Research Article

Genotypes and virulence factors of *Candida* species isolated from oral cavities of patients with type 2 diabetes mellitus

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Background/aim: This study compared the genotypes and virulence factors of *Candida* species isolated from oral cavities of healthy individuals and patients with diabetes mellitus (DM).

Materials and methods: A total of 142 healthy individuals and 73 diabetic patients participated in this study. Study populations were classified into 4 groups as follows: Group I — Healthy, without caries; Group II — Healthy, with caries; Group III — DM, with caries; Group IV — DM, without caries. Diabetic patients' blood glucose and hemoglobin A1c concentrations were determined. Identification of *Candida* species was performed with conventional methods. Biofilm production, proteinase, phospholipase, and esterase were analyzed. The genetic diversity of *Candida* species was established using rep-PCR.

Results: The most isolated species was *Candida albicans*. There were statistical differences in terms of isolated *Candida* frequency between healthy subjects and diabetic patients. There was no statistical difference between the virulence factors of groups. Twelve genotypes were determined. While there were statistical differences in aerobe biofilm production, proteinase, and phospholipase activity between genotypes, there were no statistical differences in anaerobe biofilm production and esterase activity between genotypes.

Conclusion: Diabetes has no effect on the activities of virulence factors of *Candida* species. Different genotypes of *Candida albicans* exhibited different virulence activities.

Key words: *Candida*, diabetes mellitus, genotype, virulence factors

1. Introduction

Candida species frequently appear in normal human oral microbial flora (1). However, the prevalence of *Candida* spp. depends on the sensitivity of the determination method, age, sex, presence of dentures, smoking habits, caries experience, periodontal status, antimicrobial therapies, and health problems such as immunosuppression, AIDS, and diabetes (2).

Oral fungal flora has been the subject of several studies. A strong relation has been found between the prevalence of *Candida albicans* and dental caries, particularly in children, adolescents, and young adults (3,4). These previous studies suggest that *C. albicans* could play a considerable role in the pathogenesis of caries.

Diabetes mellitus (DM) is a chronic disorder characterized by a relative or total deficiency of insulin secretion and/or accompanying resistance to the metabolic

action of insulin on target organs (5,6). Diabetes mellitus may have different and sometimes serious effects upon the tissues, and patients with poor glycemic control are particularly prone to severe and/or recurrent yeast infections. Yeast growth and adhesion may be augmented by high blood and saliva glucose concentrations, which can serve as nutrients for *Candida* species. The reduced *Candida* killing capacity of neutrophils in the presence of high glucose concentrations may lead to increased colonization of *Candida* (5,6).

The capability of *C. albicans* to persevere within the host and cause infection has been attributed to its different virulence features, which include biofilm production, dimorphism, the ability to interchange between different morphological types, interference with the immune system, synergism, and/or the production of extracellular enzymes (7).

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The biofilm production of *Candida* is an important pathogenetic factor that plays a role in the adherence to the tissue and colonization of *Candida* (8). It is also known that *Candida* spp. have the ability to grow either in aerobic or anaerobic conditions. *Candida* spp. also possess some adaptive mechanisms to survive in both situations (9).

Microorganisms have constitutive hydrolytic enzymes to facilitate penetration of host tissues. Extracellular phospholipases and proteases play an important role in destroying cell membranes that are made up of lipids and proteins (10). Due to the increasing frequency of invasive *Candida* infection, there is great interest in *Candida* virulence factors, such as phospholipase and protease production, in order to develop strategies for the control and prevention of candidiasis and also as a possible target for developing novel therapeutic approaches (11,12). Phospholipase could play an important role in the invasion of host tissues in lesions of candidiasis by destroying the epithelial cell membranes and allowing the hyphal tip to enter the cytoplasm (13). Proteinase production is considered to increase colonization and penetration to host tissues of microorganisms, and to avoid the host's immune system by degrading a number of proteins important in host defenses, such as immunoglobulins, complements, and cytokines (14).

The value of esterase production by *Candida* in pathogenic processes is less well known. Esterase is capable of hydrolyzing the ester bonds in glycerides (15).

In addition, it has been reported that the virulence of distinct genotypes of *Candida* spp. may be different (16). Although there are many studies about pathogenic fungi, it has been reported that studies on virulence factors are still needed (17,18).

The aim of this study was to compare genotypes and virulence factors of *Candida* spp. isolated from healthy people and patients with diabetes mellitus.

