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MLEE Typing of Potentially Virulent *Candida albicans* and *Candida dubliniensis* Isolated from Diabetic Patients

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Abstract

The incidence of *Candida* species, the genetic diversity and the hydrolytic exoenzyme characteristics of *C. albicans* and *C. dubliniensis* clinical isolates from patients diagnosed with diabetes mellitus under glycemic clinical control were assessed. Clinical samples were collected from the oral cavity and the isolation and identification of *Candida* species were performed by microbiological methods. The genetic diversity of clinical isolates was established using isoenzyme markers (MLEE), Nei's statistics, and clustering analysis. The virulence of oral isolates was evaluated by the in vitro production tests of aspartyl proteinases (SAPs) and phospholipases (PLs). The oral colonization incidence by the *Candida* species went by the order of 83.3%. A high prevalence of the *C. albicans* (70.4%) among the species found (*C. tropicalis, C. krusei, C. dubliniensis and Candida sp.*). A total of 84 electrophoretic types (ETs; 43.1%) was observed in the population of clinical isolates. Genetic relationship analyses showed 18 clusters (I to XVIII) and 7 taxa (A to G). The SAPs expressions was detected in all of the clinical isolates, while the PLs expressions occurred in the majority one (*C. albicans*: 92.6%; *C. dubliniensis*: 66.7%). The results showed a high prevalence of the *Candida* species, particularly the genetically diversified and potentially virulent *C. albicans* strains, in the oral cavity of diabetic patients with glucose level controlled and without oral candidiasis clinical manifestations.

Keywords: *Candida* species; Diabetes mellitus; Oral cavity; Genetic diversity; Virulence

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Introduction

Diabetes mellitus is a disease caused by metabolic disorders characterized by a reduction in partial or total of insulin production by the pancreas. It can be autoimmune with the destruction of the beta cells of pancreas responsible for the insulin synthetization or by an insufficient insulin production. As of result, the blood sugar level rises, condition known as hyperglycemia, which is harmful to the diabetic individual, especially in long-term patients since it can cause alterations in the organism [1]. The classic symptoms of the disease are polydipsia (excessive thirst), polyuria (excessive secretion of urine) and polyphagia (intense hunger), being the result of the hyperglycemia and the osmotic imbalance. The two most common types are known as type 1 diabetes mellitus and type 2 diabetes mellitus, the other forms less common are the gestational diabetes, diabetes induced by medication (corticosteroids), pancreas disease (cystic fibrosis), infection (congenital rubella) and genetic syndromes [2].

Diabetes mellitus is considered as a predisposing factor for candidiasis disease [3]. The blood sugar level in diabetic patients contributes to the growth of yeast due to the higher number of receptors available to the *Candida* [4]. There is also a decrease in the defensive capability of polymorph nuclear neutrophils and lymphocyte T associated with the hyperglycemia, which provides a favorable environment for the reproduction of the *Candida* species [5]. The correlation between diabetes and infection by the *Candida* species has been investigate in the literature,

mostly because the diabetic individuals are more susceptible to fungal infections in comparison to those without diabetes. In the colonization process and following infection, the yeast adherence to the surface of epithelial cells is recognized as an essential factor [6]. High blood and saliva sugar levels were found in diabetic patients with and without glycemic control [7,8] and, consequently, can contribute to a greater susceptibility of the host to oral infections, like the candidiasis [9]. Fungal infections are common in diabetic patients, being the candidiasis described as part of the primary infectious pathology: oral and esophageal candidiasis, pyelonephritis or cystitis [10]. The yeast and fungus are frequently cited as responsible for the severe conditions that affect the cervical ad facial areas of patients that suffer from immunological and diabetic disorders [11]. Nevertheless, the opportunistic fungal pathogens most common are the *Candida* species, with the *C. albicans* being responsible for more than 50% of the cases [12]. In addition, the risk of infection by the *Candida* species passed down to diabetic patients has a multifactorial cause. Apart from the diabetic condition, the excessive consume of sugar, bad eating habits or poor oral hygiene are factors that contribute to high saliva sugar levels, which are favorable to developing oral candidiasis. In the context, there is a direct link between glucose concentration and candidiasis [13].

The isoenzymatic typing, also known as multilocus enzyme electrophoresis (MLEE), is considered an excellent method of fingerprinting in epidemiological tracing involving microorganisms of medical interest, especially in cases of fungal infections caused by the *Candida* species. Combined with the genetic interpretation of the data, in accordance with the ploidy level of the microorganism, and systems of analysis of diversity and genetic relationship, MLEE has provided information in various levels of resolution (identification of the same strain in independent isolates, identification of microevolutionary changes in a strain, clustering of moderately related isolates, and identification of completely unrelated isolates), as well as your high reproducibility and discriminatory power [14-22].

The present study evaluated the incidence of *Candida* species in isolated from the oral cavity of patients presenting a diagnosis of diabetes mellitus and under clinical control, without clinical symptoms of oral candidoses. It also investigated the virulence potential of *C. albicans* and *C. dubliniensis* clinical isolates (using hydrolytic exoenzymes: SAPs and PLs), and the genetic polymorphism and relatedness of the *C. albicans* and *C. dubliniensis* clinical isolates, based on MLEE typing and genetic and cluster analyses.

Materials and Methods

Patients

Fifty-four patients (19 females and 35 males) presenting a diagnosis of diabetes mellitus and under clinical control, aged between 41 and 79 years (mean of 60 ± 10 years), coming from the Health Care Units of the Family Health Program (Ministry of Health, Brazil), Limeira city, São Paulo state, Brazil, were selected for the present study. The following parameters were used as inclusion and exclusion criteria: (i) patients aged 18 years and older, without any antifungal and antibiotic medications from 10 weeks prior to collection, (ii) non-pregnant women, and (iii) non-xerostomic patients, that is, patients should produce 3 to 5 mL of unstimulated saliva in 15 minutes [18]. This research was conducted in accordance with Resolution No. 466/2012 of the National Health Council and approved by the Research Ethics Committee of the FOP/UNICAMP.

