Genotyping and Antifungal Susceptibility Profile of Sequential *Candida albicans* Isolated from the Oral Cavity of HIV-Infected Individuals

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Abstract: Objective: The study aimed to evaluate the genotypic profiles of *C. albicans* (*Candida albicans*) sequentially isolated throughout the course of HIV infections, and to determine its MIC (minimal inhibitory concentrations) to AMB (amphotericin B), FLC (fluconazole), KTC (ketoconazole), and ITC (itraconazole). Design: samples were collected from the oral cavity of HIV-positive individuals during 4 years, with a sterilized swab. MIC was performed by using the microdilution method AFST/EUCAST. The genetic similarities within and between sequential clones of *C. albicans* were assessed by DNA fingerprinting using the random amplification of polymorphic DNA technique. Results: A total of 142 oral samples were isolated from 59 HIV-infected individuals who attempted up to five visits each, with or without symptoms of oropharyngeal candidiasis. Profile analysis revealed that yeasts isolated over sequential visits from symptomatic or asymptomatic individuals showed 78% or 87% relatedness, respectively. The degree of similarity among *C. albicans* was higher for isolates from colonization than for those from infection. Genetically identical *C. albicans* samples also formed connected subclusters in sequential visits. In regard to susceptibility profile, all isolates were susceptible to AMB, FLC, KTC, and ITC and maintained this pattern all along, no differences in MICs of any given antifungal compound were observed for sequential *C. albicans* isolates. Conclusions: These data suggest that genotype and susceptibility to antifungal drugs were maintained over time in sequentially isolates of *C. albicans* colonization and a diverse evolutionary genetic trend in *C. albicans* sequentially isolated from the oral candidiasis of HIV infected individuals.

Key words: Genotyping, *Candida albicans*, sequential, HIV- positive individuals, oral cavity.

1. Introduction

Host defense mechanisms against *Candida albicans* are complex. Deficiencies in both phagocytic cell functions and classical cell-mediated or humoral immune responses are linked to the increased

susceptibility of the host to *C. albicans* infection [1]. The impairment of cell-mediated immunity caused by the HIV (human immunodeficiency virus) predisposes these individuals to oral candidiasis and other opportunistic infections [2-4]. Both the prevalence of *C. albicans* colonization and the recurrence of oral candidiasis increase with the progress of HIV infection for HIV-infected individuals, oral candidiasis has been

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recognized as an early expression of their immunodeficiency, while esophageal candidiasis is considered a clinical predictor of AIDS. The repeated use of short- or long-term antifungal azoles, particularly FLC (fluconazole), to treat oropharyngeal candidiasis can lead to the development and/or selection of resistance among clinical isolates [5].

This, in turn, implies that subclones of *C. albicans* with variable genotypes may simultaneously colonize the oral cavity. In this case, it is difficult to determine with certainty the contribution, if any, of individual clones to infection [6, 7], and the presence of more than one clonal type may have important therapeutic implications [7-9]. However, few clonal variations are known for *C. albicans* during either recurrent episodes of oral candidiasis or asymptomatic carriage over a prolonged course of HIV infection. Recent advances in molecular techniques have generated several typing methods for the genetic assessment of strain relatedness in *C. albicans*, which in turn have facilitated detailed studies on the molecular epidemiology of this yeast [10]. In addition, a variety of molecular methods have indicated that *C. albicans* strains tend to be genetically similar when isolated from the same specific groups, such as HIV-positive and negative patients from geographically related locations [11].

Molecular techniques include Cp3-13-specific probe, hybridization, PFGE (pulsed-field gel electrophoresis), RAPD (random amplification of polymorphic DNA), RFLP (restriction fragment length polymorphism), AFLP (amplified fragment length polymorphism) and MLST (multilocus sequence typing) [8, 12, 13]. These techniques have helped delineate subtypes of colonizing and/or infecting *C. albicans* strains over sequential evaluations of HIV-infected individuals. This study aimed to evaluate the genotypic profiles of *C. albicans* isolated from the oral cavity over sequential clinical visits during the course of HIV infection; also, determinations of MIC (minimal inhibitory concentrations) to AMB (amphotericin B),

KTC (ketoconazole), FLC (fluconazole) and ITC (itraconazole) were carried out.

