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Characterization of Candida Species in the Oral Cavity of Diabetic Patients: A Case-Control Study

Sazan Moffaq Abdulaziz ^{1*} (<https://orcid.org/0000-0002-6135-6882>) , Rezhin Sleman Ashqi ² (<https://orcid.org/0009-0000-3528-7361>) , Saba Hawar Mustafa ³ (<https://orcid.org/0009-0000-2885-9923>) , Samyan Elham Khalid ⁴ (<https://orcid.org/0009-0003-1481-358X>) , and Author Name ⁵ 

¹Department of Medical Laboratory Technology, Erbil Technical Health and Medical College, Erbil Polytechnic University, Erbil, Iraq.

²Department of Medical Laboratory Technology, Erbil Technical Health and Medical College, Erbil Polytechnic University, Erbil, Iraq.

³Department of Medical Laboratory Technology, Erbil Technical Health and Medical College, Erbil Polytechnic University, Erbil, Iraq.

⁴Department of Medical Laboratory Technology, Erbil Technical Health and Medical College, Erbil Polytechnic University, Erbil, Iraq.

⁵Department, Faculty, University, City, Country.

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*Email address: some.one@uni.edu

*Corresponding Author



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Abstract: Oral candidiasis is a common opportunistic infection of the oral cavity caused by an overgrowth of Candida species, the most common being Candida albicans. Identification of Candida species is vital in diabetic patients more prone to oral fungal infections. Among the reasons making diabetic patients more susceptible to oral candidiasis are high levels of salivary glucose, low secretion of saliva, impaired chemotaxis, and defect of phagocytosis due to polymorphonuclear leukocyte deficiency. This study aimed to determine possible differences in total Candida count and the nature of Candida species in the oral cavity of patients with diabetes and nondiabetic individuals. Fifty samples from females and males (18-60 years) were collected from diabetic and non-diabetic patients who visited Layla Qasim Health Center.

44 (88%) out of the 50 collected samples showed positive culture. The total carriage rate was the same in both groups (88%). However, more than one Candida species were detected, Candida albicans and Candida krusei were the common ones. Candida albicans had the highest CFU/ml, followed by C. krusei, C. glabrata, and C. tropicalis. Significant differences between the mean CFU/ml of Candida albicans in the diabetic patient group (1273.22 ± 1640.87) compared to the control group (210.25 ± 404.73). The total CFU/ml was significantly higher in the study group than in the control group ($p < 0.01$).

The carriage rate of Candida species was similar between the diabetic and control groups. yet, the diabetic group exhibited a significantly higher colony-forming unit per milliliter than the control group.

Keywords: (Candida albicans; 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 (OC) [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 some of them have medical importance for example Candida albicans, Candida tropicalis, Candida pseudotropicalis, and

Candida glabrata. Oral candidiasis is a common opportunistic infection of the oral cavity caused by an overgrowth of *Candida* species, the most common being is *Candida 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 *Candida* infections and diabetic complications, aiding in the formulation of effective preventive measures [4]. *Candida albicans* (*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 rise near 629 million by the year 2045 in 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]

Among the reasons making diabetic patients more susceptible to oral candidiasis are high levels of salivary glucose, low secretion of saliva, impaired chemotaxis, and defect of phagocytosis due to polymorphonuclear leukocyte deficiency [15, 16]. In their study, Mohammadi F et al. identified that *Candida albicans* is the most prevalent species at 36.2% in diabetic patients, significantly exceeding the prevalence in healthy individuals [2].

In another study, it was investigated that the effects of oral hypoglycemic drug therapy on candidal colonization in Type II diabetic patients the mean CFU decreased from 286.50 at day zero to lower levels after treatment, with a reduction of 20 CFU at day 30 [17].

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

This study is a case-control study held 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 was filled in for each subject considering demographic and clinical criteria. Subjects in the case and control groups were subjected to age and gender matching.

2.2 Subject

Case group: 25 samples were collected from diabetic patients.

Control group: 25 samples were collected from non-diabetic individuals.

Exclusion criteria: smokers, pregnant women, people with autoimmune disorders, individuals on immune suppressive drugs, and those who took antimicrobial drugs in the previous month were excluded from the study.

2.3 Sample collection

The concentrated oral rinse method was used for sample collection, by giving the individuals 8mL of normal saline collected in sterile capped bottles and transferred to the lab for processing.

