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RESEARCH ARTICLE

Assessement of *Candida albicans* of the oral mucosa among type-2 diabetes mellitus patients attending Bahteramas Hospital, Southeast Sulawesi Province

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Abstract

Diabetes Mellitus (DM) is a disease characterized by hyperglycemia resulting from either insulin deficiency or insulin resistance. *Candida albicans* is a fungus generally found on the surface of the mucous membrane, which usually dominates and overgrows among DM patients. This study aims to identify the presence of the *C. albicans* fungus on the oral mucosa of individuals with Type-2 Diabetes Mellitus at Bahteramas Hospital, Southeast Sulawesi Province, Indonesia. A descriptive study was employed, and a purposive sampling technique was used to select a sample of 62 patients. Swab samples were cultured on Potato Dextrose Agar medium for Gram staining and carbohydrate assimilation tests. The resulting culture was tested using CHROMagar™ Candida (CAC) media. Among the 62 samples, 56 samples were identified to have a convex colony, a cream-color surface, a smooth texture, and a Gram-positive nature. The carbohydrate assimilation test showed that the indicated samples could ferment glucose and maltose, but not lactose and sucrose. Green colonies are formed through CHROMagar™ Candida differential media testing. There are 56 identification results for *C. albicans* from oral mucosal swab samples from 62 patients with Type-2 Diabetes Mellitus at the Bahteramas Hospital, Southeast Sulawesi Province, yielding six other patients with negative results.

1. INTRODUCTION

Type 2 Diabetes Mellitus (T2DM) is one of the chronic metabolic disorders that significantly weakens the immune system (1). The global prevalence of diabetes among individuals aged 20–79 in 2021 was estimated to be 10.5% (536.6 million people), and is projected to rise to 12.2% (783.2 million) by 2045 (2). This increasing prevalence underscores the gravity of the situation. One of the primary concerns for individuals with diabetes is a compromised immune system, which can increase their susceptibility to various infections. T2DM patients with metabolic decompensation experience complications related to disease progression and are more susceptible to infections, especially oral candidiasis (3).

Candidiasis is an opportunistic fungal infection caused by various *Candida* species (*Candida albicans* is the most common) (4). *Candida* colonization increases in individuals with weakened immunity and a contributing factor to the development of oral candidiasis (5). Oral candidiasis has increased significantly in recent years, and treatment difficulties are related to drug resistance (6). Exoenzyme secretion plays an important role in the virulence and pathogenesis of *Candida* species (4). Host conditions such as smoking, oral prosthesis, and Diabetes Mellitus (DM)are factors causing the development of this infection (5). The primary causative agent of this infection is *Candida albicans*, which can form biofilms and hyphae to produce hydrolytic enzymes and kandialysin, thus increasing the number and virulence of this pathogenic organism (5). Oral candidiasis often occurs in people with DM in which *C. albicans* within their oral environment tends to colonize, form biofilms, and release hydrolytic enzymes that cause inflammation (5,7).

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Type 2 diabetes mellitus (T2DM) and candidiasis are closely associated, given the respective characteristics of both diseases. However, the exact causal relationship between the two remains uncertain and requires further research (8). The more common DM has been known to induce various complications in the human body, including the growth of oral microbiota, both in terms of species and quantity (9). Oral infections caused by DM are mainly due to disturbances in the balance between various types of oral microbiota (9,10). Higher hydrophobicity potential and hemolytic activity of *Candida* species were found in diabetic patients compared to healthy individuals, suggesting that these features trigger the pathogenicity of oral candidiasis in diabetic patients (11). Viewed from a public dental health perspective, although oral candidiasis is not considered a life-threatening condition, a patients with the infection typically report symptoms that significantly affect their oral health and quality of life, including taste abnormalities, burning sensation, and tongue pain (12). In another report, suggested that the phenomenon of oral candidiasis may be caused by the increased presence of glucose in T2DM patients' saliva, which supports the growth, activity, and resistance of opportunistic microorganisms, especially *Candida* fungi. The most commonly identified species in the oral cavity, and the leading cause of oral candidiasis in T2DM patients, is *C. albicans* (5,11).

