Eurasian J. Sci. Eng., 2025, 11(2), 278-286
https://doi.org/10.23918/eajse.v11i2p19

Published by Tishk International University
Available at: https://eajse.tiu.edu.iq/

RESEARCH ARTICLE Open Access

Exploring Oral Colonization Of *Candida* Species In Diabetic Individuals In Erbil City, Iraq: A Case-Control Study

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Article History
Received: 14.04.2025
Revised: 20.06.2025
Accepted: 27.08.2025
Published: 15.09.2025
Communicated by: Dr. Orhan Tug
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Abstract:
Oral candidiasis is a prevalent opportunistic infection of the oral cavity, primarily caused by the overgrowth of *Candida* species, with *Candida albicans* being the most common. This case-control study aimed to compare oral clonization by different *Candida* species and their colony-forming units (CFU/ml) in the oral cavity of diabetic versus non-diabetic individuals. Oral rinse samples were collected from 50 individuals (25 diabetic and 25 non-diabetic) attending Layla Qasim Health Center, Erbil city, from February to March 2024. Samples were cultured on Sabouraud's dextrose agar and HiCrome^{TM} Candida differential agar for species identification. Of the 50 samples, 44 (88%) yielded positive culture for *Candida* sp. The total *Candida* carriage rate was similar in both the diabetic and control groups (88%). Multiple *Candida* species were identified, with *Candida albicans* and *Candida krusei* being the most prevalent. Diabetic patients had significantly higher colony-forming unit (CFU/ml) compared to the control group, indicating a greater fungal load despite similar carriage rates between groups (p *value*<0.01).

Keywords: *Candida albicans;* HiCromeTM Candida differential agar; *Colony forming unit; Diabetes; Oral candidiasis.*

1. Introduction

In previous decades, the rate of opportunistic fungal infections has increased worldwide. One of the common infections in this regard is oral candidiasis [1]. *Candida* species are a group of dimorphic fungi that are considered one of the most predominant causes of invasive infections. Currently, there are 200 species within the genus *Candida,* but only a few have medical importance, namely, *Candida albicans* (*C. albicans*), *Candida tropicalis* (*C. tropicalis*), *Candida pseudotropicalis* (C. *pseudotropicalis*), and *Candida glabrata* (*C. glabrata*). Oral candidiasis is a common opportunistic infection of the oral cavity caused by an overgrowth of *Candida* species, the most common being is *C. albicans*. Identification of *Candida* species is vital in diabetic patients who are more prone to oral fungal infections [2, 3]. Recognizing particular *Candida* species facilitates targeted treatment approaches. Additionally, identifying specific species contributes to comprehending the link between Candidal infection and diabetic complications, aiding in the formulation of effective preventive measures [4]. *C. albicans* is a normal microbiota of the skin and mucous membranes, causing a wide range of superficial infections to fatal systemic infections, especially among immunocompromised subjects such as patients with HIV/AIDS, diabetes, hospitalized subjects receiving long-term antibiotics, patients under organ transplant, chemotherapy, or radiotherapy [5-7]. Significantly, diabetes is one of the largest emerging threats to public health in the 21st century [8, 9]. The World Health Organization and the International Diabetes Federation predicted that the number of adults with diabetes will reach 629 million by the year 2045 worldwide [10]. It is the most common endocrine metabolic disorder [11, 12]. Approximately 85-90% of diabetic patients are diagnosed with type 2

diabetes (resulting from insulin resistance). In these patients, salivary dysfunctions such as xerostomia, decreased salivary function, lichen planus, tooth decay, and periodontal diseases are common [13, 14]. In addition to *C. albicans*, species like *C. glabrata*, *C. krusei*, *C. tropicalis*, and *C. parasilosis* are also frequently found in the oral cavity [15]. These non-albicans species are often more resistant to standard antifungal treatments and have become increasingly prevalent in immunocompromised patients[16].

Oral candidiasis commonly present with symptoms such as white curdy-like patches on the tongue, inner cheeks, or roof of the mouth that can be wiped off, leaving a red and sometimes bleeding surface. Other symptoms include a burning sensation. Soreness, altered taste, and in severe cases, difficulty in swallowing. Some patients may also experience dryness or cracking at the corners of the mouth [17].

