

# Detection of Biofilm Formation by Candida Species in the Oral Cavities of Diabetic Patients Using the Oral Rinse Technique in Erbil City

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## ABSTRACT

**Background and Objective:** Biofilm formation is a type of virulence factor of *Candida* spp. that promotes growth and protects against antifungal activity, especially in the oral cavity of diabetic patients.

**Aim:** The objective of this study is to detect biofilm formation by *Candida* spp. to determine the relationship between colony-forming units (CFU/mL) and blood sugar levels, and to assess the impact of biofilm formation on antifungal activity.

**Method:** In this current study, which includes two groups: group 1, Diabetic mellitus (55 males and 45 females) (31 Type 1 and 69 Type 2), and group 2, non-diabetic individuals (23 males and 27 females) in Erbil city. Oral rinses were collected for identification using Chromagar *Candida* and the Vitek 2 ID system. For biofilm formation, Congo Red Agar, tube adherence tests, and the Microtiter plate method were used. Antifungal discs (Voriconazole 1mg), (Fluconazole 25mg), (Miconazole 30mg), (Ketoconazole 10mg), and (Nystatin 100mg). Statistical analysis was performed using GraphPad Prism version 9.0, including independent-samples t-tests and ANOVA, and categorical variables were presented as frequencies and percentages.

**Result:** *Candida* spp. were detected in 60% (60/100) of people with diabetes and in 24% (12/50) of non-diabetic individuals. The study identified 15 of 60 isolates (25%) as biofilm producers in diabetic patients. A significant relationship between blood sugar level and oral *Candida* (CFU/mL) ( $P = 0.0001$ ). Moreover, biofilm formation increases resistance to an antifungal agent ( $P = 0.0001$ ).

**Conclusion:** Biofilm formation was associated with increased antifungal resistance. Higher blood glucose levels were associated with increased *Candida* colony counts.

**Keywords:** *Candida* spp., Biofilm, Diabetes mellitus, Oral rinse, Antifungal resistance

## Article Information

Submission Date: 30/6/2025  
Revision date: 3/8/2025  
Acceptance date: 31/8/2025  
Publishing date: June 2026

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## INTRODUCTION

Biofilm formation enables *Candida* to survive within the host and evade host defense mechanisms, thereby promoting systemic infection. It is therefore extremely important to identify the *Candida* spp. causing the infection.<sup>1</sup> Diabetes mellitus (DM) is characterized by underutilization and overproduction of glucose, resulting in hyperglycemia due to impaired insulin action, secretion, or both.<sup>2</sup> The relationship between diabetes and Oral candidiasis has been extensively researched, particularly because diabetes confer heightened vulnerability to fungal infections.<sup>3</sup> Mohammed et al. (2024) from Iraq/ Baghdad province investigated that there is a direct correlation among HbA1c, the level of blood glucose of patients and CFU/mL of *Candida* spp.<sup>4</sup> Sampath et al. (2019) from Sri Lanka revealed that regardless of the phenotypic or molecular identification methods the yeast population density in people with diabetes (>2000 CFU/mL of the oral rinse) was markedly greater than of the controls, indicating that the oral mucosa in people with diabetes provides a more conducive environment for oral *Candida* colonization.<sup>5</sup> Net et al. (2007) from America, investigated the potential role of biofilm in antifungal resistance by assessing the impact of isolated matrix material on planktonic cells, the fact that incorporation of the biofilm matrix into non-biofilm and the replication of the biofilm drug-resistant phenotype raises the hope that the matrix may bind or sequester drugs, obstructing their access to intracellular targets.<sup>6</sup> Understanding the relationship between colony-forming units of *Candida* spp., blood sugar levels, biofilm formation, and antifungal resistance is essential for the treatment of oral candidiasis.

### Aim of the study

It is to detect biofilm formation by different *Candida* spp. and to determine the ratio of *Candida* spp. in the oral cavities of diabetic patients and non-diabetic individuals. Relationship between the level of blood sugar and colony-forming unit of *Candida* spp., and to determine the impact of biofilm formation on antifungal agents.

## METHODS

The present study comprised 100 patients with diabetes mellitus, of whom 55 were male and 45 were female. Patients were categorized based on the type of diabetes: 31 had type 1 and 69 had type 2, aged between 19 and 76 years. Those pa-

tients visited the Galiawa Diabetic Health Center during the study period from June 2024 to January 2025. The study was a case-control study. Moreover, 50 non-diabetic individuals comprising 23 males and 27 females aged 19 to 53 years from Zhyan Health Center, all non-diabetic individuals, without any immunocompromised diseases. The present study was conducted with the approval of the Ethical Committee of Hawler Medical University, College of Medicine. The oral rinse technique involves giving patients 10 milliliters of sterile buffer phosphate saline (PBS) (pH 7.2) to rinse their mouths for one minute. Oral samples were promptly delivered to the microbiology lab at Hawler Teaching Hospital, and then were centrifuged at 3500 rpm for 10 minutes. After that, take 100  $\mu$ L of the precipitation and put it on Sabouraud Dextrose agar (SDA) with tetracycline (0.5 mg/mL) added as a supplement.<sup>7</sup> Inclusion Criteria: All Diabetic patients with Type 1 and 2) non-diabetic individuals (who were in good health and exhibited no symptoms or signs of systemic diseases).

**Exclusion Criteria:** For Diabetic patients, patients diagnosed with prediabetes supported by Galiawa diabetic health center, and patients infected with HIV, HBV, HBC, cancer, smokers, wearing dentures, drinkers, patients with autoimmune diseases, scleroderma, Sjögren's syndrome, and patients with a kidney transplant)

Non-diabetic individuals (RBS > 105) smoker, wearing a denture, drinker, xerostomia, autoimmune diseases, infected with HIV, HBS, HBC, cancer, patient under chemotherapy, or a kidney transplant).