Sampling and yeasts

Oral samples were harvested in the presence of a physician (10 ml sterile phosphate-buffered saline: 0.1 M NaCl, 0.1 M NaH_2PO_4, pH-7.2), properly transported (4°C) to the Laboratory of Oral Microbiology and Immunology, School of Dentistry of Piracicaba, State University of Campinas (FOP/UNICAMP), centrifuged (1700 × g for 10 min) and the sediments were resuspended in 1 mL of PBS solution, according to previously reported [18,23]. Aliquots of 100 l of each sample were inoculated onto plates containing the differential culture medium CHROMagar *Candida*® (Probac do Brasil Produtos Bacteriológicos Ltda., São Paulo, SP, Brazil) and aerobically incubated at 37°C for 48 h, using a duplicate isolation system [18,24]. The isolation and presumptive identification of some clinically important *Candida* species, as well as the count (cfu/ml) and identification of populational homogeneity (mixed yeast cultures) [22], were carried out based on colonial morphology: *C. albicans* (distinct green colonies), *C. tropicalis* (distinct dark grayish blue colonies with a dark brownish purple halo in the surrounding agar) and *C. krusei* (highly rugous characteristic, scattered colonies with pale pink centers and a white border). The *C. albicans* and *C. dubliniensis* species were confirmed by growth test at 42-45°C [25] and abundant chlamydospore production [26]. Based on the oral multicolonization patterns by *C. albicans* strains observed in several immunocompetent and immunocompromised subjects [15,18,22], the genotypes of several yeast isolates per subject (oral cavity) were analyzed using isoenzymatic markers.

Multilocus Enzyme Electrophoresis (MLEE)

Enzymes extraction procedures from freshly grown yeast cells, enzyme electrophoresis and revelation/stain, genetic interpretation of the MLEE patterns, and discriminatory power of the MLEE typing, based on the numerical index of discrimination (D)-Simpson's diversity index were accomplished using previously described methods [17-19]. The enzymatic activities analyzed were: alcohol dehydrogenase (ADH-EC 1.1.1.1), sorbitol dehydrogenase (SDH-EC 1.1.1.14), mannitol 1-phosphate dehydrogenase (M1P-EC 1.1.1.17), malate dehydrogenase (MDH-EC 1.1. 1.37), isocitrate dehydrogenase (IDH-EC 1.1.1.42), glucose dehydrogenase (GDH-EC 1.1.1.47), glucose-6-phosphate dehydrogenase (G6PDH-EC 1.1.1.49), aspartate dehydrogenase (ASD-EC 1.4.3. x), catalase (CAT-EC 1.11.1.6), peroxidase (PO-EC 1.11.1.7) and leucine aminopeptidase (LAP-EC 3.4.1.1).

Genetic and cluster analyses

Nei's statistic was used to estimate the genetic distance among

all isolates (strains) of *C. albicans* and *C. dubliniensis* [27]. Based on the matrix dij, a dendrogram was generated by the SAHN clustering method and the UPGMA algorithm. A threshold (average value:) was established to identify clusters constituted of identical and/or highly related isolates (strains) ($0 \leq dij \leq d_{ij}$), and major taxa (singular taxon, i.e., a taxonomic group of any nature or rank) constituted of moderately related ($d_{ij} < dij \leq \sum d_{ij} \pm SD$) or distantly related ($dij > d_{ij} \pm SD$) isolates, strains and/or clusters. Pearson's product-moment correlation coefficient was used to measure agreement (range of 0-1 to +1), or cophenetic correlation, between the elements dij of the genetic distance matrix and the elements dij implicit in the UPGMA dendrogram. Concordance was interpreted in the following manner: $0.9 \leq r$: very good fit; $0.8 \leq r < 0.9$: good fit; $0.7 \leq r < 0.8$: weak concordance; $r < 0.7$: very weak concordance. These analyses were performed using the program NTSYSpc v2.1. [18,19,22,27]. The percentage index of polymorphic loci (i.e., frequency of the most common allele<0.99 or 99%), the average number of alleles per locus, the average number of polymorphic alleles per locus, the number of heterozygous and homozygous alleles, and the heterozygosity of each locus were also determined as measures of diversity [28].

Virulence test

The virulence in vitro of the *C. albicans* and *C. dubliniensis* clinical isolates was determined by testing for production of hydrolytic exoenzymes, Secreted Aspartyl Proteinases (SAPs) and phospholipases (PLs), according to previously described methods [18,24,29]. Enzymatic activity (Pz) was determined by formation of a halo around the yeast colony and the results were interpreted as follows: (i) Pz=1: absence of enzyme activity (index 0); (ii) $1 > Pz \geq 0.64$: positive enzyme activity (index 1); and (iii) $Pz < 0.64$: strongly positive enzyme activity (index 2). These tests were carried out in duplicate.

Result

Oral colonization by *Candida* species

Fifty-four patients diagnosed with diabetes mellitus were analyzed for oral colonization by *Candida* species. Isolation and identification assays showed a high prevalence of *C. albicans*. *C. krusei*, *C. tropicalis*, and *Candida* sp. were only preliminarily identified using the chromogenic medium, and the identity of *C. albicans* and *C. dubliniensis* was confirmed by complementary methods. Therefore, the use of phenotypic or genotypic systems for species-level is necessary for most *Candida* species.

Oral colonization by *Candida* species was observed in 45 (83.3%) of 54 diabetic patients. Of these 45 patients with *Candida* colonization, 38 (70.4%) were colonized by *C. albicans* [i.e., 27 (50%; patients 1-13, 15-17, 19-21, 23-27, 31, 35 and 36) were exclusively colonized by *C. albicans* and 11 (20.4%) were multicolonized by *C. albicans* and *C. dubliniensis* (1 patient: 1.9%; patient 33), *C. albicans* and *C. krusei* (3 patients: 5.6%; patients 22, 28 and 30), *C. albicans* and *C. tropicalis* (2 patient: 3.7%; 14 and 18), *C. albicans* and *Candida* sp. (1 patient: 1.9%; 38), *C. albicans*, *C. krusei* and *Candida* sp. (1 patient: 1.9%; 37), *C. albicans*, *C. krusei* and *Candida* sp. (2 patients: 3.7%; patients 29 and 32) and *C. albicans*, *C. tropicalis* and *C. krusei* (1 patient: 1.9%; patient 34)] and 7 (12.9%) were colonized by *Candida non-albicans* [i.e., multicolonized by *C. krusei* and *Candida* sp. (3 patients), *C. tropicalis* and *Candida* sp. (2 patients) or *Candida* sp. (2 patients)] (Table 1).

Colonization profile	Diabetic patients ♀				Diabetic patients ♀				∑	
	CFU/ml		∑		CFU/ml		∑			
37	>350	<350	n	%	>350	<350	n	%	n	%
Candida non-albicans	1	4	5	16.6	-	2	2	8.3	5	9.3
Absence of colonization by *Candida* sp.	-	-	4	13.4	-	-	5	20.9	11	20.4
∑	10	16	30	100	6	13	24	100	54	100
Abbreviation: CFU: Colony-Forming Unit. Symbols ♂ and ♀ correspond to the male and female gender, respectively.										

Table 1: Oral occurrence of *Candida* species in patients presenting a diagnosis of diabetes mellitus and under clinical control, without clinical symptoms of oral candidoses.