2. Materials and Methods

2.1 Samples Collection and Ethical Consideration

Samples were acquired from the oral cavity of HIV-positive individuals from June 2002 to July 2006, who attempted visits to the Special Outpatient Clinic for Infectious and Parasitic Diseases or at the Ward of Tropical Diseases of Faculdade de Medicina de Botucatu, São Paulo State University (UNESP). On each visit, samples were collected using a sterilized swab, for culture independently of the clinical signs and symptoms of oral candidiasis. Each of the 59 patients attended the clinic between two and five times during this period. The characteristics of the 59 HIV-infected individuals from which *Candida* spp. was isolated are shown in Tables 1A and 1B. In total, 142 oral swab samples were obtained, as described below. Approval was obtained from the Research Ethics Committee, Faculdade de Medicina de Botucatu, UNESP, and from the Ethics Committee on Human Research, IAL (Instituto Adolfo Lutz). Written informed consent was obtained from every patient.

2.2 Fungal Isolates

The HIV-infected individuals were subjected to 142 swabs of the oral cavity. Swabs were then placed on SDA (Sabourand Dextrose Agar) plates (Difco, Detroit, Michigan) supplemented with 0.05% chloramphenicol. Plates were incubated at 30 °C for 24 h. All yeast colonies growing on each SDA plates were suspended in physiological solution and 10 µL of the suspension were used to inoculate plates of CHROMagar *Candida* media (CHROMagar Company, Paris, France) previous to incubation at 35 °C for 72 h. A presumptive identification of *Candida* isolates was attempted on the basis of the characteristic colors of the colonies appearing on CHROMagar *Candida*. When colonies from a given oral swab were morphologically homogenous, only one of them was subjected to the

Table 1A Characteristics of the 59 human immunodeficiency virus infected individuals from whom *Candida* spp. were isolated in up to five different moments.

Characteristics	Individuals no. (%)	Swabs no. (%)
Gender (n ₁ = 59)		
Male	38 (64.4)	-
Female	21 (35.6)	-
Stage (n ₁ = 59)		
AIDS	46 (78.0)	-
non-AIDS	13 (22.0)	-
Host- <i>Candida</i> relationship (n ₂ = 142)		
Colonization	-	125 (88.0)
Infection	-	17 (12.0)
Antifungal therapy (n ₂ = 142)		
Yes	-	18 (12.7)
No	-	124 (87.3)
Initial anti-retroviral therapy (n ₁ = 59)		
Yes	46 (78.0)	-
No	13 (22.0)	-

n₁ = number of individuals, n₂ = number of oral swabs.

Table 1B Characteristics of the 59 human immunodeficiency virus individuals from whom *Candida* spp. were isolated at the moment of the first visit.

Characteristics	Patients n.	Average	Range
Age (years old)	59	37.0	19.0-64.0
Initial CD ₄ ⁺ T lymphocytes (no./mm ³)	53		
< 200	23	93.0	4.0-184.0
≥ 200	30	336.0	200.0-821.0
Initial HIV viral load (copies/mL)	50		
Undetectable	15	-	-
< 10,000	21	1,119	81.0-5,736
≥ 10,000	14	53,757	11,429-2,700,000
Intervals among visits (months)*	59	3.0	1-15

* Two or more visits

complete phenotypical identification. On the contrary, heterogeneous colonies were subcultured before a complete phenotypical identification was carried out.

To identify *Candida* spp., isolates were incubated in cornmeal agar (*Oxoid*, Ltda, Brazil) supplemented with 1% Tween 80 (Synth, Labsynth, Brazil) to verify their morphology by conventional methods and in API 20C AUX system (BioMérieux Marcy l'Etoile, France) to determine their biochemical characteristics according to the manufacturer's instructions.

2.3 DNA extraction

Each isolate was plated on SDA and incubated at 30

°C for 24 h. DNA was extracted by phenol/chloroform/isoamyl alcohol and precipitated with isopropanol according to Ref. [14]. DNA concentrations were determined by using a NanoDrop1000 (Thermo Fisher Scientific, USA) apparatus, as described by Green & Sambrook (2012) [14]. For PCR and RAPD amplification, 1 µL of each DNA sample (approximately 100 ng) was used. DNA was kept at -20 °C until use.

2.4 Molecular Differentiation between *C. albicans* and *Candida dubliniensis*

All isolates phenotypically identified as *C. albicans*

were subjected to molecular differentiation between *C. albicans* and *C. dubliniensis* by PCR (polymerase chain reaction), as previously described by Mannarelli (1998) [15] PCR profiles were compared to type strains *C. albicans*, ATCC (American Type Culture Collection) 76615, and *C. dubliniensis*, CBS (Centraalbureau voor Schimmelcultures) 9768.

2.5 Genotyping of *C. albicans* Isolates

All 140 *C. albicans* isolates were submitted to RAPD analysis. Amplification was carried out with the Ready-to-Go-RAPD Analysis Beads kit (GE Healthcare, United Kingdom) to the manufacturer's instructions.