### Identification of *Candida* spp.

#### Chrom agar *Candida* (CAC) and Microscopic Examination

The morphological features of *Candida* spp. on chromogenic *Candida* agar (CAC) and SDA, such as colony shape, color, texture, and size, were used to identify *Candida* spp. then the cells were sub-cultured on CAC for 48 hr. at 37°C. and for *Candida* spp., a sterile clean coverslip was used to scrape off one of the presumed colonies, which was emulsified in a lacto-phenol cotton blue-stained drop on a microscopic slide.<sup>8</sup>

#### Vitek 2 ID-YST system (bioMérieux, Marcy-I'Étoile, France)

The liquid samples for the Vitek2 YST cards (bioMérieux, France) were obtained from overnight cultures, and the turbidity of *Candida* spp.

was measured between (1.8 to 2.2) McFarland Standard, a measurement of turbidity. Pure sub-cultures are maintained in a 0.45% (wt/vol) NaCl solution.<sup>8</sup>

#### **Germ Tube formation**

The germ-tube test is the gold standard in laboratory testing for *Candida albicans* and *Candida dubliniensis*. Add 0.5 mL of human serum to the test tube. The tube was kept at 37°C for 2 to 3 hr. examined under a microscope at magnifications of 10X to 40X.<sup>9</sup>

#### **Biofilm formation methods**

##### **Congo red agar method (CRA)**

To test biofilm production, a solid medium containing a specific supplement of brain heart infusion broth (BHI) was incubated aerobically for 24 to 48 hr at 37 °C. The presence of dark red colonies indicated a positive outcome.<sup>10</sup>

##### **Tube Adherence Test (TAT)**

This method, outlined by Millsap et al., was followed to conduct the quantitative biofilm assay. A tiny amount of *Candida* spp. from overnight culture plates was added to 10 milliliters (ml) of Sabouraud dextrose broth (SDB) containing 8 % glucose. The tubes were rinsed with PBS after 48 hours of incubation at 37°C, and then dyed with 0.1% crystal violet. A film lining the inside of the tube and its base suggested that biofilm formation was progressing well.<sup>11</sup>

##### **Microtiter plate method (MTP)**

The strains were inoculated with (SDB) supplemented with 8% glucose and incubated at 37°C for 24 hr. Biofilm development was detected by adding 100 µL of this standardized cell suspension ( $1 \times 10^6$ ) to microtiter plate wells that previously contained 100 µL of pure fresh (SDB) medium. The microtiter plates were placed in an incubator set at 37°C.

The plates were tapped 3 times with 200 µl of phosphate-buffered saline (pH 7.2), then the contents of each well were emptied. A 1% solution of crystal violet was used to stain adherent biofilms in microplates; adherent *Candida* cells were evenly stained with 1% crystal violet.<sup>25</sup> Micro-ELISA auto-reader was used to resolve the dye with 100% ethanol, and read at an optical density of 490 nm.<sup>11</sup>

#### **Antifungal sensitivity**

##### **Disc diffusion method**

A pure fresh culture of the isolate for testing was cultured on (SDA) for 24 hours at 37°C to confirm

viability and purity. An inoculum suspension standardized to a (0.5) McFarland standard by a densitometer. Then, the plates are incubated for 15 minutes following the dispensing of the disc development, and circular inhibitory zones were anticipated after 20-24 hr. at 37°C.<sup>12</sup>

#### **Statistical analysis**

The results were analyzed using GraphPad Prism version 9.0 (GraphPad Software, San Diego, CA, USA). After checking the data for normality using the Shapiro-Wilk test, differences between the two groups were analyzed using an independent-samples t-test. In comparison, comparisons among more than two groups were performed using one-way analysis of variance (ANOVA), and the data were given as Mean± Standard Deviation (SD), while category variables were presented as frequency and percentage. A p-value < 0.05 was considered statistically significant.

## **RESULTS**

Table 1: presents the demographic characteristics of this study, which included 100 participants with diabetes (31 Type 1 and 69 Type 2) and 50 non-diabetic individuals. The patient population consisted of 55 males (55%) and 45 females (45%). The non-diabetic individuals consisted of 23 males (46%) and 27 females (54%). Positive growth of *Candida* spp. about 60 (60%) in diabetic patients, while in non-diabetic individuals, about 12 (24%). Biofilm formation was detected only in DM (15 [25%] of 60 isolates) and was not produced in non-diabetic individuals. CFU/mL (Mean±SD) in DM was 523.7±351.1, while in the non-diabetic individuals it was 77.40±27.22. About the use of oral mouthwash treatment in the DM range, where 11(11%) used and 89(89%) not used, while in the non-diabetic individuals, those who used oral mouthwash were 17 (34%), and those who did not use it were 33(66%). Regarding antifungal medication use in DM, 16 (16%) were used, and 84 (84%) were not, whereas in the non-diabetic individuals, 11 (22%) were used and 39 (78%) were not.

