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Comparison of the Virulence of Different *Candida albicans* Samples Isolated from HIV-Positive Patients and Denture Stomatitis Lesions: *In Vitro* and *In Vivo* Tests

Fernanda Freire*, Felipe de Camargo Ribeiro, Damara da Silva Ávila, Cristiane Aparecida Pereira, Juliana Campos Junqueira, Antonio Olavo Cardoso Jorge

Department of Biosciences and Oral Diagnosis, Institute of Science and Technology, UNESP–University, Estadual Paulista, São José dos Campos, Brazil

Research Article

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***For Correspondence**

Department of Biosciences and Oral Diagnosis; Institute of Science and Technology, UNESP–Univ Estadual Paulista, São José dos Campos, Brazil, Tel: +55 12 39479033; Fax: +55 12 39479010.

E-mail: fefreire21@hotmail.com

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ABSTRACT

The objective of this study was to evaluate the *in vitro* and *in vivo* virulence of candidiasis induced by Candida albicans. Four clinical samples, two from HIV-positive patients (14/60) and two from denture stomatitis (DS) lesions (32/62), and one reference strain were used in this study. Initially was evaluated the *in vitro* secretion of hemolysin, lipase, phospholipase and proteinase enzymes; and cell surface hydrophobicity. Following, mice were immunosuppressed and inoculated with *C. albicans* suspensions. After six days, the animals were euthanized and the tongues were removed for macroscopic and histological analysis. All samples produced the virulence factors; however the samples from HIV-positive patients were more virulent. In the macroscopic analysis, all groups showed candidiasis lesions, without significant difference between them. The group inoculated with isolates of *C. albicans* from HIV-positive patients had higher amount of yeasts and hyphae ($p=0.0036$), and more tissue damage ($p=0.0016$). The inflammatory infiltrate showed statistical difference between the strains 14 and 62, and also between samples 62 and 32 ($p<0.0001$). Based on these results it can be concluded that the clinical samples from HIV-positive patients were more virulent. The experimental model of this work was essential to increase our understanding of the pathogenicity of *C. albicans*.

INTRODUCTION

Fungal infections in the oral cavity are mainly caused by *Candida albicans* species [1,2], which constitutes about 70% of *Candida* spp. in mouth isolated [2]. Colonization and infection by *C. albicans* yeast are mediated by the formation of a biofilm, which is composed of a heterogeneous mixture of blastoconidia, pseudohyphae and hyphae embedded in extracellular polymeric substances that form channels and pores and exhibit different phenotypic characteristics than planktonic *Candida*. Biofilm can maintain the integrity of the cells, protecting from phagocytosis and limiting the diffusion of antifungal [3]. In addition, other virulence characteristics of *C. albicans*, such as adhesion to mucosal cells, ability to convert from a single-celled yeast to a filamentous form and the secretion of hydrolytic extracellular enzymes, which are utilized in the process of host tissue invasion and liberation of nutrients, make *C. albicans* a pathogen that causes a broad spectrum of infections in different host sites [4].

C. albicans biofilms are frequently associated with the occurrence of denture stomatitis [5,6]. Denture stomatitis is a common inflammatory reaction, multifactorial etiology, which is usually associated with *Candida* species, particularly *C. albicans*, due to its high virulence, ability to adhere and form biofilms on oral cavity tissues and denture surfaces [7].

In HIV patient, oral manifestations are the most important and earliest indicators. There are seven cardinal signs of HIV infection: oral candidiasis, hairy leukoplakia, Kaposi sarcoma, linear gingival erythema, necrotizing ulcerative gingivitis, necrotizing ulcerative periodontitis, and non-Hodgkin lymphoma. The features mentioned above are present in 50% of patients with HIV infection and 80% in patients with AIDS [8]. Candidiasis is caused due to *C. albicans* [9]. There are four forms of *Candida* infection which includes pseudomembranous candidiasis, erythematous candidiasis, hyperplastic candidiasis, and angular cheilitis. It has

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been noted that one or more combination of the above said may be present in patients [8]. Low CD4 count is present in all the above four forms of candidiasis [9].

The animal model in mice has been useful in the development of experimental candidiasis, because it has no *Candida* spp. as a constituent of the microbiota and secondary immune response against this microorganism. In addition, this animal is easily obtained in large numbers, presents the immune system more similar to humans and their maintenance is cheaper [10,11].

The importance of analyzing clinical isolates is because they may differ in virulence factors. Therefore, a detailed characterization of *C. albicans* virulence factors is necessary not only to understanding the process of infection in detail but also to generating new and more effective anti-fungal compounds [12].

Thus, the objective of this study was to evaluate the production of different virulence factors *in vitro*, and compare candidiasis induced by clinical isolates of *C. albicans* in an experimental mice model.