Of the female patients, 26 (86.6%) of 30 showed oral colonization by *Candida* species. Of these 26 female patients with *Candida* colonization, 21 (70%) were colonized by *C. albicans* [i.e., 14 (46.7%; patients 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 and 31) were exclusively colonized by *C. albicans* and 7 (23.3%) were multicolonized by (i) *C. albicans* and *C. dubliniensis* (1 patient: 3.3%; patient 33), (ii) *C. albicans* and *C. krusei* (2 patients: 6.7%; patients 28 and 30), (iii) *C. albicans* and *C. tropicalis* (1 patient: 3.3%; patient 14), (iv) *C. albicans*, *C. krusei* and *Candida* sp. (2 patients: 6.7%; patients 29 and 32), and (v) *C. albicans*, *C. tropicalis* and *C. krusei* (1 patient: 3.3%; patient 34)] and 5 (16.6%) were colonized by *Candida non-albicans* [i.e., multicolonized by *C. krusei* and *Candida* sp. (2 patients), *C. tropicalis* and *Candida* sp. (2 patients) or *Candida* sp. (1 patient)] (Table 1).

Of the male patients, 19 (79.2%) of 24 showed oral colonization by *Candida* species. Of these 19 male patients with *Candida* colonization, 17 (70.8%) were colonized by *C. albicans* [i.e., 13 (54.1%; patients 15, 16, 17, 19, 20, 21, 23, 24, 25, 26, 27, 35 and 36) were exclusively colonized by *C. albicans* and 4 (16.7%) were multicolonized by (i) *C. albicans* and *C. krusei* (1 patient: 4.2%; patient 22), (ii) *C. albicans* and *Candida* sp. (1 patient: 4.2%; 38), (iii) *C. albicans* and *C. tropicalis* (1 patient: 4.2%; 18), and (iv) *C. albicans*, *C. krusei* and *Candida* sp. (1 patient: 4.2%; 37)] and 2 (8.3%) were colonized by *Candida non-albicans* [i.e.,

multicolonized by *C. krusei* and *Candida* sp. (1 patient) or *Candida* sp. (1 patient)]. Oral occurrence of *Candida* species among patient genders (male and female) presenting a diagnosis of diabetes mellitus was not significant (Teste χ^2, p<0.001) (Table 1).

Genetic diversity analysis

The izoenzymatics electrophoretic patterns of *C. albicans* (n=189) and *C. dubliniensis* (n=6) isolated from the oral cavity of 38 diabetic patients, including the *C. albicans* CBS-562 type-strain, were reproducible on different gels after three repetitions of each electrophoretic procedure. The genetic interpretation of MLEE patterns showed the following characteristics (Table 2) 13 (86.7%) enzymatic loci were polymorphic (i.e., for each enzyme locus the frequency of the most common allele was <99%) for two, three, four, five and six alleles (2 alleles: Mdh-1; 3 alleles: Cat, Gdh and Mdh-2; 4 alleles: G6pdh, Idh, Mdh-3 and Po; 5 alleles: Asd, Lap and M1p-2; 6 alleles: Adh and Sdh-1). The mean number of alleles per locus and the mean number of alleles per polymorphic locus were equals to 3.73 and 4.15, respectively. The combination of the existing alleles in 15 enzymatic loci showed 84 electrophoretic types-ETs (43.1% of the total isolates). Heterozygotes revealed two and three enzymatic bands (2 bands: Adh, Asd, Cat, G6pdh, Gdh, Idh, Lap, M1p-2, Mdh-1, Mdh-2, Mdh-3, Po and Sdh-Mdh-2). Among the homozygotes, one allele was observed in the M1p-1, Sdh-2 loci, two alleles at the Idh, Mdh-1 and Po loci, three alleles at the Asd, Cat, Gdh and Mdh-2 loci, four alleles at the loci G6pdh, Lap, M1p-2 and Mdh-3, and five alleles at the Adh and Sdh-1 loci.

In addition, monoclonal and polyclonal oral colonization patterns were found in diabetic patients based on genetic interpretation of the MLEE patterns of *C. albicans* and *C. dubliniensis* (1 isolate per ET: ET5, ET10, ET21, ET23, ET27, ET28, ET29, ET36, ET37, ET38, ET39, ET43 and ET44; 2 isolates per ET: ET2, ET3, ET4, ET7, ET13, ET16, ET18, ET19, ET26, ET32, ET34, ET35, ET40 and ET42; 3 isolates per ET: ET1, ET6, ET14, ET15, ET17, ET20 and ET31; 4 isolates per ET: ET11, ET22 and ET24; 5 isolates per ET: ET8, ET9, ET12, ET25, ET30, ET33 and ET41) (Table 2). Thirteen diabetic patients (8 women and 5 men) exhibited exclusively monoclonal pattern of oral colonization by *C. albicans*, whereas 25 patients (15 women and 10 men) exhibited monoclonal and polyclonal patterns of oral colonization by *C. albicans*. Monoclonal and polyclonal patterns of oral colonization by *C. dubliniensis* was observed only in a female diabetic patient (♀33).

Nei's statistic dij, the SAHN clustering method, and the UPGMA algorithm were used to evaluate the genetic diversity of the 189 *C. albicans* and 6 *C. dubliniensis* clinical isolates, including the *C. albicans* CBS562 type strain, isolated from the oral cavity of diabetic patients (both genders) under clinical control and without clinical symptoms of oral candidoses. The population diversity of yeasts ranged from zero to 0.3683, that is, on average, the population of clinical isolates showed from zero to 36.8 allelic substitutions for every 100 loci from a common ancestor (d_{ij} =0.049 ± 0.077).

Taking into account the genetic distance values established among the clinical isolates, 18 clusters (I to XVIII) and 7 taxa (A to G) were identified. Each cluster comprised identical (dij=0) and/or highly related (0.049 ≥ dij>0) isolates (strains), regardless of whether these isolates were epidemiologically related or not. Moderately related (0.126 ≥ dij>0.049; intra-taxon) and distantly related (dij>0.126; inter-taxa) isolates (strains) and clusters were also identified. In turn, the taxa (inter-taxa) were considered unrelated or distantly related (dij>0.126), and each taxon comprised their respective clinical isolates and/or clusters. The composition of the taxa/clusters exhibited the following characteristics:

- Taxon A: 8 clusters (44.4%), 146 isolates (74.8%), 60 ETs (71.4%), 30 patients (55.5%).
- Cluster I (71 isolates 36.4%, 29 ETs 34.5%; 17 patients 31.4%): Patients ♀1 (ET1 and ET2), ♀2 (ET3, ET4 and ET5), ♀6 (ET11), ♀12 (ET22), ♀13 (ET23 and ET24), ♀14 (ET25), ♀15 (ET26, ET27, ET28 and ET29), ♀17 (ET31 and ET32), ♀23 (ET46 and ET47), ♀24 (ET48 and ET50), ♀25 (ET51), ♀30 (ET63), ♀31 (ET64), ♀34 (ET75), ♀35 (ET76 and ET78), ♀36 (ET80 and ET81) and ♀37 (ET83).
- Cluster II (3 isolates 1.5%; 2 ETs 2.4%; 1 patient 1.8%): Patient ♀22 (ET42 and ET43).
- Cluster III (14 isolates 7.2%; 6 ETs 7.1%; 4 patients 7.4%): Patients ♀6 (ET10), ♀26 (ET52), ♀29 (ET58) and ♀30 (ET60, ET61 and ET62).
- Cluster IV (5 isolates 2.6%; 4 ETs 4.8%; 2 patients 3.7%): Patients ♀22 (ET44 and ET45) and ♀31 (ET65 and ET66).
- Cluster V (20 isolates 10.3%; 6 ETs 7.1%; 5 patients 9.2%): Patients ♀8 (ET13), ♀16 (ET30), ♀18 (ET33), ♀19 (ET35 and ET36) and ♀38 (ET84).
- Cluster VI (15 isolates 7.7%; 5 ETs 6%; 4 patients 7.4%): Patients ♀8 (ET14), ♀10 (ET17 and ET18), ♀19 (ET34) and ♀32 (ET67).
- Cluster VII (13 isolates 6.7%; 4 ETs 4.8%; 3 patients 5.5%): Patients ♀4 (ET8), ♀9 (ET15) and ♀11 (ET19 and ET20).
- Cluster VIII (2 isolates 1%; 1 ET 1.2%; 1 patients 1.8%): Patient ♀36 (ET79).
- Taxon B: 1 cluster (5.5%), 2 isolates (1.0%), 1 ETs (1.2%), 1 patient (1.8%).
- Cluster IX (2 isolates 1%; 1 ET 1.2%; 1 patient 1.8%): Patient ♀9 (ET16).
- Taxon C: 1 cluster (5.5%), 3 isolates (1.5%), 2 ETs (2.4%), 1 patient (1.8%).
- Cluster X (2 isolates 1%; 1 ET 1.2%; 1 patient 1.8%): Patient ♀20 (ET40).
- ET38 (1 isolate 0.5%; 1 ET 1.2%; 1 patient 1.8%): Patient ♀20 (ET38)
- Taxon D: 4 clusters (22.2%), 19 isolates (9.7%), 9 ETs (10.7%), 3 patients (5.5%).
- Cluster XI (5 isolates 2.6%; 2 ETs 2.4%; 1 patient 1.8%): Patient ♀3 (ET6 and ET7).
- Cluster XII (6 isolates 3.1%, being 4 *C. dubliniensis**; 3 ETs 3.6%; 1 patient 1.8%): Patient ♀33 (ET68*, ET71* and ET74*).

- Cluster XIII (6 isolates [3.1%], being 1 *C. dubliniensis*[*]; 2 ETs [2.4%]; 2 patients [3.7%]): Patients ♀5 (ET9) and ♀33 (ET70[*]).
- Cluster XIV (2 isolates [1%], being 1 *C. dubliniensis*[*]; 2 ETs [2.4%]; 1 patient [1.8%]): Patient ♀33 (ET69[*] and ET73).
- Taxon E: 2 clusters (11.1%), 10 isolates (5.1%), 2 ETs (2.4%%), 2 patients (3.7%).
- Cluster XV (5 isolates [2.6%]; 1 ET [1.2%]; 1 patient [1.8%]): Patient ♀7 (ET12).
- Cluster XVI (5 isolates [2.6%]; 1 ET [1.2%]; 1 patient [1.8%]): Patient ♀21 (ET41).
- Taxon F: 1 cluster (5.5%), 7 isolates (3.6%), 4 ETs (4.7%), 2 patients (3.7%).
- Cluster XVII (5 isolates 2.6%; 2 ETs 2.4%; 1 patient 1.8%): Patient ♀27 (ET53 and ET54).
- ET37 (1 isolate [0.5%]; 1 ET [1.2%]; 1 patient [1.8%]): Patient ♀20 (ET37).
- ET39 (1 isolate [0.5%]; 1 ET [1.2%]; 1 patient [1.8%]): Patient ♀20 (ET39).
- Taxon G: 1 cluster (5.5%), 5 isolates (2.5%), 3 ETs (3.6%), 1 patient (1.8%).
- Cluster XVIII (4 isolates [2%]; 2 ETs [2.4%]; 1 patient [1.8%]): Patient ♀28 (ET56 and ET57).
- ET55 (1 isolate [0.5%]; 1 ET [1.2%]; 1 patient [1.8%]): Patient ♀28 (ET55).
- Ungrouped isolates/ETs in taxa: 3 isolates (1.5%), 3 ETs (3.6%), 3 patients (5.5%).
- ET49 (1 isolate [0.5%]; 1 ET [1.2%]; 1 patient [1.8%]): Patient ♀28 (ET49).
- ET72 (1 isolate [0.5%]; 1 ET [1.2%]; 1 patient [1.8%]): Patient ♀33 (ET72).
- ET77 (1 isolate [0.5%]; 1 ET [1.2%]; 1 patient [1.8%]): Patient ♀35 (ET77).