**Table 1.** Demographic Characteristic of Study Population

|                            |                            | Non-diabetic individuals(50 ) | DM-Patients (100) | p. value               |
|----------------------------|----------------------------|-------------------------------|-------------------|------------------------|
| Gender                     | Female                     | 27(54%)                       | 45(45%)           | 0.298 <sup>NS</sup>    |
|                            | Male                       | 23(46%)                       | 55(55%)           |                        |
| Age (Mean±SD)              |                            | 37.78±10.96                   | 50.35±15.91       | 0.0001 <sup>++++</sup> |
| RBS (Mean±SD)              |                            | 92.82±8.44                    | 180.3±43.10       | 0.0001 <sup>++++</sup> |
| Culture growth             | No growth of Candida spp., | 38(76%)                       | 40(40%)           | 0.0001 <sup>++++</sup> |
|                            | Growth of Candida spp.     | 12(24%)                       | 60(60%)           |                        |
| CFU/mL (Mean±SD)           |                            | 77.40±27.22                   | 523.7±351.1       | 0.0001 <sup>++++</sup> |
| Bio-film formation         | Negative                   | 50(100%)                      | 85(75%)           | 0.004 <sup>++</sup>    |
|                            | Positive                   | 0                             | 15(25%)           |                        |
| Using mouth wash           | Not used                   | 33(66%)                       | 89(89%)           | 0.001 <sup>++</sup>    |
|                            | Used                       | 17(34%)                       | 11(11%)           |                        |
| Antifungal medication drug | Not used                   | 39(78%)                       | 84(84%)           | 0.0001 <sup>++++</sup> |
|                            | Used                       | 11(22%)                       | 16(16%)           |                        |

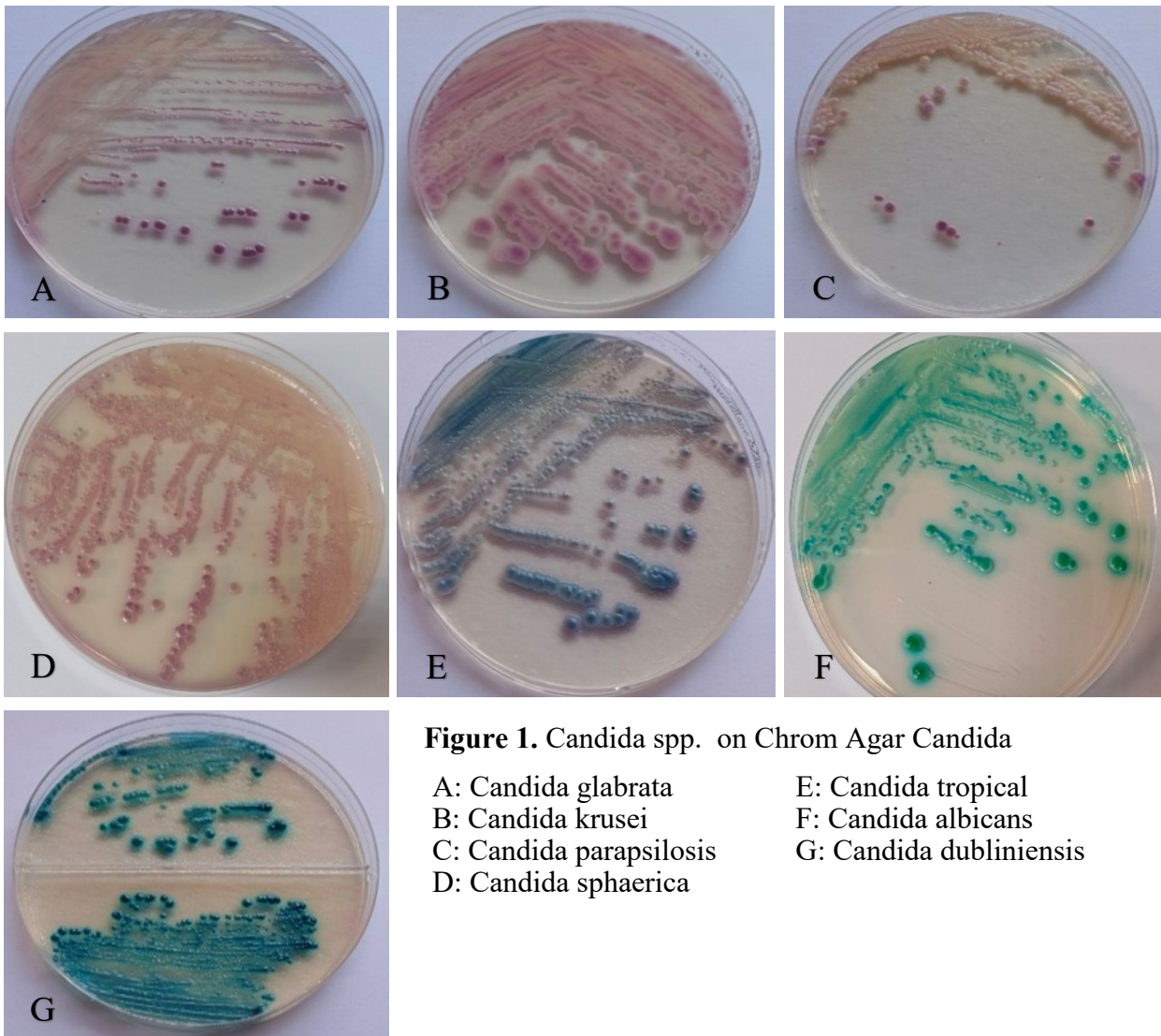
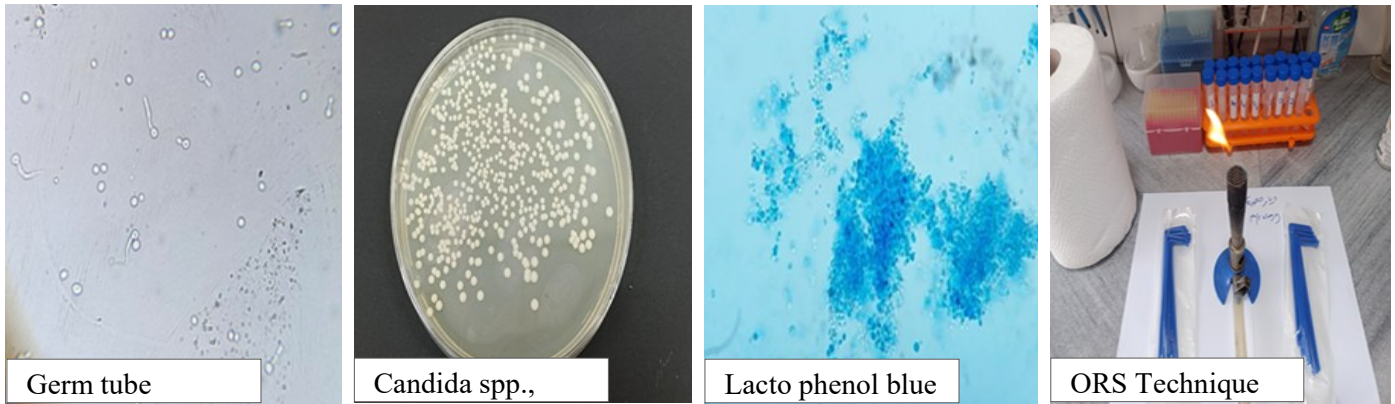
DM= Diabetes mellitus      CFU/mL = Colony forming unit