MATERIALS AND METHODS

Ethics Committee

The Animal Research Ethics Committee from the Institute of Science and Technology at UNESP, approved this study under protocol number 11/2014- CEUA/ICT-CJSC-UNESP.

Microorganisms

The Four clinical samples of *C. albicans* were used, two from HIV patients (14 and 60) and two from other individuals with denture stomatitis lesions (32 and 62). One reference strain *C. albicans* (ATCC 18804) (American Type Culture Collection-ATCC) was also included in this study. The clinical samples were previously isolated and identified [13,14], and together with the reference strain, were maintained in our laboratory stock collection at –80°C.

In Vitro Virulence Tests

For the assessment of enzymatic and hemolytic activity, each sample was seeded in Sabouraud dextrose agar (SDA; Difco, Le Pont de Claix, France) and incubated at 37ºC for 24 h. Next, the samples were equidistantly spot-inoculated (~6 mm) on the specific medium for each enzyme studied. Each sample was tested 8 times, and the plates were incubated at 37°C for 2-7 days.

Proteinase secretion was assessed using a mix of two culture media [15]. The medium A, sterilized by autoclaving, was composed of $C_6H_{12}O_6$, KH_2PO_4, $MgSO_4$ (Labsynth, Diadema, SP, Brazil) agar (Difco, Le Pont de Claix, France) and distilled water. The medium B, sterilized by filtration, was composed of bovine albumin fraction V, riboflavin, nicotinic acid, thiamine hydrochloride (Sigma-Aldrich, Milwaukee, WI, USA) and distilled water.

Phospholipase production [16] by the *Candida* isolates was assessed using SDA containing NaCl, $CaCl_2$ (Labsynth, Diadema, SP, Brazil), and sterile egg yolk emulsion without the addition of potassium tellurite (Himedia, Mumbai, India).

Lipase activity [17] was determined by growing the sample on a medium containing peptone (HiMedia), $CaCl_2$, NaCl (Labsynth), agar (Difco), Tween 80 (Sigma-Aldrich, Milwaukee, WI, USA), and distilled water.

Hemolytic activity [18] was assessed using SDA (Difco) supplemented with $C_6H_{12}O_6$ (Labsynth) and fresh sheep blood (Cecon, São Paulo, SP, Brazil).

The enzymatic and hemolytic activities were determined after the incubation period. The colony diameter and the total diameter of the colony and precipitation zone (Pz) were measured, and the enzymatic activity was scored using the method described by Price et al. [16]. The Pz value representing the ratio of the colony alone to the diameter of the colony plus the precipitation zone. The results were classified as follows: no activity (Pz=1), moderate activity ($0.64 \geq Pz < 1$) and strong activity ($Pz < 0.64$).

The CSH (Cell Surface Hydrophobicity) [19] of each sample was determined through addition of 1 ml of xylene (Sigma-Aldrich, Milwaukee, WI, USA) in each suspension of *Candida* spp. Suspension without xylene was utilized as control. After incubation in a water bath at 37°C for 40 min, the lower aqueous phase was carefully collected and measured directly by spectrophotometric readings at 520 nm. CSH was expressed as a percentage of yeast adherence to xylene and was determined by the formula [(C0) CH)/C0] × 100, where C0 is the result of the control tube and CH is the result of the test tube. Each sample was tested in triplicate. The highly hydrophobic samples were those with values greater than 50%, whereas those that were moderately hydrophobic exhibited values between 20 and 50%, and hydrophilic samples were those with values below 20%.

Induction of Oral Candidiasis

The fungal inoculum preparation: Before the oral candidosis induction, we did the collection in the mouth of the mice with swab, sowed on Sabouraud dextrose agar and it was incubated at 37°C for 48 h to verify the absence of *Candida*. The five samples were seeded onto SDA (Difco) and incubated at 37°C for 24 h. Next, each sample was grown in Yeast Nitrogen Base liquid medium (Himedia, Mumbai, India) with 100 µM of glucose added (Vetec, Rio de Janeiro, Brazil), and then incubated at 37°C

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for 18 h. Cells were collected by centrifugation and washed three times with Phosphate Buffered Saline (PBS). The pellet was resuspended in 10 ml of PBS and adjusted to 10^8 viable cells/ml after counting in a Neubauer chamber (Laboroptik GMBH, Bad Homburg, Germany).

Experimental animals: Fifty adult male mice (Mus musculus, Albinus, Swiss), weighing 30 to 60 g and with no *Candida* in their buccal cavities, were included in the study. The food and water source were ad libitum, and the animals were kept in ventilated racks with a capacity of 5 animals. Ten animals were used for the study of experimental candidiasis induced by the *C. albicans* standard sample, and forty animals in the study of experimental candidiasis induced by the clinical isolates, divided among the groups: 14, 60, 32 and 62 (n=10 for each group).