C. albicans is typically a normal part of the human oral cavity and tends to increase in cases of a compromised immune system (1). The abnormal quantity that results in T2DM sufferers remains without a thorough explanation. However, its identification is important due to the emergence of various diseases associated with immune deficiency and the broader use of immunosuppressive chemotherapy (13,14). The *Candida* strain is differs from other types, primarily due to its ability to act as an infectious agent and in its susceptibility to antifungals (13). Therefore, exploration of more identifications beyond *C. albicans* is important, as currently, limited data are available regarding the correlation and identification of oral mucosal lesions and *Candida* infections in DM patients. This research aims to fill the current gap, as minimal reports at the local or national level are inadequate to assess the prevalence of oral mucosal lesions and *Candida* infections in DM patients. This study was done in Southeast Sulawesi, Indonesia.

2. MATERIALS AND METHODS

2.1. Study Group

A total of 62 participants with DM (26 men and 36 women), aged over 40 years, were recruited from Bahteramas Regional Hospital. Eligibility was determined based on diagnostic criteria of fasting blood glucose >126 mg/dL or HbA1c >6.5%. To ensure the accuracy of our data collection, DM patients were orally interviewed using previously validated questionnaires, including the Oral Candidiasis Assessment Questionnaire, which assesses demographic characteristics and medical history.

2.2. Ethical Considerations

Respecting the autonomy of our participants, informed and voluntary written consent was obtained before any questionnaire was filled out or interviews were conducted. This ethical approach was further reinforced by obtaining approval from the Ethics Committee of Bahteramas Regional Hospital with Certificate of Research Ethical Eligibility Number: 12/KEP/RSUD/V/2024. All subjects provided their approval by filling out informed consent before further contributing samples and data.

2.3. Sample Collection

Direct sampling was performed to detect and characterize *Candida* species present in the oral lesions of diabetic patients. This method was chosen because it allows for accurate isolation of microorganisms directly from the affected site, reducing the risk of contamination from other oral surfaces. The samples were collected using a sterile swab, wiped on the oral mucosa of DM patients, then put into Potato Dextrose Broth (PDB) transport media (HiMedia, Mumbai, India). Each swab sample was duplicated and placed into two tubes of PDB media. All samples were processed at the Bina Husada Kendari Polytechnic Integrated Microbiology Laboratory.

2.4. Procedure of Culturing and Isolating *Candida* Species

Each sample were transferred to a primary isolation medium, Potato Dextrose Agar (PDA) media plate (HiMedia, Mumbai, India), with the addition of 10 mg/mL of chloramphenicol (Reg. GKL0434003401A1, Novapharin, Indonesia). This approach allows the growth of *Candida* and suppresses the growth of other oral bacterial species due to its low pH, which further increases its selectivity with the addition of antibiotics to the plate. The PDA media with samples were incubated aerobically at 37°C for 48-72 hours (12).

2.5. Identification Method

Identification of *Candida* in primary culture media was performed through several tests that aim to assess the morphology and physiological characteristics of the isolate, including colony morphology observation, gram staining, carbohydrate assimilation tests, differential media test on Candida chromogenic agar, and presumptive identification of

Candida albicans (the germ tube test and the cornmeal agar (CMA) test). All isolates that resembled *Candida*, characterized by creamy, smooth, pale, and convex colonies, were then subjected to Gram staining and carbohydrate assimilation tests using sugar media: glucose, maltose, lactose, and sucrose. During the test, the plates were incubated at 37°C for 10 days. Testing was continued using differential media on *Candida* chromogenic agar, and then the plates were incubated for 48 hours at 37°C (15).

The following assessment for *Candida albicans* is performed through the germ tube test and cornmeal agar test (Corn Meal Agar Media, CMA) (HiMedia, Mumbai, India). The germ tube test involves inducing the growth of hyphae (germ tubes) when sub cultured in liquid media containing protein (egg white) and incubated at 37°C for 2–4 hours. The results are declared positive if germ tubes are formed. Approximately 95% of *C. albicans* isolates produce germ tubes (16).

C. albicans was also identified from other species based on its ability to produce a morphology with characteristics known as Chlamydospores. Isolates were inoculated on corn meal agar media and incubated for 24, 48 and 72 hours at 25°C. Slide culture examination was carried out with isolates inoculated in a cross-hatched pattern on agar coated with a sterile cover glass and incubated for 24, 48 and 72 hours at 25°C. Then, the cultures were examined under a microscope for the presence of chlamydospores (15).

2.6. Data Analysis

The data were meticulously collected and analyzed using SPSS v21.0 software. A comprehensive logistic regression analysis was employed to examine the influence of the duration of suffering from DM and the duration of drug therapy on the identification results of *Candida albicans*. This thorough process ensures the credibility of our research.