Among the reasons making diabetic patients more susceptible to oral candidiasis are high levels of salivary glucose, low secretion of saliva, impaired chemotaxis, and a defect of phagocytosis due to polymorphonuclear leukocyte deficiency[18, 19]. In their study, Mohammadi et al. (2016) found that *C. albicans* was the most prevalent species (36.2%) in diabetic patients, significantly exceeding the prevalence rate in healthy individuals [2]. Moreover, in an experimental study, a significant decrease in the CFU during oral hypoglycemic drug therapy was reported, indicating a link between high glycemic index on *Candida* colonization rate [20].

The purpose of this study is to determine possible differences in total *Candida* count as well as the nature of *Candida* species in the oral cavity of patients with diabetes and nondiabetic individuals.

2. Methodology

2.1 Study design

A case-control study was performed in Erbil city from the 1st of February 2024 to the 7th of March 2024. Fifty samples were collected from diabetic and non-diabetic patients who visited Layla Qasim Health Center. A questionnaire form was prepared considering demographic and clinical criteria. Subjects in the case and control groups were subjected to age and gender matching. Written consent was obtained from each subject who had the will to participate in the study.

2.2 Subject

This study included 25 confirmed diabetic patients and 25 healthy individuals. Exclusion criteria were smokers, pregnant women, people with autoimmune disorders, individuals on immunosuppressive drugs, and those who took antimicrobial drugs in the previous month.

2.3 Sample collection

The concentrated oral rinse method was used for sample collection. Briefly, 8 milliliters of normal saline was transferred to a sterile capped bottle and given to each participant to rinse their mouth for 1 minute, then spill it again into the same bottle, and closed properly and transported to the microbiology lab for processing [8].

2.4 Laboratory processing

Following the collection, all the samples were subjected to centrifugation at 6000g for 5 minutes. Later, the supernatant was discarded, and the pellets were suspended in 1ml normal saline. Samples were then cultured on both Sabouraud's dextrose agar (SDA) (Oxoid S.A., Spain) and HiCrome^{TM} Candida differential agar (HiMedia Laboratories, USA) by pipetting 80 microliters from the previous suspension on the agar surface and rotating to distribute the fluid. Then plates were incubated overnight at 37°C. Following incubation, growth was examined for the characteristic colony morphology of *Candida sp.* On SDA colonies appear as creamy white, convex, 5-10 mm in diameter. On HiCrome^{TM} Candida differential agar, colonies show different colors as described by the manufacturer (table 1). Microscopical examination of each colony using Gram stain was then followed. Additionally, the germ

tube test was performed for presumptive identification of *C. albicans* and related species by incubating colonies in human serum at 37 °C for 2-3 hours and examining them under the microscope.

Table 1: Characteristics of *Candida* sp. colonies on HiCromeTM Candida differential agar (

Species	Colony morphology
Candida albicans	Light green colonies
Candida tropicalis	Blue to purple colonies
Candida krusei	Purple, fuzzy colonies
Candida glabrata	Cream to white colonies
Candida parapsilosis	White to pale pink colonies

2.5 Statistical Analysis

Data were analyzed using SPSS software. An independent t-test was used to compare the mean CFU/ml between diabetic and non-diabetic groups. A p-value < 0.05 was considered statistically significant.

3. Results

Fifty samples were collected, 25 from diabetic patients and 25 from non-diabetic healthy controls who visited Layla Qasim Health Center in Erbil city. The results showed that 48% of both groups were male and 52% were female. Their age ranged between 18-60 years. (Table 2).

Table 2: Demographic criteria of the study groups.

Characteristic	Diabetic group	Control group
Number	25	25
Age Range Mean±SD	 18-60 48±10.69	 20-60 48±10.70
Gender Male No.(%) Female No.(%)	 12 (48%) 13 (52%)	 12 (48%) 13 (52%)

Table 3 shows the rate of different *Candida* sp. carriage in the studied groups. Carriage rates of *C. albicans, C. glabrata, C. krusei, and C. tropicalis* in the diabetic group were 56%, 20%, 40% and 20%, respectively. All *Candida* species showed higher rates in the diabetic group compared to the control group.

Table 3: Oral carriage rate of different *Candida* sp. in the studied groups according to their growth on HiCrome^{TM} Candida differential agar.