The methodology described by Takakura et al. [10] was used to induce experimental candidiasis with some modifications. Briefly, the animals were immunosuppressed with 2 subcutaneous injections of prednisolone (Depo-Medrol, Laboratórios Pfizer Ltda., Guarulhos, SP, Brazil) at a dose of 100 mg/kg of body weight 1 day before and 3 days after infection with *Candida*. Tetracycline chloride (Terramicina, Laboratórios Pfizer Ltda., Guarulhos, SP, Brazil) was administered in the drinking water at a concentration of 0.83 mg/ml beginning 1 day before infection and maintained throughout the experiment. Intramuscular injection of chlorpromazine chloride (10 mg/kg of body weight; Amplictil, Sanofi Aventis, Suzano, SP, Brazil) was used to sedate the animals.

A sterile swab (Absorve, Cral, São Paulo, SP, Brazil) soaked in the *C. albicans* suspension was used to inoculate the sedated mice by rubbing the swab for 1 minute on the tongue dorsum in order to induce oral candidiasis.

The euthanasia of mice was performed within 7 days after the first immunosuppression. This procedure was performed by administration of an overdose of anesthetic. Tongues were then removed for macroscopic and microscopic analysis.

Macroscopic analysis of candidiasis on the tongue dorsum of mice: Characteristic lesions of candidiasis on the tongue dorsum were observed using a stereomicroscope (Zeiss, Göttingen, Germany). In order to quantify the number of lesions on each tongue dorsum, scores were assigned from 0 to 4: 0, normal; 1, white patches on less than 20% of the surface; 2, white patches covering between 21% and 90% of the surface; 3, white patches on more than 91% of the surface; and 4, thick white patchy pseudo membranes covering more than 91% of the surface [10].

Optical microscopy of the tongue dorsum of mice: For the purpose of microscopic analysis of the lesions, the tongues were fixed in 10% formalin for 24 hours. After embedding in paraffin, 5 µm tissue slices were cut and stained with hematoxylin-eosin (HE) and periodic acid-Schiff (PAS). The presence of candidiasis was analyzed using optical microscopy (Olympus, CX41, and Tokyo, Japan) at X400 magnification.

Candidiasis lesions were quantified by counting the number of hyphae and epithelial lesions in histological sections stained with PAS and HE, respectively. For each stain, two histological sections were randomly selected for each animal. In each histological section, 25 histologic fields were analyzed in an anteroposterior direction, resulting in a total of 50 histologic fields analyzed.

The presence of yeasts and hyphae was quantified according to the methodology of Junqueira et al. [20], attributing the following scores to histologic fields: 1, 1 to 5 yeasts/hyphae; 2, 6 to 15 yeasts/hyphae; 3, 16 to 50 yeasts/hyphae; and 4, more than 50 yeasts/hyphae. For statistical analysis, a median of the scores obtained from the 50 histologic fields was determined per animal. The intensity of the tissue lesions was evaluated by counting the number of histologic fields with the presence of epithelial lesions, such as flaking, loss of filiform papillae, loss of stratification, epithelial hyperplasia, exocytosis, spongiosis, acantholysis, hyperkeratosis, disorganization of the basal layer and intraepithelial micro abscesses development. The mean of the number of histologic sections with epithelial lesions was determined per animal for statistical analysis.

Both analyses, macroscopic and microscopic, were performed by a blinded researcher.

Statistical analysis

The data obtained were analyzed statistically with the aid GraphPad Prism 5 software (GraphPad Software, Inc, La Jolla, CA, USA). Kruskal-Wallis and Dunn's multiple comparison ($p \leq 0.05$) tests were applied to results.

RESULTS

In Vitro Virulence Tests

All samples produced the virulence factors evaluated *in vitro* **(Table 1)**. The samples that had the lowest pz values, indicating greater enzymatic activity were *C. albicans* 60 and *C. albicans* 14, both isolated from HIV-positive patients.

C. albicans 60 showed greater enzymatic activity in the secretion of lipase and proteinase enzymes. However statistically significant difference was observed in the secretion of proteinase in comparison with ATCC 18804 strain (p=0.0001), *C. albicans* sample 32 (p=0.0001), and *C. albicans* sample 62 (p=0.0382).

C. albicans 14 obtained higher enzymatic activity for hemolysin and phospholipase. Statistically difference was found in the secretion of the enzyme phospholipase compared to ATCC 18804 strain (p=0.0019); *C. albicans* sample 32 (p=0.0497) and *C. albicans* sample 62 (p=0.0105).

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Table 1. Distribution of virulence factors of *Candida albicans*.
1. Enzymatic and hemolytic classification: negative (Pz=1), moderate activity (0.6 ≥ Pz<1) and strong activity (Pz<0.64).
2 Cell surface hydrophobicity (CSH) classification: strains hydrophilic (<20%), moderate CSH (20-50%) and high CSH (≥ 50%).Significant differences in the virulence factor expression, between each C. albicans strains are indicated with different capital letters (p-value<5%, Kruskal-Wallis and Dunn's Multiple Comparison Test).