3. RESULTS AND DISCUSSION

3.1. Macroscopic and Microscopic Observations

Candidiasis of the oral cavity is the most common opportunistic infection of the oral mucosa, resulting in lesions primarily caused by *C. albicans*. This study was conducted on T2DM patients, representing individuals who suffer from a disorder of metabolic condition.

The yeasts grown from the samples were identified based on morphological criteria, using standard microbiological techniques, including culture on PDA media (macroscopic) and microscopic assay (12). From the total of 62 samples on PDA media, 56 samples showed convex macroscopic colonies with different sizes, a cream color, and a smooth surface, and all of which indicated the presence of *Candida* sp. (Table 1). This significant finding further validates the importance of our research. Morphologically, yeast colonies on PDA were white to cream, smooth, and bald (17). A study has reported that 82 Sabouraud dextrose agar (SDA) plates stained with a loopful solution resulted in creamy, opaque, smooth, and white macroscopic colonies of different sizes, which were identified as *Candida* sp. (18). Colony color was documented. Representative colonies of each *Candida* morphology type were further examined under a microscope using Gram staining (19). The results of this test showed that all colonies were Gram-positive and exhibited budding, as shown in Figure 1.

Morphologically, *Candida* colonies on PDA will appear creamy white, smooth, and convex. PDA medium is generally used as a standard culture medium for isolating *Candida sp.* due to its ability to distinguish different yeast species from the same clinical specimen (18). A study involving 130 oral swab samples on SDA found that 89% of colonies were identified as *Candida* sp., characterized by creamy and convex colonies, with differentiation between species being rare. More than one *Candida* species was identified in approximately 10% of oral samples (19).

3.2. Identification of Candida albicans Using Morphological and Biochemical Tests

Gram staining showed irregular purple-stained round-oval Gram-positive cells that did not show any characteristic features that could be used to differentiate *Candida* at the species level (20). Additional confirmatory tests were performed to identify *C. albicans* using CHROMagar, sprout tube test, and cornstarch agar (Figure 2).

A combination of morphological and biochemical criteria was used in the identification of *C. albicans* isolated in this study. The use of standard SDA or PDA media alone allows for the observation of positive or negative yeast growth without eliminating it, but will not provide sufficient information (17). Chromogenic media are often used to detect and identify *Candida* genus yeast at the species level (21). The isolated yeast strains were grown on chromogenic media (CHROMagar™ *Candida* media) to differentiate *Candida* sp. based on the color of the colonies that developed, where *C. albicans* appears green or bluish green (17). In this study, the color profile of the colonies displayed on CHROMagar was shown by green smooth colonies, which were identified as *C. albicans*, found in 56 isolates.

The Germ tube test is a standard laboratory method for identifying *C. albicans* and was used in this study. Basic mycological examination to identify *Candida* isolate species begins with screening by the ability to produce germ tubes (16), where at least 95% of *C. albicans* isolates will produce germ tubes (16). The 56 isolates are identified by positive germ tube test results, characterized by microscopic slender tubes emerging from *Candida* cells, each with a straight and thin wall, lacking a septum, and attached to the parent cell. Several studies have reported that *C. albicans* is responsive to the

germ tube test, presenting in the form of blastospores, pseudo hyphae, and hyphae, with solitary or chain buds, exhibiting narrow attachment to the parent cell, and thin walls (4,12).

The conventional fungal identification test involves inoculating *Candida* isolates from primary cultures on CMA to verify the formation of Chlamydospores, including their relationship to hyphae, pseudo hyphae, and other fungal structures (15). *C. albicans* has an elongated pseudo hyphae with grape-like blastoconidia clusters on septa. Chlamydospores are found at the tips of hyphae or their short lateral branches (22). The results of culturing the 56 isolates culture on CMA showed that chlamydoconidium formed thick-walled and large structures at the tips of true hyphae or along pseudo hyphae (Figure 2). Other studies report that *C. albicans* can be distinguished from other species by the presence of chlamydospores and pseudo hyphae, also known as pseudo mycelia. Chlamydospores are identified in large formations at the tips of hyphae or their short lateral branches, spherical in shape with thick walls, and with a diameter of 7-13 µm, which should be easily assessed with clear visibility and without any staining (4,19,22).