P	ET	NI	Cluster*	Alleles of 15 enzymatic loci**														
				Adh	Asd	Cat	G6pdh	Gdh	Idh	Lap	M1p-1	M1p-2	Mdh-1	Mdh-2	Mdh-3	Po	Sdh-1	Sdh-2
	TS			bd	cc	aa	cc	bb	bd	cc	-	bb	-	aa	aa	bb	bd	-
♀1	1	3	I	dd	ac	aa	cc	bb	bd	cc	-	bb	-	ac	aa	bc	bd	-
♀1	2	2	I	dd	ac	aa	cc	bb	bd	cc	-	bb	-	ac	aa	bb	bd	-
♀2	3	2	I	dd	cc	bb	cc	bb	bb	cc	-	bb	bb	aa	ad	bc	bd	-
♀2	4	2	I	dd	cc	bb	bb	bb	bb	cc	-	bb	bb	aa	ad	bc	bd	-
♀2	5	1	I	dd	cc	bb	cc	bb	bb	cc	-	bb	bb	aa	ad	bb	bd	-
♀3	6	3	XI	aa	cc	cc	ac	bb	bb	bb	-	bb	aa	aa	aa	bb	bd	-
♀3	7	2	XI	aa	bb	cc	ac	ac	bb	bb	-	bb	aa	aa	aa	bb	ce	-
♀4	8	5	VII	dd	ac	aa	cc	bb	bd	cc	-	bb	-	cc	ac	bb	dd	-
♀5	9	5	XIII	aa	cc	cc	cc	bb	bb	bb	-	bb	aa	aa	aa	-	bd	-
♀6	10	1	III	bd	cc	aa	cc	bb	-	cc	-	bb	-	aa	aa	bb	bb	-
♀6	11	4	I	bd	cc	aa	cc	bb	bb	cc	-	bb	-	aa	aa	bb	bb	-
♀7	12	5	XV	dd	cc	aa	cc	-	-	ee	-	-	bb	-	aa	bb	-	-
♀8	13	2	V	dd	cc	-	cc	bb	-	cc	-	be	aa	aa	ac	bb	bd	-
♀8	14	3	VI	dd	ac	-	cc	bb	-	cc	-	be	aa	aa	ac	bb	bd	-
♀9	15	3	VII	dd	ac	aa	cc	bb	bd	cc	-	bb	-	cc	ac	bb	dd	-
♀9	16	2	IX	ff	be	bb	aa	aa	ac	dd	-	cc	-	ad	ac	bb	aa	-
♀10	17	3	VI	dd	ac	-	cc	bb	-	cc	-	be	aa	aa	ac	bb	bd	-
♀10	18	2	VI	dd	ac	-	cc	bb	bb	cc	-	be	aa	aa	ac	bb	bd	-
♀11	19	2	VII	dd	ac	aa	cc	bb	bd	cc	-	bb	-	cc	ac	bb	de	-
♀11	20	3	VII	dd	ac	aa	cc	bb	bd	cc	-	bb	-	cc	ac	bc	de	-
♀12	21	1	Isolate	dd	cc	aa	cc	-	bd	cc	-	bb	aa	aa	-	bc	bd	-
♀12	22	4	I	dd	cc	aa	cc	bb	bd	cc	-	bb	aa	aa	aa	bc	bd	-
♀13	23	1	I	dd	cc	aa	cc	bb	bb	cc	-	bb	-	aa	aa	bd	bd	-
♀13	24	4	I	dd	cc	aa	cc	bb	bb	cc	-	bb	-	aa	ac	bd	bd	-
♀14	25	5	I	dd	cc	aa	cc	bb	bb	cc	-	bb	-	aa	ad	bd	bd	-
♀15	26	2	I	dd	cc	aa	cc	bb	bb	cc	-	bb	-	aa	ad	bd	bd	-
♀15	27	1	I	dd	dd	aa	cc	bb	bb	cc	-	bb	-	aa	ad	bd	bd	-
♀15	28	1	I	dd	cc	aa	dd	aa	bb	cc	-	aa	-	ac	ad	bd	cc	-
♀15	29	1	I	cc	cc	aa	dd	aa	bb	cc	-	aa	-	ac	ad	bd	cc	-
♀16	30	5	V	dd	cc	-	cc	bb	bb	cc	-	bb	aa	aa	cc	bb	bd	-
♀17	31	3	I	dd	cc	aa	cc	bb	bb	cc	-	bb	-	aa	ad	bd	bd	-
♀17	32	2	I	cc	cc	aa	cc	bb	bb	cc	-	bb	-	aa	ad	bd	bd	-
♀18	33	5	V	dd	cc	-	cc	bb	-	cc	-	bb	aa	aa	cc	bb	bd	-
♀19	34	2	VI	dd	ac	-	cc	bb	bb	cc	-	be	aa	aa	ac	bb	bd	-

♀19	35	2	V	dd	cc	-	cc	bb	bb	cc	-	be	aa	aa	ac	bd	bd	-
♀19	36	1	V	cc	cc	-	cc	bb	bb	cc	-	be	aa	aa	ac	bd	bd	-
♀20	37	1	Isolate	ab	-	aa	cc	-	-	bb	aa	bb	-	ab	aa	-	bd	-
♀20	38	1	Isolate	ab	-	aa	cc	-	bb	bb	-	bb	-	ab	aa	cc	bd	-
♀20	39	1	Isolate	bb	-	aa	cc	-	-	bb	-	bb	-	aa	-	-	bd	-
♀20	40	2	X	ab	be	aa	cd	bb	aa	bb	-	bb	-	ab	aa	cc	bd	-
♀21	41	5	XVI	cc	cc	aa	cc	-	-	ee	-	-	ab	-	aa	bb	cc	-
♀22	42	2	II	dd	cc	bb	cc	bb	bb	cc	-	-	-	bb	-	bc	bd	-
♀22	43	1	II	dd	cc	bb	cc	bb	bb	cc	-	-	-	bb	-	bb	bd	-
♀22	44	1	IV	dd	cc	bb	cc	bb	bb	cc	-	-	-	aa	dd	bb	bd	-
♀22	45	1	IV	dd	cc	bb	cc	bb	bb	cc	-	bb	-	aa	dd	bb	bd	-
♀23	46	4	I	dd	cc	bb	cc	bb	bb	cc	-	bb	-	aa	dd	bb	bd	-
♀23	47	1	I	dd	cc	aa	cc	bb	bb	cd	-	bb	-	aa	aa	bd	bd	-
♀24	48	1	I	dd	cc	aa	cc	bb	bb	cc	-	bb	-	aa	ac	bd	bd	-
♀24	49	1	Isolado	ce	be	aa	bb	cc	-	ae	-	dd	-	bb	bb	ad	ff	-
♀24	50	3	I	dd	cc	aa	cc	bb	bb	cc	-	bb	-	aa	ad	bd	bd	-
♀25	51	5	I	dd	cc	bb	cc	bb	bb	cc	-	bb	bb	aa	ad	bc	bb	-
♀26	52	5	III	dd	cc	aa	bb	bb	-	cc	-	bb	-	aa	-	bb	bb	-
♀27	53	4	XVII	ab	-	aa	cc	bb	-	bb	-	bb	-	ab	aa	-	bd	-
♀27	54	1	XVII	ab	-	aa	cc	bb	-	bb	-	bb	-	aa	aa	-	bd	-
♀28	55	1	Isolado	-	-	-	cc	bb	-	cc	-	bb	-	aa	ad	bb	-	-
♀28	56	3	XVIII	-	-	-	cc	bb	-	cc	-	bb	-	aa	ad	bb	bb	-
♀28	57	1	XVIII	-	-	-	cc	bb	-	cc	-	bb	-	aa	ad	bb	bd	-
♀29	58	4	III	bd	cc	aa	bb	bb	-	cc	-	bb	-	aa	-	bb	bb	-
♀29	59	1	Isolado	bd	cc	aa	bb	bb	-	cc	-	-	-	aa	-	bb	bb	-
♀30	60	1	III	bd	cc	aa	cc	bb	-	cc	-	bb	-	aa	-	bb	bd	-
♀30	61	2	III	dd	cc	aa	bb	bb	-	cc	-	bb	-	aa	-	bb	bb	-
♀30	62	1	III	bd	cc	aa	bb	bb	-	cc	-	bb	-	aa	-	bb	bb	-
♀30	63	1	I	dd	cc	ab	bb	bb	bb	cc	-	bb	-	aa	-	bb	bb	-
♀31	64	2	I	dd	cc	bb	cc	bb	bb	cc	-	bb	-	aa	ad	bb	bd	-
♀31	65	2	IV	dd	cc	bb	cc	bb	bb	cc	-	bb	-	aa	dd	bb	bd	-
♀31	66	1	IV	cd	cc	bb	cc	bb	bb	cc	-	bb	-	aa	dd	bb	bd	-
♀32	67	5	VI	dd	ac	-	cc	bb	bb	cc	-	be	aa	aa	ac	bb	bd	-
♀33	68***	4	XII	aa	cc	cc	cc	bb	bb	bb	-	bb	-	aa	aa	bb	bd	-
♀33	69****	1	XIV	aa	cc	cc	cc	bb	bb	bb	-	bb	-	aa	aa	bb	-	-
♀33	70****	1	XIII	aa	cc	cc	cc	bb	bb	bb	-	-	-	aa	aa	-	bd	-
♀33	71****	1	XII	aa	cc	cc	cc	bb	bb	bb	-	bb	-	aa	-	bb	bd	-
♀33	72	1	Isolado	aa	cc	-	cc	bb	bb	bb	-	-	-	-	-	bb	bd	-
♀33	73	1	XIV	aa	cc	cc	cc	bb	bb	bb	-	-	-	aa	aa	bb	-	-
♀33	74****	1	XII	aa	cc	cc	cc	bb	bb	bb	-	bb	-	aa	-	bb	bd	-
♀34	75	5	I	dd	cc	aa	dd	bb	bb	cc	-	bb	-	aa	ad	bb	bd	-
♀35	76	3	I	dd	cc	aa	cc	bb	bb	cc	-	bb	bb	aa	ac	bc	bd	-
♀35	77	1	Isolado	-	-	aa	cc	-	bb	cc	-	-	-	aa	ac	bb	-	-
♀35	78	1	I	dd	cc	aa	cc	bb	bb	cc	-	bb	bb	aa	-	bb	bd	-
♀36	79	2	XIV	dd	-	aa	cc	bb	bb	cc	-	bb	-	aa	ad	-	bd	aa
♀36	80	1	I	dd	cc	aa	cc	bb	bb	cc	-	bb	-	aa	-	bb	bd	-
♀36	81	1	I	dd	cc	aa	cc	bb	bb	cc	-	bb	-	aa	-	bb	bd	-
♀36	82	1	Isolado	dd	cc	aa	cc	bb	bb	cc	-	bb	-	aa	ad	-	bd	aa
♀37	83	5	I	dd	cc	aa	cc	bb	bb	cc	-	bb	-	aa	ad	bb	bd	-
♀38	84	5	V	dd	cc	-	cc	bb	bb	cc	-	bb	aa	aa	cc	bb	bd	-
37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37