**Table 2.** Frequency of Oral Candida spp. Isolated From the Oral Rinse of Study Population

| Candida spp.         | Non-diabetic individuals (50) |            | Diabetic patients (100) |            |
|----------------------|-------------------------------|------------|-------------------------|------------|
|                      | Number                        | Percentage | Number                  | percentage |
| Candida albicans     | 12                            | 24%        | 47                      | 78.33%     |
| Candida parapsilosis | 0                             | 0%         | 6                       | 10%        |
| Candida krusei       | 0                             | 0%         | 2                       | 3.33%      |
| Candida tropicalis   | 0                             | 0%         | 2                       | 3.33%      |
| Candida dubliniensis | 0                             | 0%         | 1                       | 1.67%      |
| Candida sphaerica    | 0                             | 0%         | 1                       | 1.67%      |
| Candida glabrata     | 0                             | 0%         | 1                       | 1.67%      |

This table shows the frequency of oral Candida spp. isolated from the oral rinse technique in the study population, 60 (60%) of 100 DM tested positive for Candida spp. compared to only 12 (24%) of 50 normal cases. The prevalence of Candida spp. specifically Candida albicans more common among diabetic patients (78.33% of isolates) in normal cases (24%). Other species, such as Can-

didia parapsilosis (10%), Candida krusei (3.33%), Candida tropicalis (3.33%), Candida glabrata (1.67%), Candida dubliniensis (1.67%), and Candida sphaerica (1.67%), were found only in people with diabetes. Candida parapsilosis was the second- most prevalent species among Candida spp.



**Figure 1.** *Candida* spp. on Chrom Agar Candida

- |                                |                                |
|--------------------------------|--------------------------------|
| A: <i>Candida glabrata</i>     | E: <i>Candida tropicalis</i>   |
| B: <i>Candida krusei</i>       | F: <i>Candida albicans</i>     |
| C: <i>Candida parapsilosis</i> | G: <i>Candida dubliniensis</i> |
| D: <i>Candida sphaerica</i>    |                                |

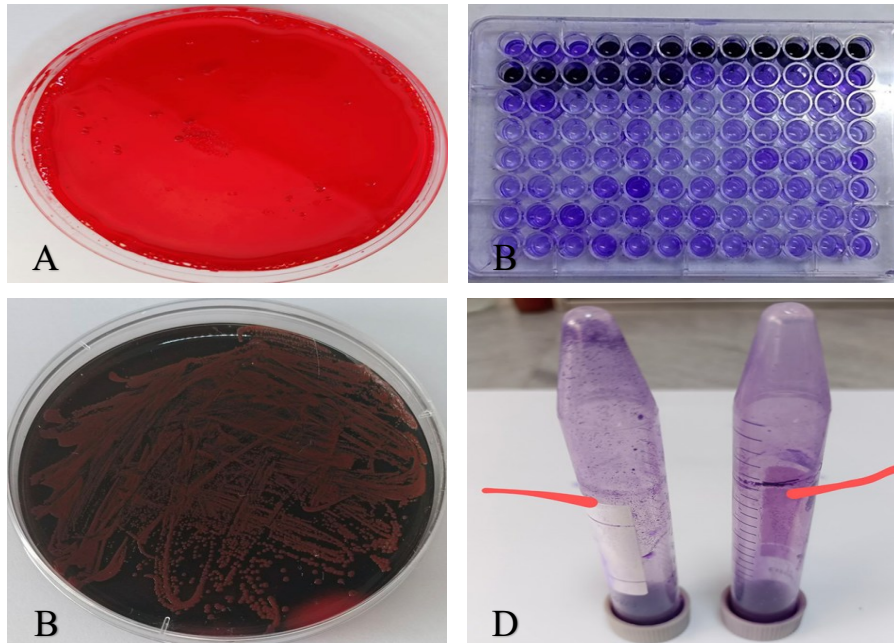
The color of *Candida* spp. depends on Chromagar company, *Candida albicans* green color, *Candida tropicalis* with metallic blue color, *Candida krusei* is pink color with fuzzy, *Candida parapsilosis* pink to white color, *Candida glabrata* pink to mauve brown, *Candida dubliniensis* dark-green to bluish-green, and *Candida sphaerica* pink to white.

Table 3 shows that, among 60 (60%) positive cultures, only 15 (25%) formed biofilms. At the same time, 45(75%) were negative for biofilm formation. It also shows a significant relationship between biofilm formation and CFU/mL of *Candida* spp. ( $p$ -value < 0.05). That means biofilm formation increased the CFU/mL of oral *Candida* spp.

