth and teeth, respectively. In contrast, lower percentages were recorded for C. tropicalis (9.5% in the mouth versus 5% in the teeth) and C. parapsilosis, which showed the lowest isolation rates (5.5% in the mouth and 2.5% in the teeth). These results reflect a clear dominance of C. albicans in both the mouth and teeth, consistent with its superior ability to colonize the mouth, thanks to multiple virulence factors such as morphomorphism, biofilm formation, and strong adhesion to dental surfaces and oral tissues. The similarity in the proportions of C. krusei and C. glabrata between the mouth and teeth, with a slightly higher number of dental isolates, suggests that the hard surfaces of teeth may provide a more favorable environment for these species, particularly within biofilms, compared to soft tissues. The relatively low isolation rates of C. tropicalis and C. parapsilosis may be attributed to their limited ability to compete with C. albicans in the oral environment or their need for specific environmental conditions to spread.

When samples were cultured on SDA medium, Candida colonies were observed to be circular or oval-convex, with a creamy white color, shiny, and smooth to the touch, as shown in Fig (4).

Microscopic examination of the colonies growing on SDA medium revealed spherical or oval cells, with some budding cells also observed, as shown in Figures (5). Candida spp were found to be positive for the cotton blue-lactophenol dye, with spherical to oval or elongated, budding cells of varying sizes observed in some Candida isolates Fig (6).

Candida species were identified using Chrom agar medium, which is based on the color of the colony. C. albicans colonies appear green, C. tropicalis colonies blue, and C. krusei colonies dull pink, while C. dublineiensis appeared whit to creamy, as shown in Fig (7).