Virulence Factor	*C. albicans*				
	***C. albicans* (ATCC)**	***C. albicans* 60**	***C. albicans* 14**	***C. albicans* 32**	***C. albicans* 62**
Hemolysin[1]	0.47 ± 0.04^{AB}	0.46 ± 0.05^{AB}	0.41 ± 0.03^{AB}	0.52 ± 0.08^{B}	0.50 ± 0.07^{AB}
Lipase[1]	0.27 ± 0.04^{AB}	0.24 ± 0.04^{B}	0.27 ± 0.05^{AB}	0.31 ± 0.07^{AB}	0.32 ± 0.08^{B}
Phospholipase[1]	0.71 ± 0.08^{A}	0.67 ± 0.02^{AB}	0.61 ± 0.05^{B}	0.68 ± 0.02^{A}	0.70 ± 0.03^{A}
Proteinase[1]	0.45 ± 0.06^{A}	0.33 ± 0.03^{B}	0.34 ± 0.03^{B}	0.44 ± 0.04^{AC}	0.39 ± 0.06^{C}
CSH[2]	29.79 ± 4.08^{A}	47.13 ± 6.13^{B}	48.84 ± 3.40^{B}	39.15 ± 3.23^{C}	40.37 ± 3.70^{C}

Regarding CSH, *C. albicans* 14 was considered more hydrophobic, together with *C. albicans* 60. Significant differences were observed between *C. albicans* 14 and ATCC strain (p=0.0001), *C. albicans* 32 (p=0.0005); and *C. albicans* 62 (p=0.0028). For *C. albicans* 60, significant differences were also observed when compared to the ATCC strain (p=0.0001); *C. albicans* sample 32 (p=0.0052), and *C. albicans* sample 62 (p=0.0234).

The samples 32 and 62, both isolated from denture stomatitis lesions also exhibited higher enzymatic activity to phospholipase and proteinase enzymes, and CSH than the ATCC strain. However statistically significant difference was observed in the secretion of proteinase by *C. albicans* sample 62 in comparison with ATCC strain (p=0,0284). The CSH also was statistically significant between *C. albicans* sample 32 and ATCC strain (p=0.0008), and between *C. albicans* sample 62 and ATCC strain (p=0.0002).

Macroscopic Analysis of *Candida albicans* Infection on the Tongue Dorsum

The macroscopic analysis showed that all groups inoculated with suspensions of *C. albicans*, showed candidiasis lesions on the tongue dorsum **(Figure 1)**. The median value of the scores assigned in the macroscopic analysis was the same for all groups. It can be observed higher scores for clinical isolates of *C. albicans* from HIV-positive patients, but there was no statistical difference between the groups (p=0.7313) **(Figure 2)**.

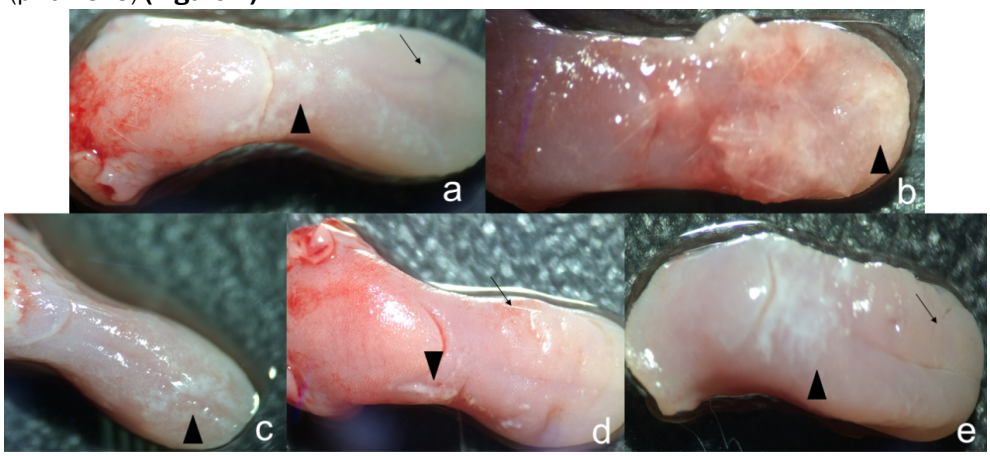

Figure 1. Macroscopic analysis of candidiasis lesions. The images show the lesions from the ATCC 18804 strain (a) and the clinical isolates 14 (b), 60 (c), 62 (d) e C32 (e), showing the presence of whitish regions (▲) and papillary atrophy (↓).