Table 1. Macroscopic and microscopic observations of oral mucosa swab samples of type-2 diabetes mellitus patients

Code	Colony Shape	Colony Color	Surface	Gram Staining
A1	Not growing	Not growing	Not growing	Not growing
A2	Not growing	Not growing	Not growing	Not growing
A3	Convex	Creamy	Smooth	Gram Positive
A4	Convex	Creamy	Smooth	Gram Positive
A5	Convex	Creamy	Smooth	Gram Positive
A6	Convex	Creamy	Smooth	Gram Positive
A7	Convex	Creamy	Smooth	Gram Positive
A8	Convex	Creamy	Smooth	Gram Positive
A9	Convex	Creamy	Smooth	Gram Positive
A10	Convex	Creamy	Smooth	Gram Positive
A11	Convex	Creamy	Smooth	Gram Positive
A12	Convex	Creamy	Smooth	Gram Positive
A13	Convex	Creamy	Smooth	Gram Positive
A14	Convex	Creamy	Smooth	Gram Positive
A15	Convex	Creamy	Smooth	Gram Positive
A16	Convex	Creamy	Smooth	Gram Positive
A17	Convex	Creamy	Smooth	Gram Positive
A18	Convex	Creamy	Smooth	Gram Positive
A19	Convex	Creamy	Smooth	Gram Positive
A20	Convex	Creamy	Smooth	Gram Positive
A21	Convex	Creamy	Smooth	Gram Positive
A22	Convex	Creamy	Smooth	Gram Positive
A23	Convex	Creamy	Smooth	Gram Positive
A24	Convex	Creamy	Smooth	Gram Positive
A25	Convex	Creamy	Smooth	Gram Positive
A26	Convex	Creamy	Smooth	Gram Positive
A27	Convex	Creamy	Smooth	Gram Positive
A28	Convex	Creamy	Smooth	Gram Positive
A29	Convex	Creamy	Smooth	Gram Positive
A30	Convex	Creamy	Smooth	Gram Positive
A40	Convex	Creamy	Smooth	Gram Positive
A41	Not growing	Not growing	Not growing	Not growing
A42	Not growing	Not growing	Not growing	Not growing
A43	Not growing	Not growing	Not growing	Not growing
A44	Convex	Creamy	Smooth	Gram Positive
A45	Convex	Creamy	Smooth	Gram Positive
A46	Convex	Creamy	Smooth	Gram Positive
A47	Convex	Creamy	Smooth	Gram Positive
A48	Convex	Creamy	Smooth	Gram Positive
A49	Convex	Creamy	Smooth	Gram Positive
A50	Convex	Creamy	Smooth	Gram Positive
A51	Convex	Creamy	Smooth	Gram Positive
A52	Not growing	Not growing	Not growing	Not growing
A53	Convex	Creamy	Smooth	Gram Positive
A54	Convex	Creamy	Smooth	Gram Positive
A55	Convex	Creamy	Smooth	Gram Positive
A56	Convex	Creamy	Smooth	Gram Positive
A57	Convex	Creamy	Smooth	Gram Positive
A58	Convex	Creamy	Smooth	Gram Positive
A59	Convex	Creamy	Smooth	Gram Positive
A60	Convex	Creamy	Smooth	Gram Positive
A61	Convex	Creamy	Smooth	Gram Positive
A62	Convex	Creamy	Smooth	Gram Positive

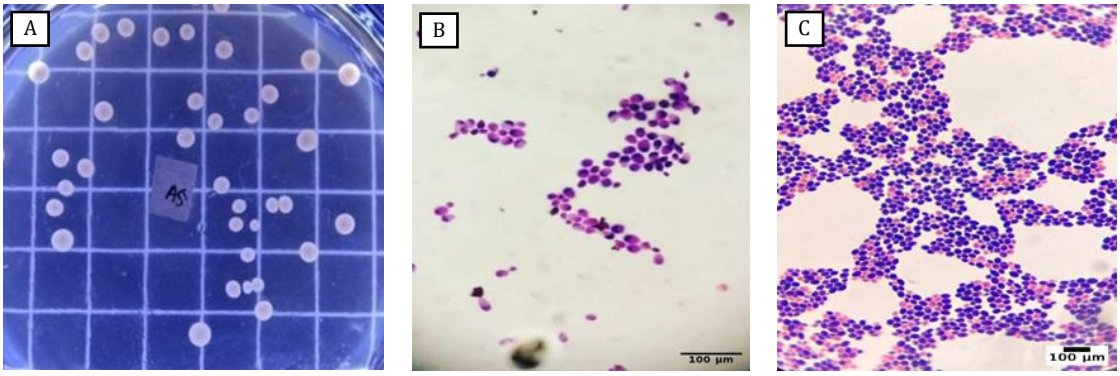

Figure 1. *Candida* species isolation results. (A) Samples indicating *Candida* characteristics on PDA media show creamy, smooth, pale, and convex colonies. (B) and (C) are Gram-positive stain with purple color appearance, observed with a 100x magnification objective lens