**Table 3.** Comparison of Biofilm Formation by the Oral Candida spp. to the CFU/mL

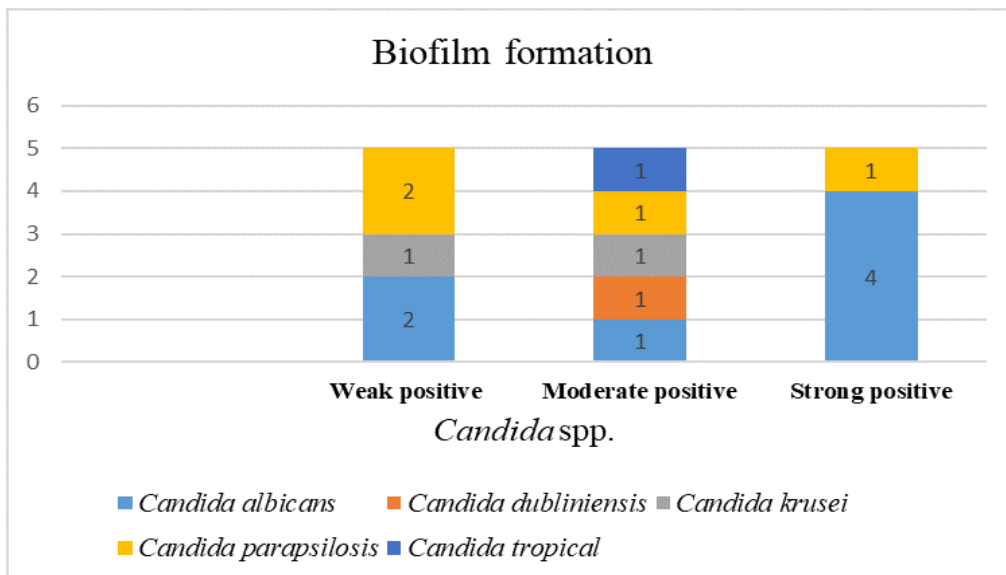
|                            | Biofilm formation |            | P-value |
|----------------------------|-------------------|------------|---------|
|                            | Negative          | Positive   |         |
| Number of isolates from 60 | 45(75%)           | 15(25%)    | 0.0001  |
| Mean±SD                    | 654.9±233.0       | 1527±460.0 |         |

\*Data was analyzed by independent samples t-test. P-value < 0.05



**Figure 2.** Biofilm Formation Methods

A: Negative biofilm formation on CRA B: Positive biofilm formation on CRA  
 C: Microtiter Plate Method D: Tube Adherence Method CRA = Congo Red Agar



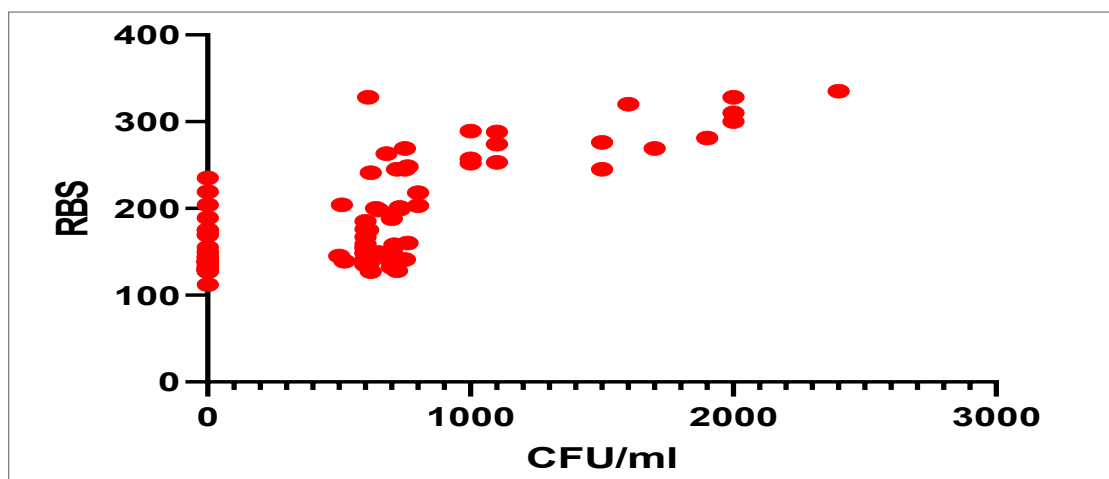
**Figure 3.** Biofilm formation by Oral Candida spp. in diabetic patients, p-value=0.483

The results show that of 15 *Candida* spp. (25%), 60 were positive for DM. *Candida albicans* is the most prevalent for biofilm formation about 7 (11.67%) and followed by *Candida parapsilosis* about 4(6.67), *Candida krusei* about 2(3.33%) *Candida dubliniensis* 1(1.67%) and *Candida tropicalis* 1(1.67%).

Table 4 shows the effect of blood sugar level on CFU/mL of oral *Candida* spp. the p-value is < 0.05, indicating a significant correlation.

**Table 4.** Correlation Between the Level of RBS and CFU/mL of Oral *Candida* spp

| CFU vs. RBS             |                  |
|-------------------------|------------------|
| Pearson correlation ®   | 0.7540           |
| 95% confidence interval | 0.6545 to 0.8278 |
| R squared               | 0.5685           |
| P value                 | <0.0001          |
| P value summary         | ****             |



**Figure 4.** Impact of level blood sugar on CFU/mL

\*Data was analyzed by Pearson correlation used for analysis  
RBS = Random blood sugar

This result shows that the CFU/mL range is associated with blood sugar level.