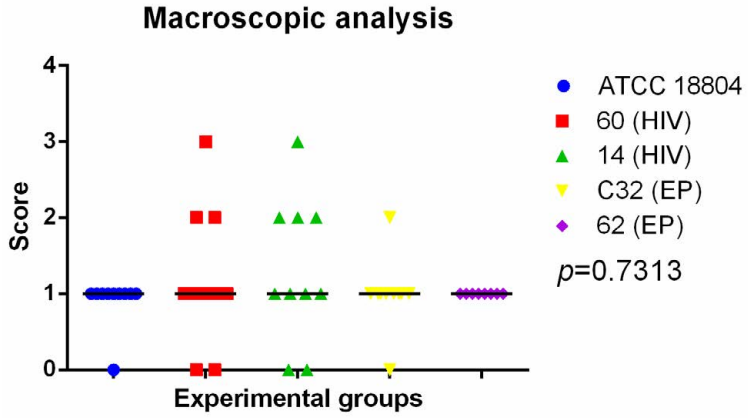

Figure 2. Scores and medians obtained from the macroscopic examination of the tongue dorsum of groups infected with the ATCC 18804 strain and the clinical isolates. (Kruskal–Wallis=0.7313). GraphPad Prism 5 software (GraphPad Software, Inc, La Jolla, CA, USA) was used to create this artwork.

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Microscopic Analysis of *Candida albicans* Infection on the Tongue Dorsum

The Microscopic analysis was performed by quantification of yeasts and hyphae, epithelial changes and the extent of the inflammatory infiltrate in the connective tissue.

In candidiasis lesions, yeasts and hyphae were observed limited to the keratin layer of the tongue dorsum, mainly concentrated in the simple conical papillae and sometimes around the dorsum of the tongue. When there was a higher concentration of *Candida*, noticed the presence of polymorphonuclear leukocytes in the epithelium forming intraepithelial microabcesses and the presence of inflammatory infiltrate in the connective tissue.

The presence of yeasts and hyphae was quantified in 50 histologic fields for each animal. The group inoculated with clinical isolates of *C. albicans* from HIV-positive patients (14 and 60) had higher amount of yeasts and hyphae **(Figure 3)**, with statistical difference between the groups analyzed (p=0.0036), which showed higher pathogenicity for these isolates **(Figure 4)**.

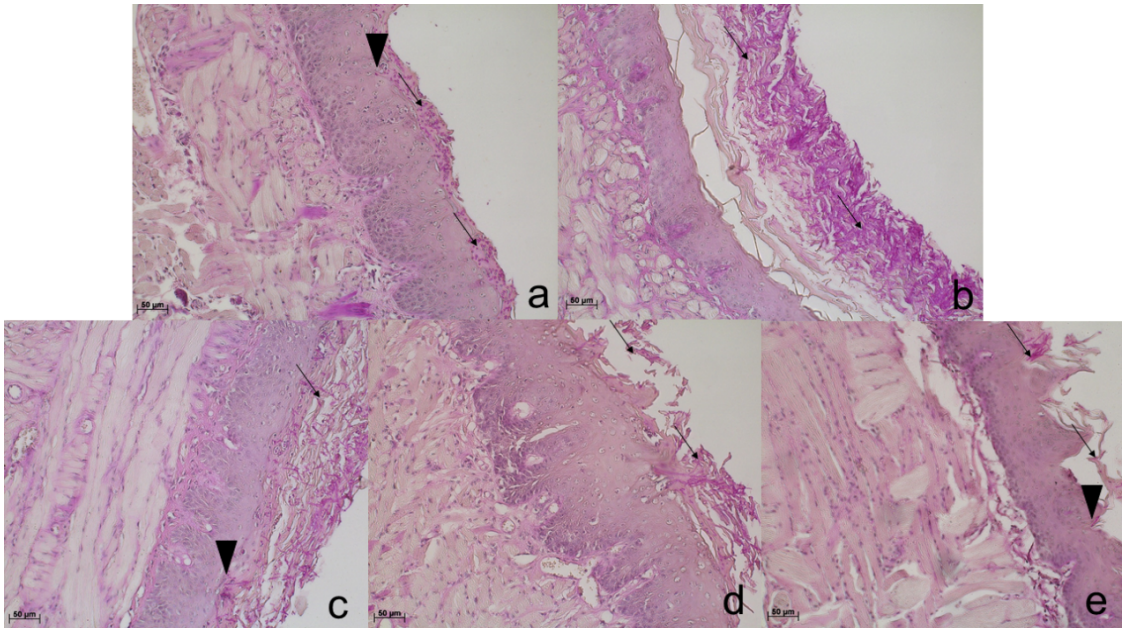

Figure 3. Sagittal incision of the tongue dorsum of mice infected with the ATCC 18804 strain and the clinical isolates. Yeasts and hyphae in keratin (↓) and in the epithelium (▼) in the ATCC 18804 strain (a) and in the clinical isolates 14 (b), 60 (c), 62 (d) e C32 (e). PAS; magnification: 200X.