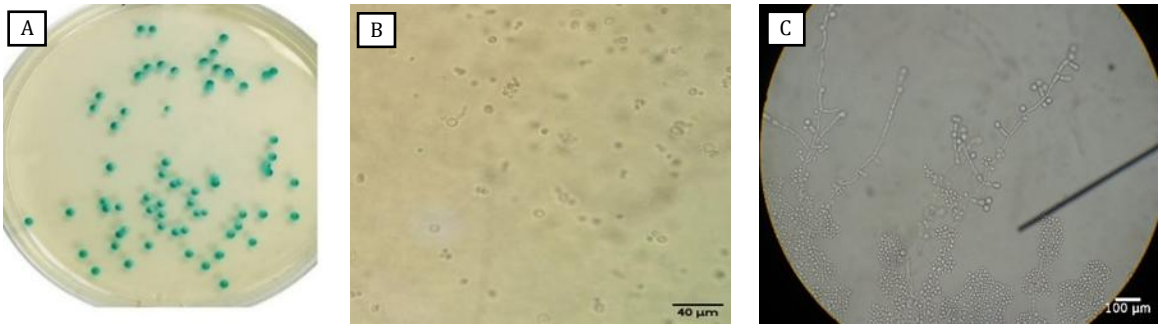

Figure 2. Results of the identification of *C. albicans* on (A) CHROMagar media according to the media's label instructions (Himedia), showing light green smooth colonies, which are identified as *C. albicans*. (B) Germ tubes, involving the induction of hyphae growth when sub cultured in liquid media for 2-4 hours, where sprouts are formed. (C) Corn meal, identifying chlamydospores, which are indicated by their large size and thick walls, observed with a 100x magnification objective lens

3.3. Carbohydrate Assimilation Test Results

The carbohydrate assimilation test measures the yeast's ability to utilize specific carbohydrates as the primary source of carbon in cellular respiration. A positive reaction result is indicated by a change in pH observed in the tested media, using sugar as the base material. This examination utilizes an incubation time of 10 days at 37°C. Production results are indicated by the presence of gas and compared to the standard pH, which is an indication of the occurrence of a fermentation process (9). The carbohydrate assimilation test follows the identification of sugar using sugar media, including glucose, maltose, lactose, and sucrose. The results are presented in Table 2.

Based on Table 2, sample code A3 – A62 can ferment glucose and maltose, as indicated by a change in pH, characterized by a color change in the confectionery medium from green to yellow. Concurrently, lactose and sucrose fermentation did not occur, as the color of the sugar medium remained unchanged. Based on these tests, the microorganism tested is identified as *C. albicans* (16). To further support this finding, the test was continued by using *Candida* CHROMagar™ media.

CHROMagar™ *Candida* is a new differential culture medium which is known to facilitate the isolation and identification of *C. albicans, C. tropicalis*, and *C. krusei*, as well as showing polyfungi populations in clinical samples (15,23). Based on previous research, the sample showed green colonies formed after 48 hours of incubation using *Candida* CHROMagar™ media (24). *Candida* isolation and culture on agar medium, such as SDA, or culture on differential media, such as chromogenic media, usually does not allow species identification, thereby overcoming the limitations of conventional culture identification procedures (23).

Both the result test of CHROMagar™ *Candida* and the subsequent tests, which show the formation of green colonies after 48 hours incubation, strongly indicate the presence of *C. albicans*, as presented in Figure 1. Of 62 samples from patients with T2DM, 56 samples (90,32%) showed *C. albicans* colonies on the oral mucosal swabs, while 6 (9,67%) patients presented no colony growth, as shown in Table 3. DM is a metabolic disease that predisposes individuals to fungal infections, including those associated with *C. albicans* infection, which is caused by its immunosuppressive effect in DM

patients. The relationship between diabetes and candidiasis is mainly due to the increased susceptibility of diabetic patients to fungal infections when compared to those without the disease. Several mechanisms are associated with higher *Candida* sp. infection in DM patients, depending on the site of infection, which can be either local or systemic infection (25).