Candida sp.	Diabetic group		Control group	
	No.	***%***	***No.***	***%***
C. albicans	*14*	*56*	*11*	*44*
C. glabrata	*5*	*20*	*3*	*12*
C. krusei	*10*	*40*	*7*	*28*
C. tropicalis	*5*	*20*	*3*	*12*

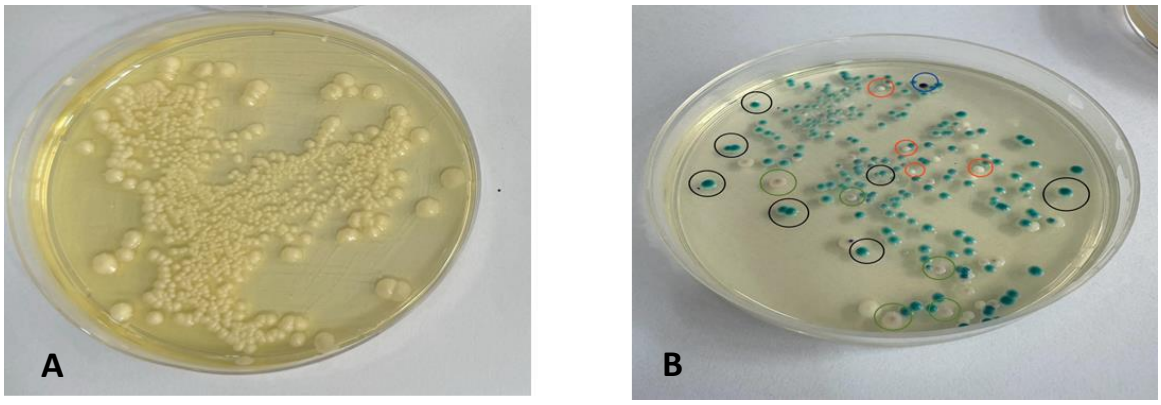

Figure 1: Characteristic *Candida* sp. colonies on (A) SDA and (B) HiCrome™ Candida differential agar.

Figure 1 shows the characteristic *Candida* sp. colonies on SDA as white to cream-colored, smooth, glabrous colonies and have different sizes that cannot be differentiated into specific species (A). In addition, growth of *Candida* sp. on HiCrome™ Candida differential agar (B) shows different colony morphologies, each of which is characteristic of a specific species.

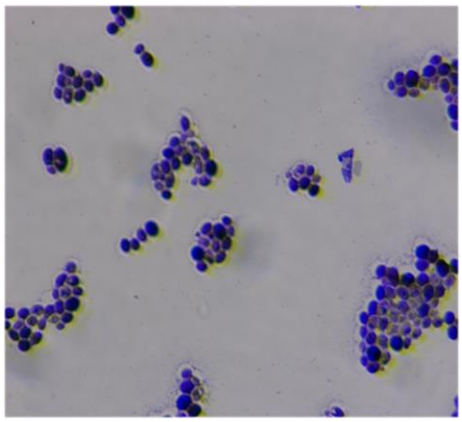

Figure 2: Gram-stained smear of *Candida* sp. from colonies on SDA showing typical morphology.

Figure 2 shows a Gram-stained smear prepared from the growth of *Candida* sp. colonies on SDA appear as gram-positive, round or oval-shaped cells with smaller daughter cells budding off from the larger parent cells. Cells are seen in clusters.

Table 4 presents the multi-pattern occurrence of *Candida* sp. in the oral cavity of the studied subjects. *C. albicans* + *C. krusei* constituted the common pattern in both groups, with a doubled number in the diabetic group. The *C. krusei* + *C. tropicalis* pattern was double in the diabetic group. The only triple occurrence was composed of *C. albicans* + *C. krusei* + *C. glabrata,* which was twice in the diabetic group compared to the control group. Other patterns that showed no difference between study groups were *C. albicans* + *C. glabrata, C. albicans* + *C. tropicalis, and C. glabrata* + *C. tropicalis*.

Table 4: Frequency of subjects carrying more than one *Candida* sp.