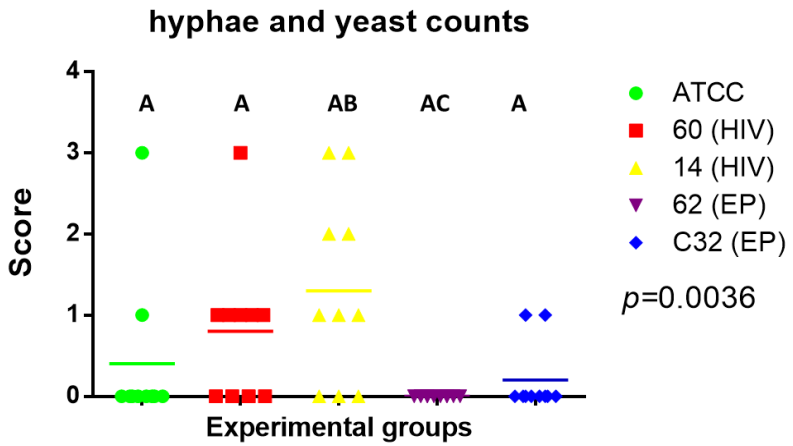

Figure 4. Scores and medians of the number of hyphae and yeast derived from the 50 histologic fields analyzed from the ATCC 18804 strain and the clinical isolates. (Kruskal – Wallis=0.0036). Values followed by different capital letters indicate a significant difference between the groups. GraphPad Prism 5 software (GraphPad Software, Inc, La Jolla, CA, USA) was used to create this artwork

Epithelial changes were quantified, revealing numerous injuries of the epithelium such as flaking, loss of filiform papillae, loss of stratification, epithelial hyperplasia, exocytosis, spongiosis, acantholysis, hyperkeratosis, disorganization of the basal layer and intraepithelial micro abscesses development. Clinical samples of *C. albicans* from HIV-positive patients (14 and 60) showed more tissue damage than the other groups **(Figure 5)**, having difference statistics (p=0.0016) **(Figure 6).** Already in the clinical samples from denture stomatitis lesions (32 and 62) few epithelial changes were observed regarding the other groups **(Figure 5)**.

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The results in the assignment of scores for the inflammatory infiltrate, showed statistical difference between the groups 14 and 62 and also between samples 62 and 32, with a value of p<0.0001 **(Figure 7)**.

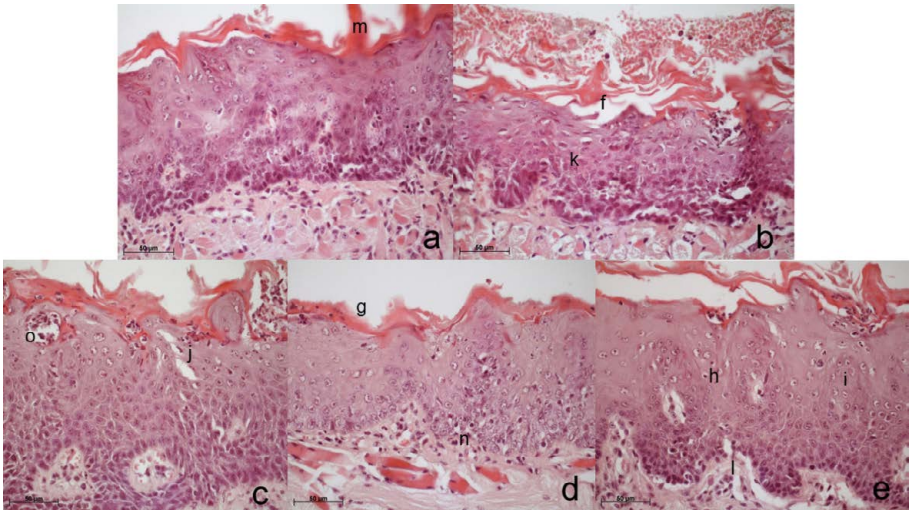

Figure 5. Sagittal incision of the tongue dorsum of mice infected with the ATCC 18804 strain and the clinical isolates, displaying a mild and moderate inflammatory infiltrate in the ATCC 18804 strain (a) and in the clinical isolates 14 (b), 60 (c), 62 (d) e C32 (e). It can be observed the presence of all lesions studied: flaking (f), loss of filiform papillae (g), loss of stratification (h), epithelial hyperplasia (i), exocytosis (j), spongiosis (k), acantholysis (l), hyperkeratosis (m), disorganization of the basal layer (n) and intraepithelial micro abscesses development (o). HE; magnification: 400X.