3.4. Prevalence of *Candida albicans* in Type 2 Diabetes Mellitus Patients

C. albicans is part of the permanent natural microflora of the oral cavity. However, when the ecosystem changes, *C. albicans* grows vigorously and infection can occur in people with metabolic disorders such as in patients with type 2 DM (9,26). Thus, the efficient identification of *C. albicans*, which is currently available through various techniques for isolating of *Candida* in the oral cavity, is presented in this study. Species identification is crucial for accurate oral cavity diagnosis, which facilitates the prevention and treatment of oral candidiasis (13).

A previous study reported that the prevalence rate of *C. albicans* infection in DM patients was 67% compared to 51% in healthy people (27). Most other studies show higher rates of oral candidiasis (40-92%) in patients with DM, compared to healthy people (16-49%) (25,27). Oral *Candida* infestation in diabetic cases in Sri Lanka is significantly higher than in healthy individuals without DM (26).

Table 2. Carbohydrate assimilation test results

Biochemical Test				
Sample Code	Glucose	Maltose	Lactose	Sucrose
A3	+	+	-	-
A4	+	+	-	-
A5	+	+	-	-
A6	+	+	-	-
A7	+	+	-	-
A8	+	+	-	-
A9	+	+	-	-
A10	+	+	-	-
A11	+	+	-	-
A12	+	+	-	-
A13	+	+	-	-
A14	+	+	-	-
A15	+	+	-	-
A16	+	+	-	-
A17	+	+	-	-
A18	+	+	-	-
A19	+	+	-	-
A20	+	+	-	-
A21	+	+	-	-
A22	+	+	-	-
A23	+	+	-	-
A24	+	+	-	-
A25	+	+	-	-
A26	+	+	-	-
A27	+	+	-	-
A28	+	+	-	-
A29	+	+	-	-
A30	+	+	-	-

Biochemical Test				
Sample Code	Glucose	Maltose	Lactose	Sucrose
A31	+	+	-	-
A32	+	+	-	-
A33	+	+	-	-
A34	+	+	-	-
A35	+	+	-	-
A36	+	+	-	-
A37	+	+	-	-
A38	+	+	-	-
A39	+	+	-	-
A40	+	+	-	-
A44	+	+	-	-
A45	+	+	-	-
A46	+	+	-	-
A47	+	+	-	-
A48	+	+	-	-
A49	+	+	-	-
A50	+	+	-	-
A51	+	+	-	-
A53	+	+	-	-
A54	+	+	-	-
A55	+	+	-	-
A56	+	+	-	-
A57	+	+	-	-
A58	+	+	-	-
A59	+	+	-	-
A60	+	+	-	-
A61	+	+	-	-
A62	+	+	-	-

Note: (+) Positive, (-) Negative

Table 3. Demographics of the patients sample and yeast culture

N = 62	Demographics and Yeast Culture
Gender	Male 26 (41,9%)
	Female 36 (58,1%)
Mean Age	55 years (45 – 65)
Average duration of suffering from Diabetes Mellitus	6,2 years (3 – 10)
Average duration of drugs therapy	4 years (2 – 8)
Positive yeast culture *C. albicans*	56 (90,33%)
Negative yeast culture *C. albicans*	6 (9,67%)

The results of the germ tube test revealed a gap between the stem cells (yeast cells) and the hyphae that formed on their surface, known as sprouts. A germ tube is a pseudo hyphae formed from the extension of the blastospore. A germ tube test was performed to differentiate *C. albicans* species from other *Candida* species. There are only two types of *Candida* that can form germ tubes, namely *C. albicans* and *C. tropicalis*. The germ tube test was performed using media containing protein factors, such as serum and plasma, which were incubated for 3 hours. In this study, egg white was used as a medium for testing germ tubes. Egg white is an inexpensive and readily available medium that contains protein essential for the growth of organisms, including *Candida* (22,28). The transition from blastospore to hyphae begins with the formation of a germ tube, which will become mycelium. Germ tube shape is associated with *Candida* virulence and increases the ability of *Candida* to adhere to and invade the mucosal and skin epithelium (20). The transition from yeast to hyphal form is a transition to the pathogenic form (29). The ability to form a germ tube is mainly found in *C. albicans* (19).