2. Materials and methods
A total of 215 subjects (142 healthy subjects and 73 with type 2 DM) were included in this study. The mean age of the healthy subjects (71 women and 71 men, ages 16 to 60) was 26 years old, and for those with type 2 DM (49 women and 24 men, ages 20 to 66) it was 46 years old.

Examination of the oral cavity was carried out by one of the authors (AEŞ) under artificial light. Dental caries were evaluated using dental mirrors, dental probes, and radiographs. Periodontal status was evaluated using periodontal probes and radiographs. The oral mucosa was also examined for the presence or absence of candidiasis.

Individuals with the following conditions were included in the study: over 16 years of age, not having taken antifungal medication or antibiotics within the 10 weeks prior to sample collection, with no periodontal problems (no bleeding from the gingival sulcus on gentle probing and no alveolar bone loss as radiographically determined), no oral candidiasis, no teeth fillings, no prosthetic treatments (bridges, crowns, or partial dentures), no orthodontic appliances, and no tobacco smoking habits.

Diagnosis of DM was made according to the criteria of the American Diabetes Association and European Association for the Study of Diabetes (ADA-EASD). In DM patients, glycemic control, as assessed by hemoglobin A1c (HbA1c) (glycosylated hemoglobin), and fasting blood glucose were used as measures of recent diabetic control. Blood glucose of >200 mg/dL and HbA1c of >9 were considered as signs of poorly controlled diabetes.

The presence of clinical and/or radiographic cavitation into dentin was considered as carious tooth (19,20). The individuals were divided into groups according to the presence or absence of caries on teeth:

Group I: Healthy, without caries (n = 95),
Group II: Healthy, with caries (n = 47),
Group III: Diabetes mellitus, with caries (n = 53),
Group IV: Diabetes mellitus, without caries (n = 20).

2.1. Identification of *Candida* spp.
The microbiological samples were collected by swabbing with a dry sterile cotton stick from the middle of the dorsum of the tongue (1). All the samples were processed within 2 h in the mycology laboratory of the Department of Microbiology at the Faculty of Medicine, Erciyes University. All samples were inoculated on 2 Sabouraud dextrose agar (SDA, Oxoid, UK) containing chloramphenicol (50 mg/L) medium in aerobic incubation at 25 °C and 35 °C for 24–72 h. All isolated strains were identified using macroscopic and microscopic morphology, the germ tube test, chlamydospore production, urease activity, and carbohydrate assimilation with the API 32C system test kits (BioMerieux, Marcy l'Etoile, France) (21,22).

2.2. Yeast suspensions for enzymatic activities
Yeast suspensions were prepared from the yeast isolates included in the study to evaluate phospholipase, proteinase, esterase, and biofilm formation. A loopful of the stock culture was streaked onto SDA (Oxoid) with chloramphenicol and incubated at 37 °C for 24–48 h. Yeasts were harvested and suspended in sterile phosphate-buffered solution at turbidity equal to an optical density (OD) of 0.5 McFarland. The final suspension was adjusted to contain $2.5–5 \times 10^6$ yeast cells/mL (9,23,24).

2.3. Determination of proteinase activity
Test isolates were tested in triplicate, during 2 independent experiments, to verify the enzymatic activity of proteinases. Extracellular proteolytic activity (SAP) of the isolates was assessed in bovine serum albumin agar according to Staib (24) and Cassone et al. (25). Ten microliters of previously prepared suspension from isolates was transferred to the

test medium. The plates were incubated at 37 °C for 48 h and 72 h to examine the proteinase activity. The ratio of the diameter of the colony to that of the clear zone of proteolysis (in millimeters) was used as an index to represent the extent of proteinase activity by the different *Candida* spp. isolates. *C. albicans* ATCC 10231 and ATCC 10261 were used as controls.

2.4. Determination of phospholipase activity

The phospholipase production of the isolates was assayed according to Price et al. (23) and Polak (26). The test medium consisted of malt agar containing 1 M sodium chloride, 0.005 M calcium chloride, and 2% egg yolk. Each strain was inoculated in triplicate. Each culture was then incubated at 37 °C for 48 h. Phospholipase activity was expressed as the ratio of the diameter of the colony to the diameter of the colony plus the precipitation zone (in millimeters) (23). As a control, *C. albicans* ATCC 10231 and ATCC 24433 strains were also used in the experiments.