Candida sp.	Diabetic group	Control group	Total No.
C. albicans + *C. krusei*	4	2	6
C. albicans + *C. glabrata*	1	1	2
C. albicans + *C. tropicalis*	1	1	2

C. albicans + *C. krusei* + *C. glabrata*	2	1	3
C. krusei + *C. tropicalis*	2	1	3
C. glabrata + *C. tropicalis*	1	1	2
Total	11	7	18

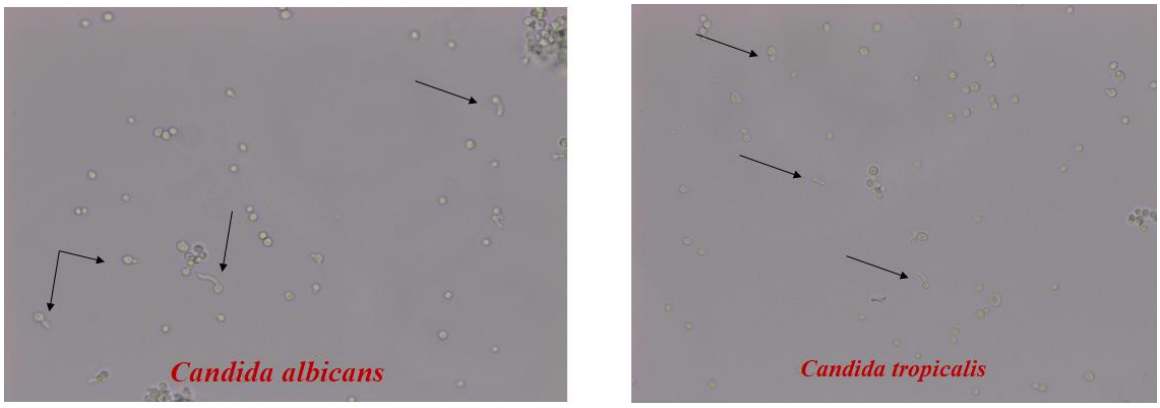

Figure 3: Both *Candida albicans* and *Candida tropicalis* were germ tube test positive as shown by arrows.

The results of the germ tube test are shown in Figure 3, in which both *Candida albicans* and *Candida tropicalis* produced germ tubes, a finding that necessitates confirmation by either some biochemical tests or growth on HiCrome™ Candida differential agar.

Table 5 shows the CFU/ml of *Candida* sp. in the studied groups. *C. albicans* had the highest CFU/ml, followed by *C. krusei, C. glabrata, and C. tropicalis*. There was a significant difference between the mean CFU/ml of *C. albicans* and the other *Candida* species between the diabetic and the control groups (t-test $p < 0.01$). Yet, Table 6 presents a comparison between the studied groups in terms of both the total carriage rate and the total CFU/ml. The total carriage rate was the same in both groups (88%). However, the total CFU/ml was significantly higher in the diabetic group compared to the control group ($p < 0.05$).

Table 5: CFU/ml of *Candida* sp. in the studied groups.

Candida sp.	Diabetic group		Control group		t-test p value
	CFU/ml		CFU/ml		
	Range	*Mean±SD*	*Range*	*Mean±SD*	
C. albicans	*0 - 2438*	*1273.22±1640.87*	*0 - 1950*	*210.25±404.73*	$p < 0.01$
C. glabrata	*0 - 250*	33.80±*69.81*	*0 - 250*	*2.87±53.00*	$p < 0.01$
C. krusei	*0 - 2250*	314.81±*572.50*	*0 - 575*	*71.33±140.07*	$p < 0.01$
C. tropicalis	*0 - 475*	*63.80±124.85*	*0 - 75*	*6.52±15.21*	$p < 0.01$

Table 6: Comparison between the studied groups in total carriage rate and total CFU/ml.

Characteristic	Diabetic group	Control group
Total carriage rate no. (%)	22/25 (88%)	22/25 (88%)
CFU/ml Total Mean±SD	 27526.83 1273.22±1640.86*	 19553 814.48±801.60

* Significant difference between the two means ($p < 0.05$)

4. Discussion

This study aimed to assess the prevalence of *Candida* species carriage among diabetic patients (case group) and healthy individuals (control group). It also sought to identify various *Candida* species in both groups and compare CFU/ml between them. Participants were carefully chosen and matched based on their demographic and clinical characteristics, ensuring equal distribution of diabetic and non-diabetic subjects (Table 1).