Table 2: Allelic profiles of 84 electrophoretic types (ET; strains) of *C. albicans* and *C. dubliniensis* isolated from the oral cavity of patients presenting a diagnosis of diabetes mellitus and under clinical control, without clinical symptoms of oral candidoses.

CP	CI	ET	Pz	SAP	CP	CI	ET	Pz	SAP	CP	CI	ET	Pz	SAP
			PL					PL					PL	
♀1	A	ET1	1	1	♀9	A	ET15	2	2	♂17	A	ET31	1	2
	B	ET1	1	1		B	ET15	2	2		B	ET32	1	2
	C	ET1	1	1		C	ET15	2	2		C	ET31	1	2
	D	ET2	1	1		D	ET16	2	2		D	ET32	1	2
	E	ET2	1	2		E	ET16	2	2		E	ET31	2	2
♀2	A	ET3	2	1	♀10	A	ET17	2	2	♂18	A	ET33	2	1
	B	ET4	2	1		B	ET17	1	2		B	ET33	2	2
	C	ET3	2	2		C	ET17	1	2		C	ET33	2	1
	D	ET5	2	2		D	ET18	1	2		D	ET33	2	1
	E	ET4	2	2		E	ET18	2	2		E	ET33	2	2
♀3	A	ET6	1	2	♀11	A	ET19	1	2	♂19	A	ET34	2	1
	B	ET6	1	2		B	ET19	1	2		B	ET34	1	1
	C	ET6	0	1		C	ET20	1	2		C	ET35	2	2
	D	ET7	1	2		D	ET20	1	2		D	ET36	2	2
	E	ET7	2	2		E	ET20	2	2		E	ET35	2	2
♀4	A	ET8	2	2	♀12	A	ET21	2	2	♂20	A	ET37	1	2
	B	ET8	2	2		B	ET22	2	2		B	ET38	2	2
	C	ET8	1	2		C	ET22	2	2		C	ET39	1	2
	D	ET8	1	1		D	ET22	2	2		D	ET40	2	2
	E	ET8	2	2		E	ET22	2	2		E	ET40	1	2
♀5	A	ET9	1	2	♀13	A	ET23	2	2	♂21	A	ET41	0	2
	B	ET9	1	1		B	ET24	2	2		B	ET41	0	1
	C	ET9	2	1		C	ET24	2	2		C	ET41	0	1
	D	ET9	1	1		D	ET24	2	2		D	ET41	0	1
	E	ET9	2	2		E	ET24	1	2		E	ET41	0	1
♀6	A	ET10	2	1	♀14	A	ET25	2	2	♂22	A	ET42	1	2
	B	ET11	2	2		B	ET25	2	2		B	ET43	1	2
	C	ET11	1	2		C	ET25	2	2		C	ET44	1	2
	D	ET11	1	2		D	ET25	2	2		D	ET45	1	2
	E	ET11	1	2		E	ET25	1	2		E	ET42	1	2
♀7	A	ET12	2	2	♂15	A	ET26	1	2	♂23	A	ET46	1	2
	B	ET12	2	2		B	ET27	1	2		B	ET46	2	2
	C	ET12	2	2		C	ET28	1	2		C	ET47	2	1
	D	ET12	2	2		D	ET26	1	1		D	ET46	2	1
	E	ET12	2	2		E	ET29	1	2		E	ET46	2	1
♀8	A	ET13	2	2	♂16	A	ET30	2	2	♂24	A	ET48	2	2
	B	ET14	2	2		B	ET30	2	2		B	ET49	0	2
	C	ET13	2	2		C	ET30	2	2		C	ET50	2	1
	D	ET14	2	2		D	ET30	2	1		D	ET50	2	2
	E	ET14	2	2		E	ET30	2	2		E	ET50	2	2
♂25	A	ET51	2	2	♀30	A	ET60	1	2	♀34	A	ET75	2	2
	B	ET51	2	2		B	ET61	1	2		B	ET75	2	2
	C	ET51	2	2		C	ET62	0	1		C	ET75	1	1
	D	ET51	1	2		D	ET61	1	2		D	ET75	2	1
	E	ET51	2	2		E	ET62	2	2		E	ET75	1	1
♂26	A	ET52	1	2	♀31	A	ET64	2	2	♂35	A	ET76	1	1
	B	ET52	1	2		B	ET65	2	2		B	ET77	0	1
	C	ET52	2	2		C	ET65	1	2		C	ET78	0	1
	D	ET52	1	2		D	ET64	1	1		D	ET76	1	1
	E	ET52	2	2		E	ET66	2	2		E	ET76	1	1