2.5. Determination of esterase activity

The esterase activity of the isolates was assessed in Tween 80 agar according to Slifkin (27). Ten microliters of previously prepared suspension from each isolate was carefully inoculated on the Tween 80 opacity test medium and then incubated at 37 °C for 48 h and 72 h to examine the esterase activity. This activity was considered positive in the presence of a halo pervious to light around the inoculation site (27). Test isolates were tested in triplicate, during 2 independent experiments, to verify the enzymatic activity of esterase.

2.6. Determination of biofilm formation

Biofilms of *Candida* spp. were permitted to grow in wells of microtiter plates (IWAKI, Tokyo, Japan) as previously described (28). The plates were incubated at 37 °C in both aerobic and anaerobic conditions for 24 h to induce biofilm formation. The OD was measured using a microtiter reader (Biotek ELx808, USA) at 450 nm. Three wells were used for each strain, and the arithmetical mean of 3 readings was used in analyses. *Candida albicans* ATCC 90028 was employed as the control strain. Sterile brain-heart infusion broth without microorganisms was employed as the negative control. The cutoff value was determined by arithmetically averaging the OD of the negative control well and by adding 2 standard deviations. Samples with an OD higher than the cutoff value were considered positive,

whereas those with lower values than the cutoff were considered negative.

2.7. Determination of genotyping of *Candida* spp.

All the isolates obtained from DM patients and healthy individuals were genotyped by repetitive sequence-based polymerase chain reaction (rep-PCR). The rep-PCR-based fingerprint patterns of all isolates were obtained in gel-like images by using DiversiLab 2.1.66 web-based interpretation software. For analysis, the Pearson correlation coefficient was used in the similarity calculation, and the unweighted pair group method with arithmetic mean (UPGMA) was used to automatically compare the rep-PCR profiles. Strains of *C. albicans* with a similarity of 95% or above were accepted as main clones, and those in the main clone category having a similarity of 97.5% or above were accepted as subclones. Isolates with a similarity of 95% or below were accepted as different clones (9). In the present study, *C. albicans* ATCC 90028 was employed as the reference strain.

This study was approved by the ethics committee of Erciyes University. All patients gave written informed consent.

2.8. Statistical analysis

All statistical analyses were performed using SPSS 15.0. Normal distributions of the variables were analyzed by visual (histogram and o-probability graphics) and analytic methods (Shapiro–Wilk test). The Mann–Whitney U test, two-proportion test, and Fisher exact test were used, as the data were ordinal. $P < 0.05$ was considered significant.

3. Results

The values of blood glucose and HbA1c of Groups III and IV are presented in Table 1. There were no statistically significant differences between values of blood glucose and HbA1c of Groups III and IV.

Candida was isolated in 21% of Group I, 42.5% of Group II, 37.7% of Group III, and 60% of Group IV (Table 2). There were statistically significant differences between Groups I and II, Groups I and III, and Groups I and IV ($P < 0.05$). There were no statistically significant differences between Groups II and III, Groups II and IV, and Groups III and IV ($P > 0.05$).

The 72 *Candida* species isolated from the oral samples included *C. albicans* (n = 65), *C. glabrata* (n = 5), *C. kefyr* (n

Table 1. Values of blood glucose and HbA1c of Groups III and IV.

Group	Blood glucose level			HbA1c		
	Median	25%	75%	Median	25%	75%
Group III	218	151	238	8.6	6.3	9.4
Group IV	161	131.2	214.2	7.1	6.4	8.9

Table 2. *Candida* isolation rates and *Candida* species acording to groups.

Groups	With *Candida*	Without *Candida*	*C. albicans*	*C. glabrata*	*C. kefyr*	*C. parapsilosis*
Group I (n = 95)	20 (21%)	75 (79%)	18	2	_	_
Group II (n = 47)	20 (42.5%)	27 (57.5%)	20	_	_	_
Group III (n = 53)	20 (37.7%)	33 (62.3%)	15	3	1	1
Group IV (n = 20)	12 (60%)	8 (40%)	12	_	_	_
Total (n = 215)	72	153	65	5	1	1

= 1), and *C. parapsilosis* (n = 1) (Table 2). The percentages of *Candida albicans* obtained from diabetic patients and from healthy individuals were 84.3% and 95%, respectively.