3.5. Statistical Evaluation of Factors Associated with *Candida albicans* Colonization

Based on previous research, the ability of *C. tropicalis* to form a germ tube similar to that of *C. albicans,* and its morphology is as comparable that of *C. albicans* germ tube; both are difficult to distinguish. The only difference is that *C. albicans* forms a large number of germ tubes while *C. tropicalis* forms a smaller number (1-2 in the field of view) (17,19).

The results of the binary logistic regression analysis are presented in Table 4. The model examined the association between the duration of DM and the duration of drug therapy, with a focus on identifying *C. albicans*. The analysis showed that neither variable was statistically significantly associated with *C. albicans* detection ($p > 0.05$). Specifically, DM was not a significant predictor (OR = 0.95; 95% CI [0.70, 1.30]; $p = 0.760$), and Drug Therapy also showed no significant association (OR = 1.16; 95% CI [0.74, 1.81]; $p = 0.520$). These findings indicate that the likelihood of *C. albicans* identification did not differ significantly based on the duration of DM or the duration of drug therapy among the study participants. This study obtained identification results for *C. albicans* in 90.3% of the T2DM patients. This finding is consistent with several studies, including one that reported no significant difference in the mean number of *Candida* colonies between age and DM status (30).

Several studies have also reported no significant relationship between the level of colonization and DM, regardless of the type of DM or the duration of suffering from DM (11,31,32). This is because *Candida* species are part of the normal mucosal flora of healthy individuals, where their presence in the oral cavity cannot trigger infection. HbA1c values were not analyzed, which limits the ability to assess the impact of glycemic control on oral *Candida* colonization. In this study, neither the duration of diabetes nor the type of therapy demonstrated a significant association with *Candida* detection. This suggests that chronicity alone does not significantly influence oral colonization when glycemic levels remain elevated over time. Persistent hyperglycemia, regardless of disease duration, may create a favorable environment for fungal growth. Additionally, unmeasured factors such as oral hygiene practices, denture use, diet, and salivary flow may have contributed to colonization patterns. They could overshadow the effects of disease chronicity or treatment regimen (5,33). The detection of *Candida* in asymptomatic patients indicates that subclinical colonization is common in individuals with DM (12). This finding supports the need for routine oral screening even in the absence of symptoms, as undetected colonization could predispose to opportunistic infections under favorable conditions.

A key limitation of this study is the absence of a healthy control group, which restricts direct comparison of oral *Candida* colonization between diabetic and non-diabetic individuals. Although previous studies have reported similar trends and found no significant difference in colony counts when adjusting for age and other variables, inclusion of a control group in the present study would have strengthened the validity of our findings. Recruitment challenges and resource constraints prevented the inclusion of healthy participants in this study. Future research should incorporate a control group to allow more robust comparisons and confirm whether observed colonization rates are specific to diabetes or influenced by other confounding factors.

Table 4. Factors Associated with the identification of *Candida albicans*: Results from binary logistic regression analysis

Variables in the Equation						
Variable	**Coefficient**	**S.E.**	**Wald Statistic**	**p-value**	**Odds Ratio (OR)**	**95% CI for OR**
Diabetes Mellitus	-0.049	0.160	0.093	0.760	0.952	[0.70, 1.30]
Drug Therapy	0.147	0.228	0.414	0.520	1.158	[0.74, 1.81]
Constant	1.997	0.780	6.546	0.011	7.364	-

4. CONCLUSIONS

Type 2 Diabetes Mellitus (T2DM) is a risk factor for opportunistic infections, including oral candidiasis. However, not all oral lesions are caused by *Candida*, thus necessitating further examination with appropriate procedures to identify of *Candida*. Our comprehensive study, which included thorough examination and statistical analysis, reports the identification of *C. albicans* in T2DM patients, showing positive results for *C. albicans* in 56 of 62 patients. The results of statistical tests showed that there was no significant relationship between the duration of suffering from DM and the duration of therapy for *Candida* colonization.

Author contributions: F, AF: Conceptualization, Methodology, Investigation, Data collection, and Original draft preparation. AU: Data analysis, Validation, Visualization. MAS: Formal Analysis.

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Ethics statement: All clinical isolates and study protocols used in this research study were approved by the Ethics Committee of Bahteramas Regional Hospital with Certificate of Research Ethical Eligibility Number: 12/KEP/RSUD/V/2024.

Conflict of interest: The authors declare no conflicts of interest.

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