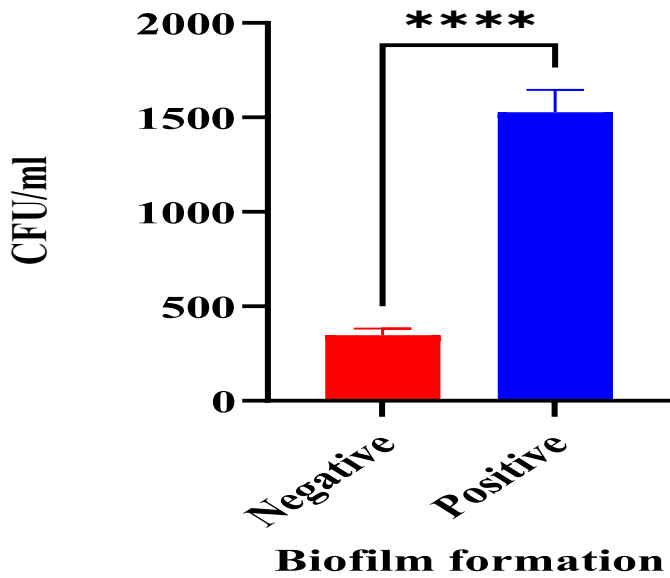
Table 5 shows the effect of positive and negative biofilm formation on antifungal disc activity. *Candida* spp. with biofilm formation showed higher resistance to antifungal discs than those without

biofilm formation. Biofilm formation increases the resistance of *Candida* spp. (p-value < 0.05). The result showed that biofilm has the highest resistance against (Nystatin 100 mg), followed by (Miconazole 30 mg), (Fluconazole 25mg), (Ketoconazole 10 mg), and (Voriconazole 1 mg)

**Table 5.** Effect of Biofilm Formation on Antifungal Agents

|                  | Type of antifungal agents |             |            |             |            |             |            |             |             |             |
|------------------|---------------------------|-------------|------------|-------------|------------|-------------|------------|-------------|-------------|-------------|
|                  | VOR 1mg                   |             | FLU 25mg   |             | KTC10mg    |             | MIC 30mg   |             | NYS100mg    |             |
|                  | R                         | S           | R          | S           | R          | S           | R          | S           | R.          | S.          |
| Number of values | 8                         | 52          | 9          | 51          | 8          | 52          | 10         | 50          | 38          | 22          |
| Mean±SD          | 1888±285.0                | 687.9±175.1 | 1757±474.5 | 702.2±210.3 | 1888±285.0 | 716.7±182.9 | 1770±365.3 | 693.4±137.0 | 998.7±518.9 | 655.5±91.12 |
| p. value         | 0.0001                    |             | 0.0001     |             | 0.0001     |             | 0.0001     |             | 0.003       |             |

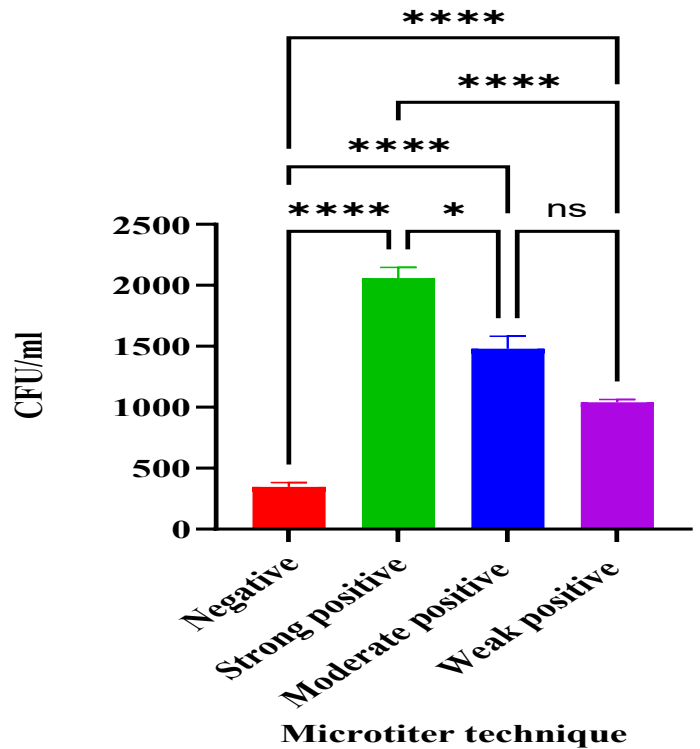
R = Resistance, S= sensitive



**Figure 5.** Association between biofilm and CFU/mL

The result shows a positive increase in CFU/mL of Oral *Candida* spp. in DM, indicating that biofilm formation protects *Candida* spp. from antifungal activity and extends their growth range. Figure 6 shows the level of biofilm formation, from weak to strong. The result showed that strong biofilm increases the CFU/mL of Oral Can-

*didia* spp. in DM > 1500. p-value < 0.05, while negative biofilm formation showed the lowest range of CFU/mL < 500.



**Figure 6.** Microtiter technique for evaluation of biofilm range



**Figure 7.** Antifungal Sensitivity

A: *Candida albicans* biofilm Formation positive show resistance  
 B: *Candida albicans* Biofilm Negative (VOR 1 = Voriconazole 1mg), (NYS100mg = Nystatin 100mg), (MIC30mg= Miconazole 30mg), (KTC 10mg = Ketoconazole), (FLU25mg = Fluconazole 25mg).

These figures show differences between *Candida* spp., with biofilm formation affecting antifungal disc resistance, while without biofilm formation, *Candida* spp. show sensitivity.