Candida was isolated at a rate of 40% of individuals with caries and 27.8% of individuals without caries. However, there was no statistically significant difference between groups (P > 0.05).

Candida was isolated at a rate of 43.8% from diabetic patients and 28.2% from healthy individuals. There was a statistically significant difference between groups (P < 0.05).

There were 27 patients who had glucose levels higher than 200 mg/dL out of a total of 73 DM patients. Of these 27 patients, *Candida* was isolated from 12 (44.4%) of them. There were 19 patients who had HbA1c levels higher than 9 out of a total of 73 DM patients. Of these 19 patients, *Candida* was isolated from 11 (62.9%) of them. Although isolated *Candida* rates were higher in patients with blood glucose of >200 mg/dL and HbA1c of >9 than in patients who had blood glucose levels of <200 and HbA1c levels of <9, we did not see any statistically significant differences in both groups (P > 0.05).

There were no statistically significant differences between virulence factors of Groups I, II, III, and IV (P > 0.05) (Table 3).

3.1. Genotyping

Dendrogram analysis and virtual gel image of rep-PCR fingerprint patterns of *Candida* spp. (*C. albicans*, *C. glabrata*, *C. kefyr*, and *C. parapsilosis)* and genetic proximity of *C. albicans* isolated from groups with the DiversiLab software, version 3.3. can be seen in Figures 1–4.

There were 12 main clones (A to L), and from 6 of these main clones, subclones were determined (Table 4). Two subclones in main clone A (A1, A2), 3 subclones in main clone B (B1, B2, B3), 2 subclones in main clone C (C1, C2), 2 subclones in main clone D (D1, D2), 3 subclones in main clone E (E1, E2, E3), and 3 subclones in main clone H (H1, H2, H3) were determined. However, in this study, clones of 27 species could not be determined. In Group I, 3 main clones (A to C) and 7 subclones (A1, A2, B1, B2, B3, C1, C2) were determined, and clones of 3 species could not be determined. In Group II, 4 main clones (D to G) and 5 subclones (D1, D2, E1, E2, E3) were determined, and clones of 7 species could not be determined. In Group III, 2 main clones (H, I) and 3 subclones (H1, H2, H3) were determined, and clones of 11 species could not be determined. In Group IV, 3 clones (J to L) were determined, and the clones of 6 species could not be determined.

Table 3. Virulence factors of *Candida* species according to groups.

Groups	Slime				Esterase		Proteinase		Phospholipase	
	Aerobe		Anaerobe							
	P	N	P	N	P	N	P	N	P	N
Group I	4	16	-	20	3	17	6	14	11	9
Group II	2	18	2	18	2	18	8	12	14	6
Group III	3	17	1	19	7	13	9	11	13	7
Group IV	-	12	-	12	1	11	2	10	8	4
Total	9	63	3	69	13	59	25	47	46	26

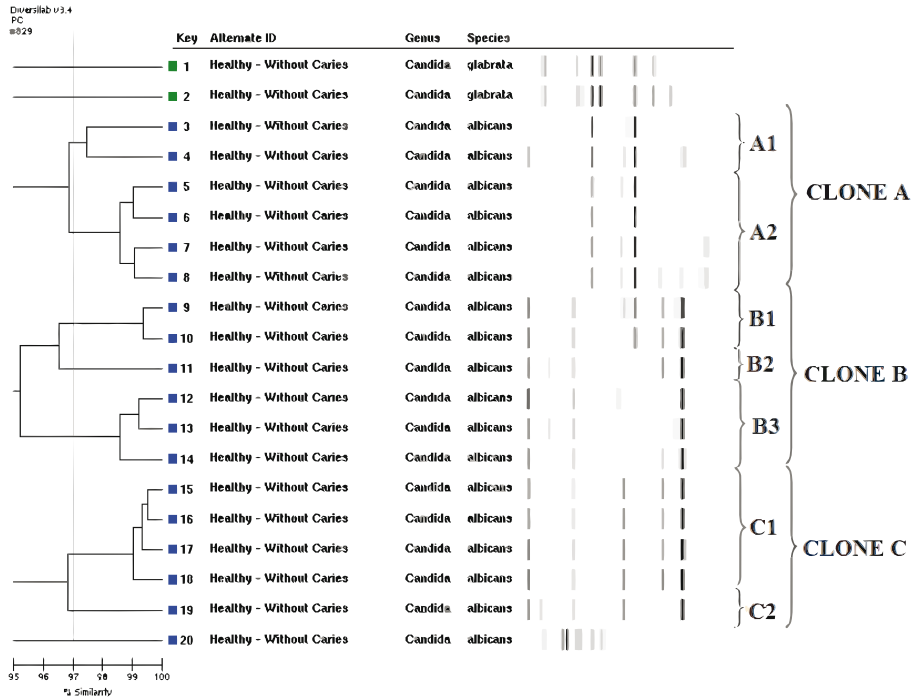N: Negative number, P: Positive number.