2.4 Laboratory processing

following 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) and CHROMagar Candida by pipetting 80 microliters from the previous suspension on the agar surface and rotating them to distribute the fluid. Then the 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. On CHROMagar Candida colonies show different colors as described by the manufacturer in the table below:

Table 2.1 Characteristics of *Candida sp.* colonies on CHROMagar Candida

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

3 Results

Fifty samples were collected, twenty-five from diabetic patients and twenty-five from non-diabetic patients who visited Layla Qasim Health Center. The results showed that 48% of both groups were male and 52% were female. Their age ranged between 18-60 years. (Table 3.1).

Table 3.1: Demographic criteria of the study groups.

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

Table 3.1 shows the rate of different *Candida sp.* carriage in the studied groups in which the rate of *C. albicans*, *C. glabrata*, *C. krusei*, and *C. tropicalis* carriage in diabetic patients and control groups were 56%, 20%, 40%, 20% and 44%, 12%, 28%, and 12%, respectively. All the species showed higher carriage rates in the diabetic group.

Table 3.2: Rate of different *Candida* sp. carriage in the studied groups according to their growth on CHROMagar *Candida*.

<i>Candida</i> sp.	Diabetic patients		Control	
	No.	%	No.	%
<i>C. albicans</i>	14	56	11	44
<i>C. glabrata</i>	5	20	3	12
<i>C. krusei</i>	10	40	7	28
<i>C. tropicalis</i>	5	20	3	12

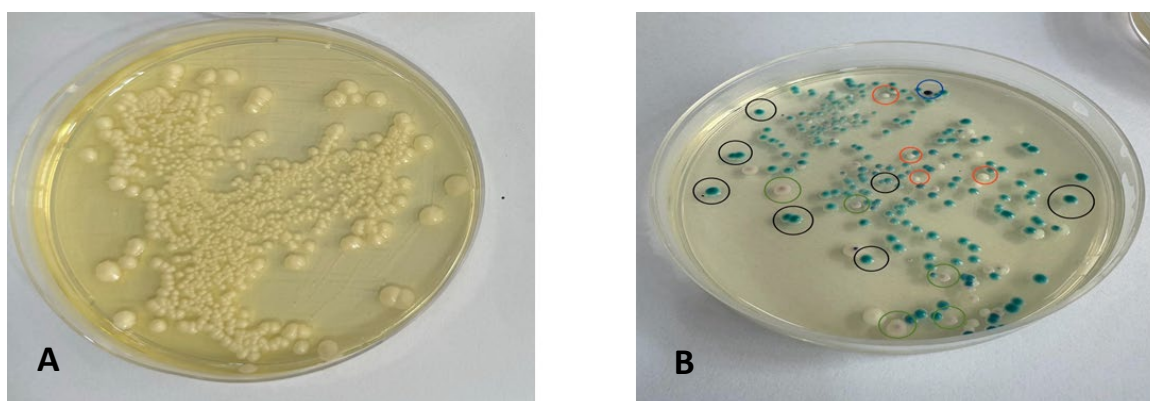


Figure 3.1: Characteristic *Candida* sp. colonies on (A) Sabouraud's dextrose agar and (B) CHROM agar *Candida*.

Figure 3.1 shows the characteristic *Candida* sp. colonies on (A) Sabouraud's dextrose agar appear as white to cream-colored, smooth, glabrous colonies and have different sizes that cannot be differentiated into specific species. In addition, growth of *Candida* sp. on CHROM agar *Candida* (B) shows different colony morphologies each of which is characteristic of a specific species. **Figure 3.2** shows a Gram-stained smear prepared from growth of *Candida* sp. colonies on SDA shows gram-positive round or oval-shaped cells with smaller daughter cells budding off from the larger parent cells, cells are seen in clusters.

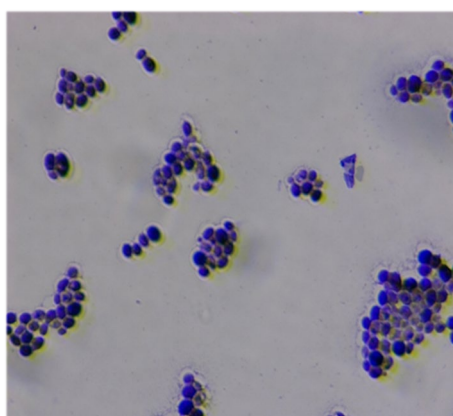


Figure 3.2: Gram-stained smear prepared from growth of *Candida* sp. colonies on SDA.