## DISCUSSION

Biofilms are a critical virulence factor produced by *Candida* spp. in the oral cavity, particularly in diabetic individuals who exhibit increased susceptibility to persistent *Candida* colonization and infection. A significant will increase in colony-forming units per milliliter is induced by the conducive environment for *Candida* spp. established by hyperglycemia associated with diabetes mellitus, elevated blood glucose levels facilitate increased *Candida* adhesion and growth on oral surfaces by providing a nutritional supply.<sup>6</sup> This investigation revealed that the prevalence of *Candida* spp. was higher in DM than healthy individuals. This observation aligns with the findings from Iraq, by Al-Harba et al.<sup>13</sup> Glucose in saliva acts as a nutrient for *Candida* spp. and inhibits the phagocytic function of neutrophils, thereby facilitating *Candida* colonization.<sup>14</sup> The findings of our study revealed that *Candida albicans* was the predominant isolated species, consistent with the study in Iraq/Duhok province by Sultan and Saadullah.<sup>15</sup> The findings of our investigation revealed a statistically significant association between the prevalence rate of CFU/mL and blood sugar levels this outcome aligns with the findings of Shenoy et al.<sup>16</sup> Sashikumar et al. expressed satisfaction with the substantial positive association between salivary glucose and CFU/mL of *Candida* spp. across the entire study population, corroborating findings by other researchers that elevated *Candida* levels correspond to increased salivary glucose concentrations.<sup>17</sup> Thomson et al. discovered a link between the manifestation of *Candida* infection signs and symptoms and yeast counts above 400 CFU/mL in saliva.<sup>18</sup> The current findings indicate greater colonization with *Candida* spp. in diabetic patients than in healthy individuals, corroborating prior investigations. Research conducted by Lotfi-Kamran et al.<sup>19</sup> Studies by Sampath et al. revealed that a substantial proportion of people with diabetes exceeded the threshold for oral yeasts compared with the control group.<sup>5</sup> The findings of our investigation indicated biofilm formation by *Candida* spp. exclusively in patients with diabetes and absent in non-diabetic individuals. This finding is corroborated in Iraq/Sulaimani province by Mohammed et al.<sup>20</sup> The initial phase of *Candida* colonization and infection involves yeast adherence to epithelial cell surfaces.<sup>16</sup> Rajendran et al. discovered a substantial correlation between HbA1c levels and biofilm production.<sup>21</sup> The findings of our

investigation indicated that *Candida* spp. exhibiting biofilm development and influencing antifungal efficacy, corroborating the results of Kumar et al.<sup>22</sup> The findings of our investigation indicate that *Candida albicans* exhibited strong biofilm development. This outcome aligns with the findings from Iraq/Baghdad province by Rubaie and Qaysi.<sup>23,24</sup> The present study showed *Candida* spp. with biofilm formation is more resistant against antifungal agents than without biofilm formation. This result is consistent with the findings from Brazil by Yamauchi.<sup>25</sup> The current study demonstrates that biofilm formation exhibited greater resistance to nystatin 100mg. This outcome is consistent with Chandra et al.<sup>26</sup>

## Limitation

The main limitation of the present study was a sample size of non-diabetic individuals, because of exclusion criteria applied during non-diabetic individual's selection like (RBS > 105), smoker, wearing a denture, drinker, xerostomia, and non-diabetic individuals with certain condition like autoimmune diseases, infected with HIV, HBS, HBC, cancer, patient under chemotherapy, or a kidney transplant, reduced the number of non-diabetic individuals.

## CONCLUSION

This study demonstrated that biofilm formation was associated with increased antifungal resistance. And the colony-forming unit of *Candida* spp. increases with blood sugar levels. Biofilms contribute to the persistence of *Candida* infections, resulting in substantial clinical and economic challenges. Also, the isolation of *Candida* spp. occurs more frequently in diabetic patients than in non-diabetic individuals. *Candida* spp. are more prevalent in patients with DM than in non-diabetic individuals. Consequently, characterization of biofilms is crucial for identifying therapeutic strategies to prevent and treat chronic infections. We recommend studying other virulence factors that enhance the growth and pathogenicity of *Candida* spp. in the oral cavities of diabetic patients, thereby contributing to the development of antifungal resistance.

## Acknowledgment

The authors are thankful to the staff of the Galiawa Center for Diabetic patients, Hawler Medical Scientific Research and Department of Medical

Laboratory Erbil hospital in Erbil city, Iraq

### Conflict of Interest

The authors declare no conflicts of interest.

### Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

### REFERENCES

1. Darmani H, Al-Saleh DARH. Oral rinses: some kill and some cripple *Candida albicans*. *Med Princ Pract*. 2024;33(4):338-46. DOI: 10.1159/000538368
2. Karina D, Heldayani I, Hidayat W. Oral opportunistic infection induced by stress and silent type 2 diabetes mellitus in young adult patient: a case report. *Int Med Case Rep J*. 2025;59-66. DOI: 10.2147/IMCRJ.S488127
3. Belazi M, Velegraki A, Fleva A, Gidakou I, Papanau L, Baka D, et al. Candidal overgrowth in diabetic patients: potential predisposing factors. *Mycoses*. 2005;48(3):192-6. DOI: 10.1111/j.1439-0507.2005.01124.x
4. Mohammed MJ, Al-Mizraqchi AS, Ibrahim SM. Oral findings, salivary copper, magnesium, and leptin in type II diabetic patients in relation to oral *Candida* species. *Int J Microbiol*. 2024;2024:8177437. DOI: 10.1155/2024/8177437
5. Sampath A, Weerasekera M, Dilhari A, Gunasekara C, Bulugahapitiya U, Fernando N, et al. Type 2 diabetes mellitus and oral *Candida* colonization: analysis of risk factors in a Sri Lankan cohort. *Acta Odontol Scand*. 2019;77(7):508-16. DOI: 10.1080/00016357.2019.1607547
6. Nett J, Lincoln L, Marchillo K, et al. Putative role of  $\beta$ -1,3 glucans in *Candida albicans* biofilm resistance. *Antimicrob Agents Chemother*. 2007;51(2):510-20. DOI: 10.1128/AAC.01056-06
7. AL-Dabbagh AHA, Ajah HA, Salman JAS. Detection of virulence factors from *Candida* spp. isolated from oral and vaginal candidiasis in Iraqi patients. *Arch Razi Inst*. 2023;78(1):465-74. DOI: 10.22092/ARI.2022.359464.2420
8. Stefaniuk E, Baraniak A, Fortuna M, Hryniewicz W. Usefulness of CHROMagar *Candida* medium, biochemical methods—API ID32C and VITEK 2 compact and two MALDI-TOF MS systems for *Candida* spp. identification. *Pol J Microbiol*. 2016 Jan 1;65(1):111-4. DOI: <https://doi.org/10.5604/17331331.1197283>
9. Rachel R, Anuradha M, Leela KV. Biofilm formation and antifungal susceptibility of *Candida* species in vulvovaginal candidiasis. *J Pure Appl Microbiol*. 2024;18(1). <https://doi.org/10.22207/JPAM.18.1.20>
10. Saxena N, Maheshwari D, Dadhich D, Singh S. Evaluation of Congo red agar for biofilm detection. *J Evol Med Dent Sci*. 2014;3(59):13234-8. DOI: 10.14260/jemds/2014/3761
11. Millsap KW, Bos R, van der Mei HC, Busscher HJ. Adhesive interactions of yeast and bacteria on silicone. *Antonie Van Leeuwenhoek*. 2001;79(3-4):337-43. DOI: 10.1023/a:1012013101862
12. Humphries R, Bobenchik AM, Hindler JA, Schuetz AN. Changes in CLSI M100 antimicrobial standards. *J Clin Microbiol*. 2021;59(12):e00213-21. DOI: 10.1128/JCM.00213-21
13. Al-Harba HR, Nasir-Alla N, Jabir AA. Correlation between oral *Candida albicans* and IL-23 in diabetic patients. *Med J Babylon*. 2024;21(4):915-20. DOI: [https://doi.org/10.4103/mjbl.mjbl\\_517\\_23](https://doi.org/10.4103/mjbl.mjbl_517_23)