Figure 1. Dendrogram analysis and virtual gel image of rep-PCR fingerprint patterns of *Candida* spp. and genotype of *C. albicans* isolated from Group I with the DiversiLab software, version 3.3.

Figure 2. Dendrogram analysis and virtual gel image of rep-PCR fingerprint patterns of *Candida* spp. and genotype of *C. albicans* isolated from Group II with the DiversiLab software, version 3.3.
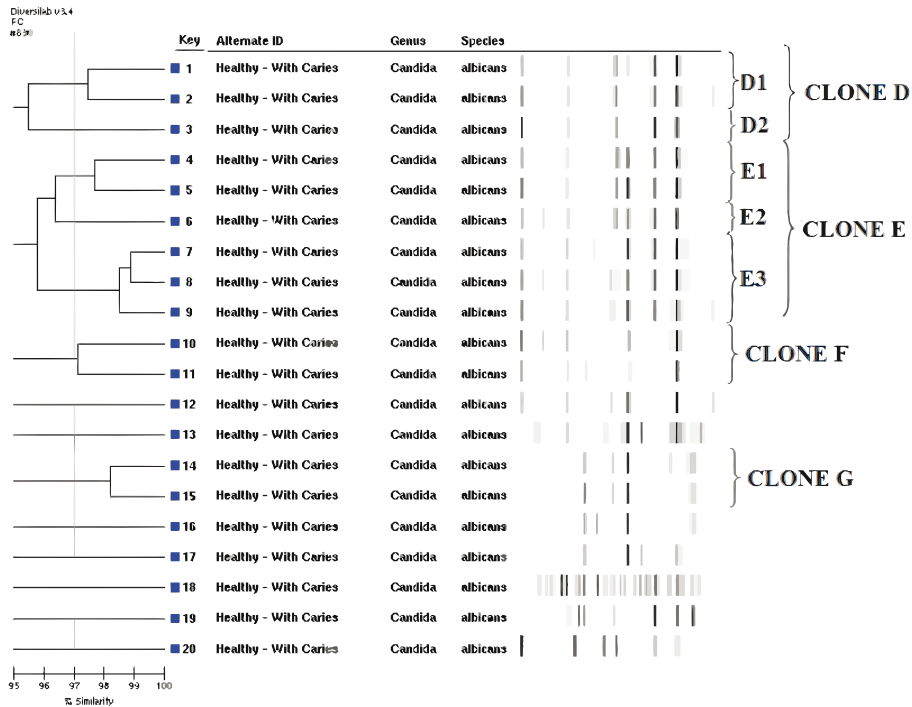
Figure 3. Dendrogram analysis and virtual gel image of rep-PCR fingerprint patterns of *Candida* spp. and genotype of *C. albicans* isolated from Group III with the DiversiLab software, version 3.3.