Table 3.3 shows multi-pattern of the presence of more than one *Candida* sp. in one subject. *C. albicans* + *C. krusei* constituted the common pattern in both groups with doubled number in diabetic group. *C. krusei* + *C. tropicalis* pattern was double in diabetic group. The only triple form of occurrence of *Candida* sp. was *C. albicans* + *C. krusei* + *C. glabrata* which was twice in diabetic group compared to control group. No difference was found in the carriage number of each of *C. albicans* + *C. glabrata*, *C. albicans* + *C. tropicalis* and *C. glabrata* + *C. tropicalis* patterns in both studied groups.

Table 3.3: Frequency of subjects carrying more than one *Candida* sp.

<i>Candida</i> sp.	Diabetic patients No.	Control No.	Total
<i>C. albicans</i> + <i>C. krusei</i>	4	2	6
<i>C. albicans</i> + <i>C. glabrata</i>	1	1	2
<i>C. albicans</i> + <i>C. tropicalis</i>	1	1	2
<i>C. albicans</i> + <i>C. krusei</i> + <i>C. glabrata</i>	2	1	3
<i>C. krusei</i> + <i>C. tropicalis</i>	2	1	3
<i>C. glabrata</i> + <i>C. tropicalis</i>	1	1	2
Total	11	7	18

The results of the Germ tube test are shown in **Figure 3.3**, in which both *Candida albicans* and *Candida tropicalis* were Germ tube test positive this means that this method should be replaced by biochemical tests or growth on CHROMagar *Candida*.

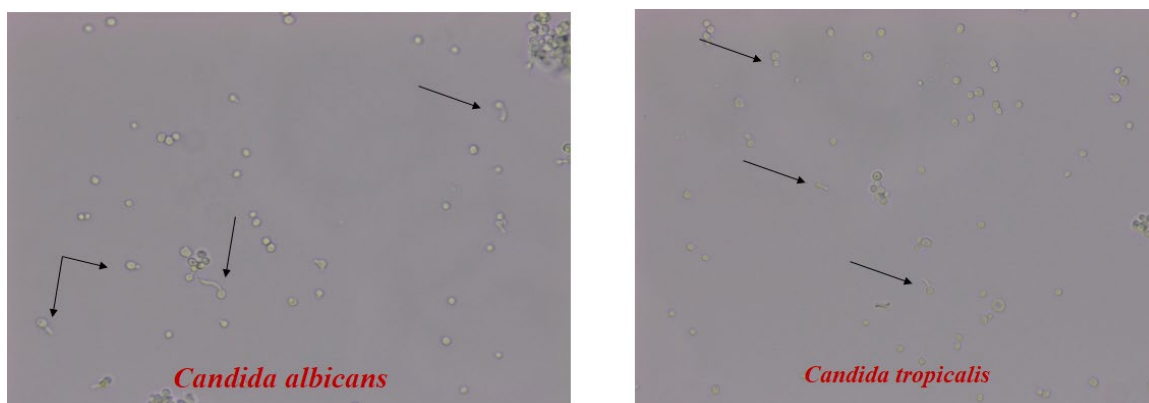


Figure 3.3: Both *Candida albicans* and *Candida tropicalis* were Germ tube test positive as shown by arrows.

Table 3.4 shows the CFU/ml of *Candida sp.* in the studied groups. *Candida 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 *Candida albicans* and the other *Candida* species ($p < 0.01$). Significant differences between the mean CFU/ml of *Candida albicans* in the Diabetic group (1273.22 ± 1640.87) compared to the control group (210.25 ± 404.73) (t-test, $P < 0.01$). Similarly, significant differences were observed for *Candida tropicalis* (Diabetic patients: 63.80 ± 124.85 , Control: 6.52 ± 15.21) ($P < 0.01$). Also, significant differences were observed for *C. krusei* (Diabetic patients: 314.81 ± 572.50) compared to control (71.33 ± 40.07) ($p < 0.005$). And significant differences for *C. glabrata* (Diabetic patients: 33.80 ± 69.81) compared to the control (2.87 ± 3.00) ($p < 0.0012$).

Table 3.4: CFU/ml of *Candida sp.* in the studied groups.