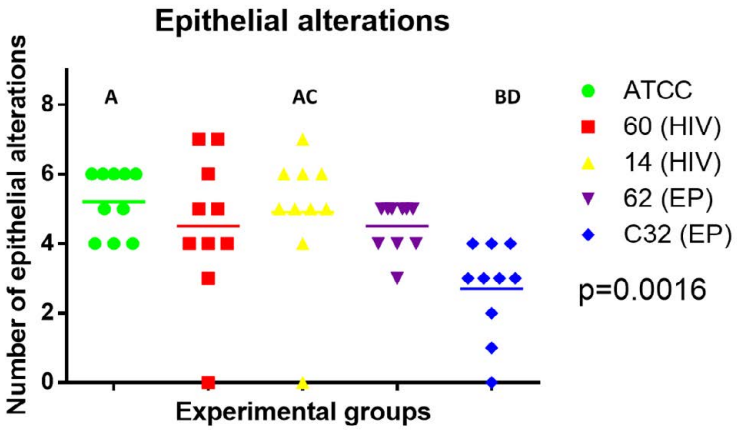

Figure 6. Number of epithelial changes and medians observed in candidiasis microscopic lesions on the tongue dorsum of mice infected with the ATCC 18804 strain and the clinical isolates. (Kruskal – Wallis = 0.0016). Values followed by different capital letters indicate a significant difference between the groups. GraphPad Prism 5 software (GraphPad Software, Inc, La Jolla, CA, USA) was used to create this artwork

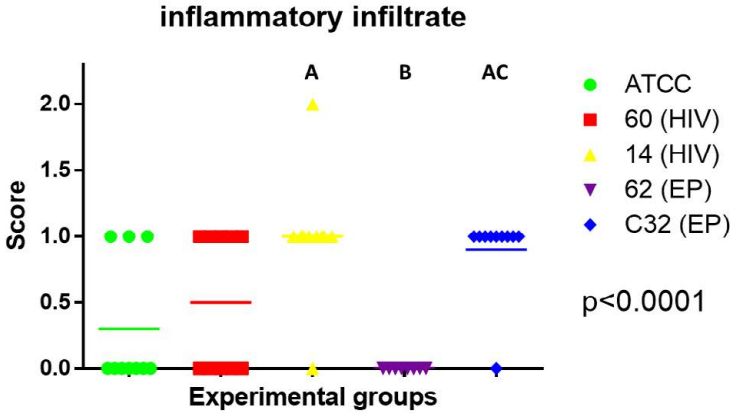

Figure 7. Scores and medians of the extent of the inflammatory infiltrate in the connective tissue observed in microscopic candidiasis lesions in the dorsum of the mice´s tongue infected with the ATCC 18804 strain and the clinical isolates (Kruskal – Wallis<0.0001). Values followed by different capital letters indicate a significant difference between the groups. GraphPad Prism 5 software (GraphPad Software, Inc, La Jolla, CA, USA) was used to create this artwork.

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DISCUSSION

Candida spp. are commensal found in 30-50% of human oral cavity. However, under certain conditions, commensal fungus can become opportunistic pathogens and cause surface and/or systemic mycoses. As most common opportunistic infection, oral candidiasis had its incidence increased rapidly for several decades due to the increasing number of compromised hosts such as patients with organ transplants, HIV-infected, undergoing chemotherapy or due to misuse of antibiotics [21,22].

Pereira et al. [14] isolated, quantified, identified, and compared opportunistic microorganisms from prosthesis-fitting surfaces, the hard palate, and mouth rinses of individuals wearing removable maxillary prosthesis with (50) and without (50) lesions of denture stomatitis. *C. albicans* was the most frequently isolated yeast species in both groups, following by *C. tropicalis* and *C. glabrata*. In a similar study, Martins et al. [23], isolated and determined the incidence of oral *Candida* spp. in dental prostheses. Oral swabs were collected from the dental prostheses of 66 patients and *Candida albicans* was the most frequently isolated microorganism (63%). For this reason, the present study used two *C. albicans* clinical isolates from denture stomatitis injury to verify its virulence in oral candidiasis formation.

Junqueira et al. [13] collected candidiasis samples from 60 seropositive HIV patients, identified by the API20C system and found that the most commonly isolated species were *C. albicans* (51.56%) followed by non-albicans *Candida* spp. (43.73%). Also, showed that these species were resistant to fluconazole and amphotericin B, important antifungal for the treatment of candidiasis. In a similar study conducted by Maheshwari et al. [24], a total of 128 *Candida* isolates were obtained from 88 HIV seropositive cases and 7 different *Candida* species were identified. *C. albicans* was the most common species isolated (50%). Gemaque et al. [25] investigated the prevalence of contagious infectious diseases in 107 patients at the University Hospital of the Federal University of Pará and verified that tuberculosis was the most prevalent disease (40,2%), followed by AIDS (32,7%). And the most common injuries in these patients were periodontal disease (57,5%) and oral candidiasis (22,8%). Berberi and Noujeim [26] in a descriptive cross-sectional study conducted for a two years period, 50 patients with HIV infection were evaluated. All patients showed at least one oral manifestation. The most common oral lesion identified was pseudo membranous candidiasis accounting for 76% (38/50) followed by periodontal disease 34% (17/50), herpetic lesions and hairy leukoplakia 10% for each (5/50), gingivitis 8% (4/50), oral ulceration 8% (4/50), Kaposi's sarcoma 6% (3/50) and Non-Hodgkin lymphoma 2% (1/50). Thus, also were used clinical *C. albicans* samples from HIV positive patients to verify their virulence *in vitro* and in oral candidiasis formation, besides that was compared its virulence with the virulence from samples of denture stomatitis injury and the standard ATCC 18804 strain.