Fig 4: Candida spp. on SDA

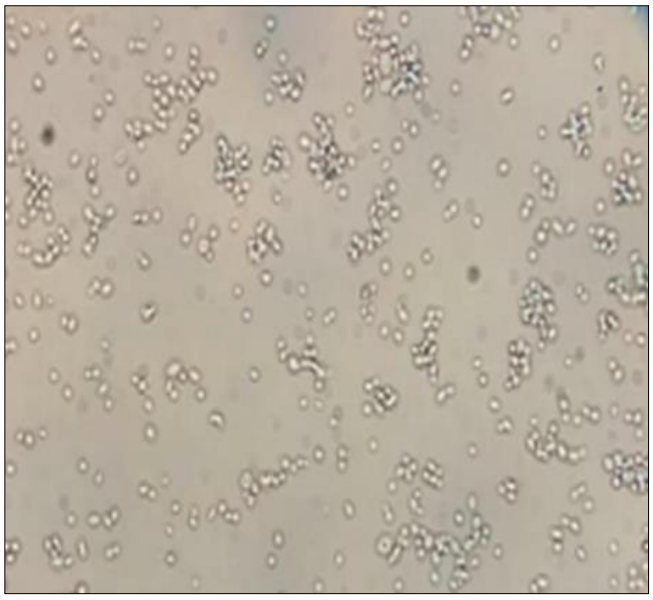

Fig 5: Candida spp under a microscope

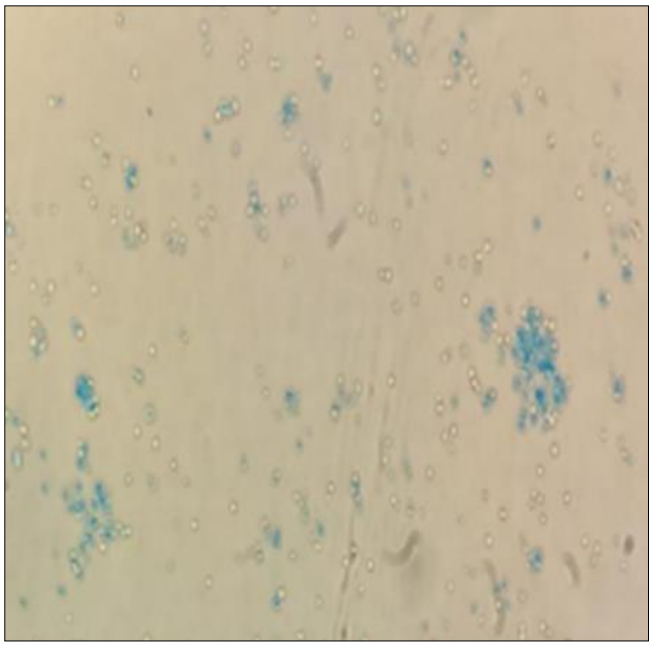

Fig 6: Candida spp stained with lactophenol blue

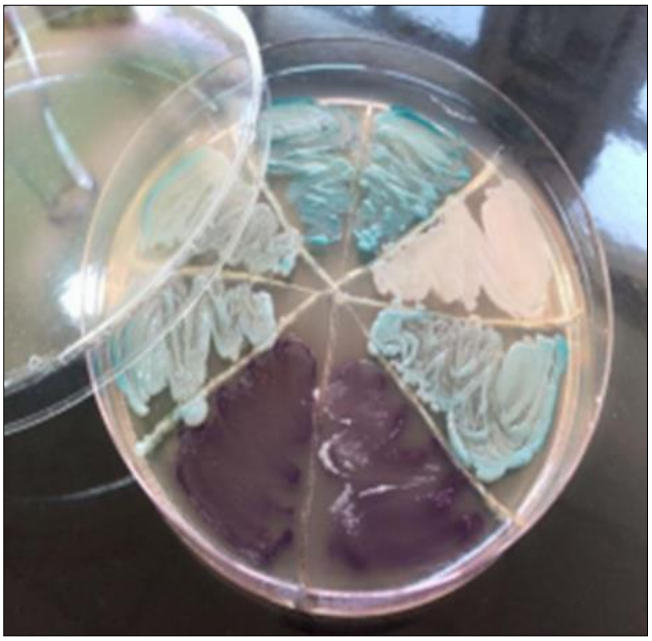

Fig 7: Diagnosis of Candida species using Chromium Agar at 37°C and incubation for 48 hours. C. albicans, C. tropicalis, C. krusi, C. parapisilosis and C, galabrats

Discussion

These results clearly indicate that the presence of braces is associated with a significantly higher percentage of positive swabs compared to teeth without braces. This is attributed to the fact that orthodontic appliances, whether fixed or removable, provide additional surfaces and retention areas that facilitate the accumulation of plaque and food debris, creating a suitable environment for the growth of microorganisms. Furthermore, the difficulty of cleaning around the wires and brackets reduces the effectiveness of daily oral hygiene practices, even for diligent individuals, which explains the higher percentage of positive results in this group. In contrast, teeth without braces showed lower proportions of positive smears, reflecting easier oral cleaning and fewer areas of microbial retention. The present study agree with [13] that showed increase. Certain changes in the oral environment were brought about by orthodontic therapy, particularly with fixed appliances. These changes included a decrease in pH, an increase in the buildup of dental plaque, and an increase in the amount of bacteria in saliva [14, 15]. The current study contradicts [16], which found no statistically significant rise in C. albicans colonization during orthodontic treatment. This suggests that the presence, absence, or degree of C. albicans colonization was unaffected by the permanent equipment.

The results indicate a clear variation in the distribution of positive samples according to age and the presence of braces. It was observed that the younger and middle-aged age groups using braces recorded higher percentages of positive samples. This may be attributed to the fact that braces provide a suitable environment for plaque accumulation and make oral hygiene more difficult, especially for children and adolescents who may not fully adhere to oral hygiene practices. This result agree with [17], findings show young individuals in southeast Turkey have a high frequency of oral Candida spp. and that the use of braces increases Candida counts

The high percentage among patients with chronic diseases may be due to the association of these conditions with weakened general immunity or the use of long-term medications that affect the oral environment. CARVALHO *et al* [18] showed a strong correlation between the greater prevalence rates of Candida carriage and density in diabetes individuals and poor glycemic

control. Furthermore, a significant incidence of Candida dubliniensis was discovered in diabetes patients; this species may be mistakenly identified as Candida albicans due to its similar morphology.

These results indicate that the vast majority of the sample relied on foods rich in sugars in their diet, along with other food components, which may have a direct impact on oral health and microbial balance. Frequent consumption of sugars is a major factor in promoting the growth of pathogenic microorganisms by providing a suitable nutrient medium for them. The present study agree with [19] that showed a positive association between sugars and caries.

According to the current study, Candida albicans was the more prevalent species, followed by Candida krusei, Candida glabrata, and Candida parapsilosis, which had the smallest isolation frequencies. The current study concurs with [20], which shown that, following the introduction of orthodontics, Candida albicans colonized the oral cavity at a rate of 72.5%, followed by Candida glabrata and Candida parapsilosis. The current study contradicts [21], which found no evidence of an increase in C. albicans colonization following orthodontic therapy.

Conclusion

The results of this study demonstrate that orthodontic appliances significantly increase fungal colonization in the oral cavity compared to teeth without braces. Higher isolation rates were observed in younger age groups and in individuals with certain risk factors such as smoking, pregnancy, and medical treatments. *Candida albicans* was the predominant species isolated from both oral and dental samples. These findings highlight the importance of proper oral hygiene and regular follow-up during orthodontic treatment to reduce the risk of fungal overgrowth.

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