Figure 4. Dendrogram analysis and virtual gel image of rep-PCR fingerprint patterns of *Candida* spp. and genotype of *C. albicans* isolated from Group IV with the DiversiLab software, version 3.3.
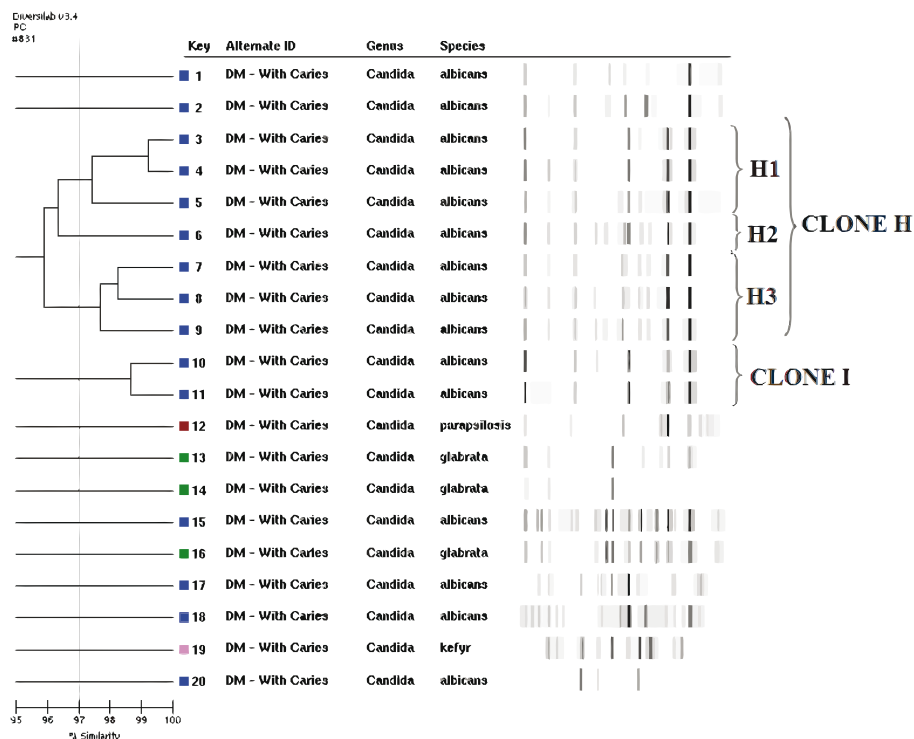
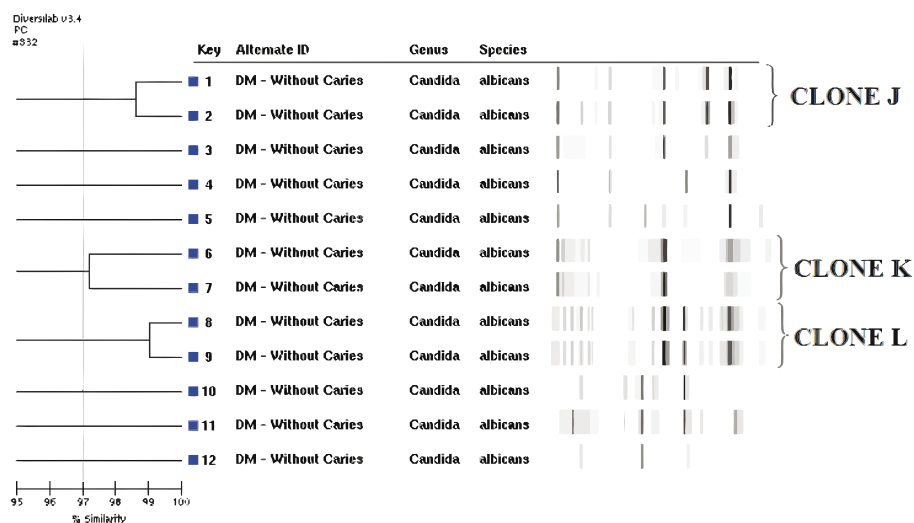### 3.2. Virulence factors and genetic proximity

There were statistically significant differences in aerobe biofilm production in clone A and clone B (P < 0.005), but there was no statistically significant difference in the other clones (P > 0.05). Moreover, there were statistically significant differences in phospholipase production (P < 0.05) between clone A and clones B, C, D, E, F, H, and J; between clone B and clone L; and between clone C and clone K (P < 0.05); but there was no difference between other clones (P > 0.05). In proteinase activity, there were

Table 4. The genetic proximity and virulence factors of *C. albicans*.