The transformation from a harmless commensal to a virulent pathogen is attributable to an extensive repertoire of selectively expressed virulence determinants [27]. In the present study, we evaluated *in vitro* five different attributes of virulence: secretion of hemolysin, lipase, phospholipase and proteinase enzymes; and CSH. All samples studied produced the virulence factors, however, the sample isolated from HIV patients were considered most virulent than ATCC and samples isolated from lesions of DS. These samples showed strong enzymatic activity, and were considered the most hydrophobic.

Adherence to the host tissue, which is required for colonization and subsequent infection, is cited as the first stage of the infection process for the members of the genus *Candida* [28]. The extracellular hydrolytic enzymes studied in the present study are believed to play an important role in *Candida* overgrowth because these facilitate adherence, tissue penetration and the subsequent invasion of the host. Proteinase, phospholipase and lipase degrade several physiologically important substrates, such as albumin, immunoglobulin, phospholipids and skin proteins, thereby contributing to rupture of the epithelial cell membrane, allowing the penetration of hyphae into the cytoplasm during the process of infection [29,30]. In addition to hydrolytic extracellular enzymes was also evaluated the hemolytic capacity of the samples of *C. albicans* in blood-enriched medium. Under these culture conditions, all *C. albicans* samples demonstrated strong hemolytic activity. *C. albicans* destroys erythrocytes to obtain iron to producing substances known as hemolysin. Furthermore, the secretion of hemolysin followed by iron acquisition facilitates hyphal invasion and the development of disseminated candidiasis [4].

Another virulence factor evaluated in the present study was CSH, which is connected with adhesion and pathogenic processes of *C. albicans*. All samples studied were considered hydrophobic; however, the *C. albicans* samples isolated from HIV patients were more hydrophobic than those of lesions of DS and ATCC. CSH is an important virulence property conferred by the mannosylated surface proteins that coat fungal cells. Some of these proteins confer to the fungal cells the capacity to adhere to host cells or to inanimate substrata and increase resistance to macrophages and germination competence, which are essential for the establishment of chronic lesions [31].

In the assay of induction of oral candidiasis performed in this study, the tongues of mice in all groups inoculated with suspensions of *C. albicans* exhibited macroscopic lesions characteristic of candidiasis, as the presence of whitish regions with papillary atrophy areas located mainly in the region of the simple conical papillae. The median value was the same for all groups. It can be observed higher scores for the HIV samples groups, but there was no statistical difference between the groups. Studies using the same methodology of this also observed features candidiasis pseudomembranous injuries on the dorsum of the mice tongues [32-37].

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In the microscopic analysis were found many yeasts and hyphae on the dorsum of the mice tongues and numerous epithelial lesions, especially in groups inoculated with *C. albicans* from HIV positive patients, indicating its higher virulence. Mild inflammatory infiltrate was observed in most histological sections with the presence of polymorphonuclear and mononuclear. Were also observed intraepithelial microabscess underlying the yeast and hyphae located in the keratin layer. Studies using the same methodology of this also observed macroscopic and histological lesions features of candidiasis pseudomembranous on the dorsum of the mice tongues [32-39].

The experimental model of this work was essential to increase our understanding of the pathogenicity of *C. albicans*, once the characteristics of this yeast and the host responses are expressed at the development of clinical disease. Based on this study it can be assumed that the clinical samples from HIV-positive patients are more virulent. However, further studies are needed to evaluate the molecular changes and the dynamics of the immune response in infection by *C. albicans* seeking a greater understanding of human disease and the development of new therapies for candidiasis lesions.

The *C. albicans* samples from HIV-positive patients were considered more virulent than ATCC strain and samples isolated from lesions of DS in the *in vitro* study. The experimental oral candidiasis in immunosuppressed mice was lower in infections caused by standard strain ATCC 18804 and clinical samples from denture stomatitis lesions than in infections caused by clinical isolates of *C. albicans* from HIV-positive patients. Thus, it can be assumed that the clinical samples from HIV-positive patients are more virulent.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ETHICAL APPROVAL

All applicable institutional guidelines for the care and use of animals were followed.

All procedures performed in studies involving animals were in accordance with the ethical standards of the institution (Animal Research Ethics Committee from the Institute of Science and Technology at UNESP) under the protocol number 11/2014-CEUA/ICT-CJSC-UNESP.

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