<i>Candida sp.</i>	Diabetic patients		Control		t-test <i>P</i> -value
	Range	Mean±SD	Range	Mean±SD	
<i>C. albicans</i>	0 - 2438	1273.22±1640.87	0 - 1950	210.25±404.73	0.000**
<i>C. glabrata</i>	0 - 250	33.80±69.81	0 - 250	2.87±53.00	0.0012*
<i>C. krusei</i>	0 - 2250	314.81±572.50	0 - 575	71.33±140.07	0.005*
<i>C. tropicalis</i>	0 - 475	63.80±124.85	0 - 75	6.52±15.21	0.000**

*Significant difference between the two means (P -value < 0.0)

**highly significant difference between the two means (P -value < 0.01)

Table 3.5 shows the comparison between the studied groups in total carriage rate and total CFU/ml. The total carriage rate was the same in both groups (88%). However, the total CFU/ml was significantly higher in the study group than in the control group ($p < 0.01$).

Table 3.7: Comparison between the studied groups in total carriage rate and total CFU/ml.

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

**highly significant difference between the two means (P -value < 0.01)

4 Discussion

This study aimed to estimate the carriage rate of *Candida* sp. among patients with diabetic patients (case) and healthy individuals (controls), Identify different *Candida* sp. in both the case and control groups, and Compare the Colony Forming Unit (CFU)/ml in both the study and control groups. The study groups were carefully selected and matched based on demographic and clinical criteria, ensuring an equal number of diabetes and non-diabetic individuals [Table 3.1]. 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 [18]. Furthermore, because *Candida* species prefer high-sugar environments, elevated glucose levels in diabetes individuals provide a great environment for their growth and proliferation [19]. Our results showed that the total carriage rate for Diabetic patients and the control group were similar, in which both group's carriage rates for *Candida* species were (88%). However, the total CFU/ml was significantly higher in the study group (DM) than in the control group, with significant differences between the mean CFU/ml of *Candida albicans* in the Diabetic patients group (1273.22 ± 1640.87) compared to the control group (210.25 ± 404.73) (t-test, $P < 0.01$). Similarly, significant differences were observed for *Candida tropicalis* (Diabetic patients: 63.80 ± 124.85 , Control: 6.52 ± 15.21) ($P < 0.01$). Also, significant differences were observed for *C.krusei* (Diabetic patients: 314.81 ± 572.50) compared to control (71.331 ± 40.07) ($p < 0.005$). And significant differences for *C. glabrata* (Diabetic patients: 33.80 ± 69.81) compared to the control (2.875 ± 3.00) ($p < 0.0012$). To compare our results with other research, 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 [20] this could be due to the method of sample collection or the number of subjects used for the study. In the present study for the CFU/ml, there is a significant difference seen between both groups. Although *C. albicans* has the highest prevalence in the oral cavity among *Candida* species, the incidence of oral candidiasis with other species such as *C. glabrata* and *C. krusei* that are less susceptible toazole compounds has grown during the last two decades [21]. As in this present study, we can respectively confirm that besides *C. albicans*, *C. glabrata* and *C. krusei* are also present in significant numbers. Also, we have confirmed that the Germ tube test is not enough for the identification of *Candida albicans* because *Candida tropicalis* is also germ tube positive [21, 22] in that case, using CHROMagar or other biochemical tests should be helpful.

5 Conclusion

The carriage rate of *Candida* species was found to be similar between the diabetic and control groups. However, the colony-forming units per milliliter (CFU/ml) in the diabetic group were significantly higher compared to the control group. The *Candida* species identified included *C.albicans*, *C.tropicalis*, *C.krusei*, and *C. glabrata*, with *C.albicans* and *C. krusei* showing the highest carriage rates in both groups. Additionally, more than one-third of the subjects were found to carry more than one *Candida* species in their oral cavity. Among the identified species *C.albicans*, and *C. tropicalis* were germ tube test positive. The study also highlighted that a concentrated oral rinse proved to be an ideal method for screening *Candida* species in the oral cavity. More than one-third of the subjects were found to carry more than one *Candida* sp. in their oral cavity.

6 Recommendations

1. Screening for candida sp. carriage rate and CFU/ml in the oral cavity in diabetic patients is necessary to prevent Candidiasis.
 2. Applying the concentrated oral rinse for collecting Candida sp. from the oral cavity.
 3. Never rely on germ tube tests alone to identify *C. albicans*. The use of CHROM agar Candida or API or VITEK 2 system is essential for definitive diagnosis.
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