Clones	Clone number	Slime				Esterase		Proteinase		Phospholipase	
		Aerobe		Anaerobe							
		P	N	P	N	P	N	P	N	P	N
A	6	4	2	-	6	-	6	-	6	-	6
A1	2	2	-	-	2	-	2	-	2	-	2
A2	4	2	2	-	4	-	4	-	4	-	4
B	7	-	7	-	7	3	4	3	4	7	-
B1	3	-	3	-	3	1	2	2	1	3	-
B2	1	-	1	-	1	-	1	-	1	1	-
B3	3	-	3	-	3	2	1	1	2	3	-
C	5	-	5	-	5	-	5	3	2	5	-
C1	4	-	4	-	4	-	4	3	1	4	-
C2	1	-	1	-	1	-	1	-	1	1	-
D	3	-	3	-	3	-	3	-	3	3	-
D1	2	-	2	-	2	-	2	-	2	2	-
D2	1	-	1	-	1	-	1	-	1	1	-
E	5	1	4	1	4	1	4	4	1	5	-
E1	1	1	-	1	-	-	1	-	1	1	-
E2	1	-	1	-	1	-	1	1	-	1	-
E3	3	-	3	-	3	1	2	3	-	3	-
F	2	-	2	-	2	-	2	2	-	2	-
G	1	-	1	-	1	-	1	-	1	-	1
H	7	1	6	-	7	4	3	4	3	6	1
H1	3	-	3	-	3	2	1	1	2	2	1
H2	1	1	-	-	1	-	1	1	-	1	-
H3	3	-	3	-	3	2	1	2	1	3	-
I	2	-	2	-	2	1	1	1	1	1	1
J	2	-	2	-	2	-	2	-	2	2	-
K	2	-	2	-	2	-	2	1	1	1	1
L	2	-	2	-	2	-	2	-	2	-	2

N: Negative number, P: Positive number.

only differences between clone A and clone E and between clone A and clone F (P < 0.05); there was no difference between other clones (P > 0.05). There was no statistically significant difference in any clone in anaerobe environment biofilm production and esterase activity (P > 0.05).

4. Discussion

This study compared genotypes and virulence factors of *Candida* spp. isolated from healthy individuals and patients with type 2 DM.

In the present study, the most frequently isolated species was *Candida albicans*. There were statistically significant differences in terms of isolated *Candida* frequency between healthy subjects and diabetic patients. There was no difference between the groups in terms of virulence factors. Twelve genotypes (A to L) for strains of *Candida albicans* were determined. Genotypes G and L did not show any virulence activity. In addition, in anaerobe biofilm production and esterase activity, there was no significant difference between genotypes, but in biofilm aerobe, phospholipase, and proteinase activity there were differences between genotypes.

Few studies have investigated genotypes and virulence factors of oral isolates of patients with DM, and authors have not always examined more than one or two virulence attributes at a time (17,18,29).

Candida spp. are capable of colonizing the hard surface of the teeth, invading the dentinal tubules, participating in the formation of microbial biofilm, and producing large amounts of acids that are responsible for demineralization of tooth enamel and dissolution of hydroxyapatite (30–32). *C. albicans* has been isolated from dentine caries lesions in children with high frequencies ranging from 71% to 97% (21). Furthermore, Beighton et al. (33) reported the relatively high prevalence of *Candida* spp. (58.5%) in 82 root caries lesions. However, the predominant oral habitats of *C. albicans* are carious lesions, from which this fungus has been isolated with high frequencies (up to 97%) (34). In the present study, in all study groups, *Candida* was isolated at a rate of 40% of individuals with caries and at a rate of 27.8% of individuals without caries. Also, in our study, the prevalence of *Candida* spp. was higher in healthy individuals with caries than in healthy individuals without caries, as other studies have indicated before. In diabetic patients, *Candida* prevalence in patients with and without caries was similar.

Diabetic patients exhibit high susceptibility to *Candida* species, mainly *C. albicans* and other fungal infections (17). A literature review presented rates of oral carriage of *Candida* in diabetic patients between 18 and 80% (35). In this study, in all study groups, *Candida* was isolated at a rate of 43.8% of diabetic patients and at a rate of 28.2% of healthy individuals. In previous studies, it was shown

that *C. albicans* was the most common pathogen, being isolated intraorally in 17%–75% of healthy individuals and more than 80% of diabetic patients (2,17). In our study, we found these percentages to be 95% in healthy individuals and 84.3% in diabetic patients.

Yeast growth and adhesion may be enhanced by high glucose concentration in the blood and saliva, which can serve as nutrients for *Candida* organisms. The reduced *Candida* killing capacity by neutrophils in the presence of high glucose concentrations may also account for any increased colonization by *Candida* (5). Furthermore, in diabetic patients, poor glycemic control is a critical factor that promotes oral yeast colonization in these patients (35). In our study, the frequency of *Candida* was higher in poorly controlled diabetic patients than in regular diabetic patients. These differences were not statistically significant, but it was concluded that disruption of diabetes regulation may lead to the tendency for *Candida* infection to increase.