♂27	A	ET53	1	2	♀32	A	ET67	1	2	♂36	A	ET79	2	2
	B	ET53	2	2		B	ET67	2	2		B	ET80	2	2
	C	ET54	1	2		C	ET67	1	2		C	ET79	2	2
	D	ET53	2	2		D	ET67	2	2		D	ET81	2	2
	E	ET53	1	2		E	ET67	1	2		E	ET82	1	2
♀28	A	ET55	1	1	♀33	A**	ET68	2	2	♂37	A	ET83	2	2
	B	ET56	1	1		B	ET68	2	2		B	ET83	2	2
	C	ET56	1	1		C**	ET68	1	1		C	ET83	1	1
	D	ET56	1	1		D	ET68	1	1		D	ET83	1	1
	E	ET57	1	2		E**	ET69	2	2		E	ET83	2	2
♀29	A	ET58	2	1		F**	ET70	1	2	♂38	A	ET84	1	2
	B	ET58	2	1		G**	ET71	0	2		B	ET84	0	2
	C	ET59	2	2		H	ET72	0	2		C	ET84	0	2
	D	ET58	2	2		I	ET73	2	2		D	ET84	2	2
	E	ET58	2	2		J**	ET74	0	2		E	ET84	0	2

CP: Code of Patient; CI: Code of Isolate; ET: Electrophoretic Type. *Pz=1: absence of enzymatic activity (index 0); 1>Pz ^{3} 0.64: positive enzymatic activity (index 1); and Pz<0.64: strongly positive enzymatic activity (Index 2). ** Isolates identify as *C. dubliniensis*.

Table 3: Exoenzyme activities in vitro profile (Pz) of Secreted Aspartyl Proteinases (SAPs) and phospholipases (PLs) of 189 *C. albicans* isolates (n ETs) and 6 *C. dubliniensis* isolates (n ETs) from the oral cavity of diabetic patients under clinical control and without clinical symptoms of oral candidoses.

Virulence profile in vitro (SAPs and PLs)

A total of 189 *C. albicans* isolates (100%) showed positive activities for secreted aspartyl proteinases (SAPs). Indices 1 and 2 were observed in 46 (24.3%) and 143 (75.7%) *C. albicans* isolates, respectively. Pz values ranged from 0.33 to 0.88 (mean of 0.58 ± 0.19). A total of 175 *C. albicans* isolates (92.6%) showed positive activities for phospholipases (PLs). Indices 1 and 2 were observed in 74 (39.2%) and 101 (53.4%) *C. albicans* isolates, respectively. Pz values ranged from 0.56 to 1.00 (mean of 0.72 ± 0.29). A total of 6 *C. dubliniensis* isolates (100%) showed positive activities for SAPs. Indices 1 and 2 were observed in 1 (16.7%) and 5 (83.3%) *C. dubliniensis* isolates, respectively. Pz values ranged from 0.26 to 0.91 (mean of 0.55 ± 0.22). A total of 4 *C. dubliniensis* isolates (66.7%) showed positive activities for PLs. Indices 1 and 2 were observed in 2 (33.3%) and 2 (33.3%) *C. dubliniensis* isolates, respectively. Pz values ranged from 0.53 to 1.00 (mean of 0.76 ± 0.20) (Table 3).

Discussion

A high incidence (83.3%) of oral colonization by *Candida* species was observed in patients diagnosed with diabetes mellitus (mean of 60 ± 10 years), with blood glucose level controlled and without clinical manifestation of oral candidiasis, coming from the Health Care Units of the Family Health Program (Ministry of Health, Brazil), Limeira city, São Paulo state, Brazil. The *Candida* species was greatly prevalent (70.4%) among the preliminarily isolated species (*C. albicans*, *C. tropicalis*, *C. krusei* and *Candida* sp.) and characterized by microbiological tests (*C. albicans* and *C. dubliniensis*). The majority of these patients showed oral monocolonization by *C. albicans*, that is, around 50% of the patients were colonize exclusively by *C. albicans*, whereas about 20% were multicolonized by *C. albicans* and other species and near 12% were colonize by the C. non-albicans species. The *C. dubliniensis* species was identified in the oral cavity of one diabetic patient (♀33) and associated with *C. albicans*, being that both species were found in high concentration. There was no visible difference in the incidence of oral colonization by the *Candida* species among the male and female patients. The density of the oral colonization of each *Candida* species ranged differently (♀350 ou♀350 UFC/mL) among the diabetic patients, despite the clinical signs and symptoms of oral candidiasis been nonexistent. The gathered information on oral colonization by *Candida* species in diabetic patients with controlled blood sugar and asymptomatic, regarding candidiasis disease, are aligned with the researches which demonstrates that the abundance of the species in the oral cavity without necessarily having clinical manifestation of candidiasis [18,30,31]. Additionally, clinical sampling methods, which consists in the use of appropriate oral rinse followed by proper care and transport, can be aligned with the microorganism colonization results of high density. The clinical sampling method used in the present research has been considered appropriate and passive for the isolation and characterization of the oral yeasts [18,32]. Many studies point the high frequency of the *Candida* species in diabetic patients with blood sugar level controlled or systemic condition and comorbidity. The *C. albicans* species has been noted to be extremely present in the oral cavity of these patients, whether it be in monocolonization or in association with other species of the same genus (*C. dubliniensis, C. glabrata, C. guilliermondii, C. kefyr, C. krusei, C. lipolytica, C. metapsilosis, C. orthopsilosis, C. parapsilosis, C. stellatoidea and C. tropicalis*) [30,33-37].

The typing by the MLEE method and the genetic interpretation of the isoenzyme patterns of *C. albicans* (n=189) and *C. dubliniensis* (n=6) clinical isolates revealed 84 electrophoretic types (ETs; 43.1% of the total isolates) and monoclonality or polyclonality (i.e., two or more ETs colonizing the same patient) in the colonization profiles by the *C. albicans* (and in a smaller proportion and incidence for the *C. dubliniensis* species) in the oral cavity of diabetic patients.