14. Balan PB, Gogineni S, Kumari NS, et al. *Candida* carriage and saliva growth in diabetes. *J Dent Res Dent Clin Dent Prospects*. 2015;9:274-9. doi: 10.15171/joddd.2015.048
15. Sultan NH, Saadullah AA. Determination of Factors Affecting the Isolation of *Candida* Species Among Diabetic Patients with Oral Candidiasis in Duhok Governorate, Iraq. *Journal of Education & Science*. 2025 Dec 1;34(4):1. <https://doi.org/10.33899/jes.v34i4.49244>
16. Shenoy MP, Puranik RS, Vanaki SS, Puranik SR, Shetty P, Shenoy R. A comparative study of oral candidal species carriage in patients with type1 and type2 diabetes mellitus. *J Oral Maxillofac Pathol*. 2014;18(Suppl 1):S60-S65. DOI: 10.4103/0973-029X.141361
17. Sashikumar R, Kannan R. Salivary glucose and oral candidal carriage in type II diabetics. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2010;109(5):706-11. DOI: 10.1016/j.tripleo.2009.12.042
18. Thomson WM, Chalmers JM, Spencer AJ, Slade GD. Medication and dry mouth: findings from a cohort study of older people. *Journal of public health dentistry*. 2000 Mar;60(1):12-20. DOI: 10.1111/j.1752-7325.2000.tb03286.x
19. Pallavan B, Ramesh V, Dhanasekaran BP, Oza N, Indu S, Govindarajan V. Comparison and correlation of candidal colonization in diabetic patients and normal individuals. *Journal of Diabetes & Metabolic Disorders*. 2014 Jun 4;13(1):66. DOI: 10.1186/2251-6581-13-66
20. Mohammed S, Shekhany K, Jalal P, Fattah CH. Identification and genotyping of *Candida* species involved in oral candidiasis among diabetic patients. *Sulaimani Dent J*. 2022;9(1):9. DOI 10.17656/sdj.10148
21. Rajendran R, Robertson DP, Hodge PJ, et al. Hydrolytic enzyme production in *C. albicans* biofilms in diabetics. *Mycopathologia*. 2010;170(4):229-35.
22. Kumar D, Banerjee T, Chakravarty J, Singh SK, Dwivedi A, Tilak R. Identification, antifungal resistance profile, in vitro biofilm formation and ultrastructural characteristics of *Candida* species isolated from diabetic foot patients in Northern India. *Indian Journal of Medical Microbiology*. 2016 Jul 1;34(3):308-14. DOI: 10.4103/0255-0857.188320
23. AL-Rubaie SR, Al-Qaysi SA. Detection of Some Virulence Factors in *Candida albicans* Obtained from Different Clinical Specimens of Iraqi Patients. *Ibn AL-Haitham Journal For Pure and Applied Sciences*. 2024 Apr 20;37(2):1-1.. DOI: <https://doi.org/10.30526/37.2.3315>
24. Samad AA, Ahmed JM, Izzat KZ, Abdulqadir SS, Muhammed MQ, Bakir MA. Knowledge, Attitude and Practice of Infection Control Among Dental Students at College of Dentistry - Hawler Medical University. *EDJ*. 2024 Mar. 28;6(3):283-9. <https://doi.org/10.15218/edj.2024.33>
25. Yamauchi LM. Biofilm Formation, Virulence Factors and Antifungal Susceptibility of *Candida* spp. Isolated From the Oral Cavity of Diabetes Mellitus Patients. *J Clin Immunol Microbiol*. 2022;3(3):1-2 DOI: <http://dx.doi.org/10.46889/JCIM.2022.3306>
26. Chandra J, Mukherjee PK, Leidich SD, Faddoul FF, Hoyer LL, Douglas LJ, Ghannoum MA. Antifungal resistance of candidal biofilms formed on denture acrylic in vitro. *Journal of dental research*. 2001 Mar;80(3):903-8. DOI: 10.1177/00220345010800031101