Because *Candida* is most intensely colonized in the tongue compared with the rest of the oral mucosa, *Candida* is often isolated from there (1). In almost one-third of dentate carriers, the tongue is the only positive oral site. Therefore, the specimen collection method from the dorsal tongue applied in this study was found to be a simple, fast, and convenient method for isolating *Candida* spp. in the oral cavity.

Several virulence factors of *Candida* have been discovered, including biofilm formation, phenotypic diversity, and production of extracellular enzymes, such as phospholipase, proteinase, and esterase (17).

Biofilm formation is a major virulence factor in the pathogenicity of *Candida* (36). The biofilm consists of cells that are in relationships with surface, yeast, and filaments, which are surrounded by an extracellular matrix. Biofilms are the reservoir of the infections; they cause the spread of the infections (36,37). In this study, biofilm formation was observed in all groups, but there were no statistically significant differences between the biofilm production of groups in both aerobic and anaerobic conditions.

Enzymatic activity may play an essential role in the capacity of *Candida* to establish itself as a colonizing and/or infectious microorganism. Proteinase production is important for increasing the ability of certain organisms to colonize and penetrate the host tissue and destroy the host immune system, breaking a significant number of important proteins for immunity (38). Lipolytic enzymes, such as phospholipases and esterases, are associated in *Candida* infections with host cell penetration, adhesion to epithelial cells, invasion of reconstituted human oral epithelium, and even host signal transduction pathways (39). Previous studies have confirmed that high phospholipase production is linked to a higher degree of pathogenicity (40,41). Different frequencies of enzymatic

activity have been reported in *Candida* spp. isolated from different anatomic sites. For example, Oksuz et al. (42) found phospholipase activity to be higher in isolates from oral (59%) and fecal samples (42.8%), and protease activity in isolates from urogenital (55.1%) and skin samples (58.8%). In addition, Manfredi et al. (18) showed that the analysis of proteinase production for *Candida* isolates from diabetic patients and isolates from nondiabetic subjects did not reveal any significant differences between the two groups. In this study, we determined enzymatic activities such as that of proteinases, esterase, and phospholipases of *Candida* species isolated from DM and healthy groups, but there were no significant differences between groups.

In the groups taking part in this research, the genetic proximities of *C. albicans* were detected. There were 12 main clones, and subclones were determined from 6 of these main clones to be isolated *C. albicans*. In the healthy group without caries, there were 3 clones (A, B, C); in the healthy group with caries, there were 4 clones (D, E, F, G); in the DM group with caries, there were 2 clones (H, I); and in the DM group without caries, there were 3 clones (J, K, L). However, Boriollo et al. (17) showed that the genetic diversity observed in the total yeast population revealed 4 large, genetically distinct groups (A to D).

The virulence of distinct genotypes of *Candida* spp. may be different (16). In the present study, clones G and L did not show any virulence activity. Furthermore, in this study, in the aerobic environment, in aerobic biofilm production there was a statistically significant difference only between two clones. In anaerobic biofilm production and esterase activity, there was no statistically significant

difference, but in phospholipase and proteinase activity there was a statistically significant difference between clones. In a previous study that included genetic and phenotypic evaluation of *C. albicans* strains isolated from the subgingival biofilm of diabetic patients with chronic periodontitis, it was found that genotype A expressed higher phospholipase activity than genotype B (16). Moreover, the authors emphasized that genotype A was more virulent than genotype B. In another previous study, it was reported that *C. albicans* genotype B showed a significant increase in phospholipase and proteinase activity when compared to genotypes A or C (43). In 2012, İnci et al. (9) investigated some virulence factors in *Candida* species isolated from patients with suspected invasive fungal infection (isolated from blood, bronchoalveolar lavage, tissue sample, and peritoneal, pleural, and cerebrospinal fluid) and sought to identify their relationships with *Candida* genotypes. They found no difference in virulence factors (biofilm, phospholipase, esterase, proteinase) evaluated among the *C. albicans* genotypes. De Cassia Mardegan et al. (44) analyzed *C. albicans* oral strains from caries-free and caries-active healthy children and suggested that the enzymatic profile does not depend on the genotypic characteristics of the isolates.

In conclusion, our results show that diabetes and caries have no effects on activities of virulence factors of *Candida* spp. isolated from oral cavities. Different genotypes of *C. albicans* showed different virulence activities. However, to confirm our results, more patients in each group and more advanced studies are needed.

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