However, identical ETs were not observed colonizing two or more intra-family unrelated diabetic patients. These results suggest a high genetic diversity in the *C. albicans* population isolated from oral cavity of the diabetic patients, which is also associated to a mono or polyclonal oral colonization standard. Furthermore, this information instigates an idea of an intrapersonal genotypic identity (i.e., exclusive yeast genotypes in each diabetic patient) associated to *C. albicans* strains possibly selected or better adapted in the oral ecological niches. This method demonstrated a high reproducibility value (i.e., data were reproducible on different gels after three repetitions of each assay) and discriminatory power (i.e., index of discrimination or Simpson's diversity index equal to 0.988). The Simpson's diversity index has been suggested to the methodological and discriminatory studies involving the genotypic and phenotypic typing of the *C. albicans*. The results found in the present research about the MLEE method and the epidemiological studies substantiate with other findings in existing literature [17-21]. In addition, the diversity patterns of the population based on *C. albicans* clinical isolates (i.e., the percentage index of polymorphic loci, the average number of alleles per locus, the average number of polymorphic alleles per locus, the number of heterozygous and homozygous alleles, and the heterozygosity of each locus) were consistent with their diploid nature and compatible with other epidemiological studies involving *C. albicans* and immunocompetent and immunocompromised patients [16,17,19-21].

The genetic relationship analyses of the *C. albicans* clinical isolates population using the Nei's statistic dij, the SAHN clustering method (UPGMA algorithm) and the dendrogram (rjk=0.89763; considered as good fit), revealed a variation from zero to 0.3683, that is, on average, the population of clinical isolates showed from zero to 36.8 allelic substitutions for every 100 loci from a common ancestor (dij mean equal to 0.049 $\pm$ 0.077). A total of 18 clusters (I to XVIII) and 7 taxa (A to G) were identified, being the taxon A consisting of a greater number of isolates (74.8%), strains (71.4%), clusters (44.4%) and patients (55.5%), followed by the D, E, F, G, C, B taxa and other ETs not grouped in taxon. The clusters identified in the dendrogram exhibited high or low frequency of isolates of *C. albicans*. Each cluster comprised identical isolates (dij=0; isolates belonging to a given ET) and/or highly related isolates (0.049 $\geq$ dij > 0) coming from (i) diabetic patients epidemiologically unrelated, regardless of gender (male and female) or of characteristics of the opportunistic pathogen (i.e., exoenzymes production), and (ii) a same diabetic patient. These findings reinforce the hypothesis of high genetic diversity among the *C. albicans* population isolated from the oral cavity of diabetic patients with blood sugar controlled and without clinical manifestations of candidiasis. Furthermore, it also suggests that the majority of these patients share highly related strains, however not genetically identical. Events of direct or indirect transmissibility of *C. albicans* strains among individuals could be involved, as well as microevolutionary process would potentially result in strains better adapted to adverse conditions in diabetic patients [19,22]. On the other hand, some diabetic patients may harbor *C. albicans* strains genetically related to those strains most commonly dispersed in the diabetic patient population. In addition to the high discriminatory power and reproducibility, MLEE has been considered an excellent method of fingerprinting in the epidemiological tracing of human fungal infections, especially involving *Candida* species, and represents a valuable complement to current molecular typing methods. Additionally, MLEE provides information such as identification of the same strain in independent isolates, identification of microevolutionary changes in a strain (i.e., highly related but not identical isolates), clustering of moderately related isolates, and identification of completely unrelated isolates [14,15,17,19,21,22].

Proteolytic or lipolytic exoenzymes seem to play an important role in pathogenicity of *Candida* species. The occurrence of the SAP expression was observed in all clinical isolates of *C. albicans* and *C. dubliniensis*, being the index 2 (strongly positive enzymatic activity) highly frequent (*C. albicans*: 75.7%; *C. dubliniensis*: 83.3%). The PLs expression was observed in the majority of the clinical isolates of *C. albicans* (92.6%) and *C. dubliniensis* (66.7), being the index 1 and 2 variably expressive (*C. albicans*: 53.4%; *C. dubliniensis*: 33.3%). No correlation was observed between the exoenzyme activity profiles (SAPs e PLs) and *C. albicans* strains, clusters of highly related *C. albicans* strains and gender of diabetic patient (male or female). These findings corroborate with several studies in the literature about the exozyme expression profiles (SAPs e PLs) of clinical isolates of *C. albicans* and its fingerprinting profiles and genetic relationship patterns, the patient's clinical characteristics such as the diabetes mellitus [38], caries-free and caries-active healthy children [39], periodontal pocket, gingival sulcus and oral mucosa [40], patients diabetic and non-diabetic [18] and vaginal candidiasis [21].

Conclusion

In the present research, a high incidence of *Candida* species, specially *C. albicans* strains genetically diversified and potentially virulent (elevated expression of SAPs and PLs exoenzymes), was found in the oral cavity of diabetic patients under glycemic control and without clinical manifestations of oral candidiasis, with unique colonization patterns or mixed (two or more *Candida* species) and regardless of the patient's gender. There are major important preventive measures that diabetic patients can take to prevent infection by *Candida* species and its consequences for health and well-being. Nevertheless, in eventual cases of clinical manifestations of oral candidiasis in this group of patients, these findings (monocolonization and multicolonization patterns by *Candida* species) apprise a broad-spectrum of antifungal therapy, since some species of *Candida* may have intrinsic resistance to certain antifungals. In addition, is it extremely important to offer a combined treatment that gathers de service of health professionals such as doctors and dental surgeons to provide a better quality of life for the patients.

The MLEE typing of *C. albicans* and *C. albicans. C. krusei, C. tropicalis C. dubliniensis* demonstrates high reproducibility and discriminatory power. The population of oral clinical isolates of *C. albicans* has high genetic diversity and mono or polyclonal colonization patterns, possibly associated with an intrapersonal genotype identity (genotypes exclusive of diabetic patients).

Different clusters (moderately related or genetically related a part from one another) with variable frequencies of clinical isolates or strains (ETs) of *C. albicans* are identified by the genetic relationship analysis. Clinical isolates clustered (highly related and/or identical isolates) suggest that most of the intra-family unrelated diabetic patients share highly related strains, but not genetically identical, regardless of the patient's gender. Less frequently, clusters of highly related clinical isolates of *C. albicans* and/or *C. dubliniensis* are found exclusively in some diabetic patients, possibly due to being more adapted and/or selected to the host's intrinsic oral conditions.

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Conflicts of Interest

The authors declare no competing interests.

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