Several factors related to the altered immune response, glucose levels, and oral environment in diabetes patients can be linked to their higher susceptibility to oral candidiasis. Diabetes mellitus affects the immune system, which may result in decreased phagocytosis, decreased cytokine production, and impaired neutrophil function, all essential for the immune system's defense against fungal infections [21]. Furthermore, because *Candida* species prefer high-sugar environments, elevated glucose levels in diabetes individuals provide a great environment for their growth and proliferation [22].

Our findings indicated that the overall *Candida* carriage rates were identical for both diabetic patients and the control group, with each showing a carriage rate of 88%. However, the total CFU/ml was significantly elevated in the diabetic group compared to the control group. Specifically, the mean CFU/ml of *Candida* albicans was markedly higher in the diabetic group (1273.22 ± 1640.87) compared to the control group (210.25 ± 404.73) (t-test, $P < 0.01$).

An important observation in this study was the presence of mixed infections in several participants. A total of 18 subjects carried more than one species of *Candida*, with diabetic individuals showing a higher frequency (11 out of 25) compared to the control group (7 out of 25). The most common mixed pattern was *Candida* albicans and *Candida* krusei. This suggests that diabetes not only increases fungal load but may also alter the oral environment to support colonization, which could complicate treatment due to variable antifungal resistance profiles among species. These findings align with studies such as Taei et al. (2019), which reported rising cases of polyfungal oral infections [23].

To compare our results with other studies, the carriage rate in our study in both groups was similar, but in some other studies, the carriage rate of diabetic patients was higher than in the non-diabetic group [24]. On the other hand , our findings are in agreement with studies such as those conducted by Al-Attas and Amro (2010) in Saudi Arabia, which reported similar carriage rates but higher fungal loads in diabetic patients.[8]. This variation could be attributed to the sampling method or the number of subjects included in the study. In the current analysis, a significant difference in CFU/ml was detected between the two groups, with *C.albicans* being the major isolate. *C.glabrata* and *C.krusei* were also present in considerable numbers. Although *C.albicans* remains the most commonly isolated species in the oral cavity, the incidence of oral candidiasis caused by species like *C.glabrata and C.krusei*, which are less responsive to azole treatments, has been increasingly reported over the past two decades [23]. Furthermore, according to our findings, a correction should be made to our knowledge and to the microbiological methodology of manual C.albicans identification, as using the germ-tube test alone is insufficient for presumptive identification of C.albicans, as C. tropicalis can also produce a germ tube. Using HiCrome™ Candida differential agar or other biochemical tests will be helpful [23, 25].

Despite the valuable results gained from this study, there were a few limitations. The low sample size might limit the generalizability of the findings to the broader population. This study helps to understand the relationship between diabetes and *Candida* colonization in the oral cavity. By identifying higher CFU/ml in diabetic patients, the research highlights the increased fungal load that may predispose this population to oral candidiasis. The study also emphasizes the need for screening for fungal infections in diabetic patients and considering preventative measures as part of their routine care.

5. Conclusion

The carriage rate of *Candida* species was comparable between diabetic and non-diabetic groups, but the CFU/ml was significantly higher in the diabetic group. The identified species included *C.albicans, C.tropicalis, C.krusei, and C.glabrata, with C.albicans* and *C.krusei* showing the highest prevalence. Additionally, over one-third of participants had more than one *Candida* species in their oral cavity. *C.albicans* and *C.tropicalis* were germ tube producers. A concentrated oral rinse was shown to be an effective screening method for oral *Candida* species. Screening for *Candida* sp. carriage rate and CFU/ml in the oral cavity in diabetic patients is necessary to prevent Candidiasis. Confirmatory methods such as HiCrome™ Candida differential agar or API 20 Candida, or VITEK 2 system are recommended for definitive diagnosis.

6. Author's contributions

Sazan Moffaq Abdulaziz contributed to the conceptualization of the study, methodology design, and final recheck. Heshu Jalal Ahmed prepared the final draft of the manuscript. Rezhin Sleman Ashqi was responsible for conducting laboratory procedures and managing data. Saba Hawar Mustafa assisted in sample collection and photographic documentation. Samyan Elham Khalid contributed to the literature review and the first draft of the manuscript. All authors have read and approved the final version of the paper.

7. Conflict of Interest

The authors declare no conflict of interest.

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