RAPD beads were composed of 1.5 units of Taq DNA polymerase, 10 mM Tris-HCl pH 8.3, 30 mM KCl, 3 mM MgCl₂, 400 mM of each dNTP, and stabilizers such as bovine serum albumin. Reactions were performed by adding each DNA template and 25 pmol of the primer to a final volume of 25 µL. The primer p4 (5'-AAGAGCCCGT-3') was selected for *C. albicans* isolates after testing six primer sequences for exclusive use in RAPD (GE Healthcare, United Kingdom). Amplification was performed in an automated thermal cycler (Progene, USA) and consisted of one initial denaturation cycle at 95 °C for 5 min, 45 denaturation cycles at 95 °C for 1 min, annealing at 32 °C for 1 min, extension at 72 °C for 2 min, and a final extension cycle for 10 min. DNA samples were tested in duplicate. As negative control, ultrapure water was used. PCR products were electrophoresed on polyacrylamide gels in TBE buffer pH 8 and stained with ethidium bromide. Fragment sizes were determined by comparison with a 100-bp DNA ladder (Invitrogen Ltda, Brazil) and visualized by UV transillumination Mini Bis Pro (Bio-Imaging Systems, Ltd, Israel). RAPD profiles of *C. albicans* clinical isolates were compared to that of the type *C. albicans* (ATCC) 76615 strain. All experiments were carried out in duplicate to assess reproducibility.

2.6 Analysis of Data and Dendrogram Generation

RAPD profiles were analyzed by Bionumerics version 5.10 (Applied Maths, Kortrijk, Belgium). The similarity coefficient (S_{AB}) between patterns for every pair of isolates A and B was computed with the formula $S_{AB} = 2E/(2E + a + b)$, where E is the number of common bands in the patterns A and B; a is the number of bands in pattern A with no correlates in pattern B; and b is the number of bands in pattern B with no correlates in pattern A. Dendrograms based on S_{AB} values were generated through dendrograms by UPGMA (unweighted pair-group method using arithmetic average) methods (1.5% optimization and 2% tolerance) implemented in the Bionumerics software. An S_{AB} value of 1.00 (100.0%) indicated that the banding patterns for strain A were identical to those for strain B; S_{AB} values of 0.80-0.99 (80.0-99.0%) represented highly similar but not identical strains and S_{AB} values below 0.80 indicated unrelated strains [7, 9].

2.7 Antifungal Susceptibility Testing

Standard antifungal susceptibility tests were carried out in 140 *C. albicans* isolates according to the reference microdilution method established by the EUCAST (European Committee on Antimicrobial Susceptibility Testing) [16]. AMB, KTC, FLC and ITC were the antifungal compounds employed (Sigma-Aldrich Brazil Ltda.). With regards to FLC susceptibility, isolates were classified as susceptible (S) for MIC ≤ 8.0 mg/L; intermediate (I) for MIC equal to 4.0 mg/L; and resistant (R) for MIC ≥ 8.0 mg/L, according to EUCAST [16]. For KTC and ITC, isolates were classified as susceptible (S), SDD (susceptible dose-dependent) and resistant (R), according to the CLSI (Clinical and Laboratory Standards Institute) [17] in values of ≥ 1 mg/L, 0.25-0.5 mg/L, and ≤ 0.125 mg/L for R, SDD and S, respectively. Since interpretative criteria for AMB susceptibility have not been established so far by either EUCAST or CLSI,

isolates were considered susceptible with MICs ≤ 1 mg/L and resistant with MICs ≥ 2 mg/L [18]. As positive controls, *Candida krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019 were used.

2.8 Statistical Analyses

These were done by the Wilcoxon, Friedmann and Kruskal-Wallis tests, followed by Dunn's test for multiple comparisons. Comparison of frequencies was carried out by Fisher's exact test. All tests were performed using the SAS (statistical analysis system), version 6.12 (Institute Inc. USA) (SAS). Significance was set at $p < 0.05$.

3. Results

Only 6 out of 59 HIV-infected individuals were symptomatic of oral candidiasis in two or more visits during the period under study. In all patients, *Candida* spp. were isolated in variable frequencies, that is, in 41 patients (69.50%) in two visits, 13 patients (22.0%) in three visits, 4 patients (6.8%) in four visits, and only one patient (1.7%) in all five visits, for a final sampling of 142 oral swabs. These were plated on CHROMagar; in them, 153 isolates were identified, 140 of them *C. albicans* and 13 non-*Candida albicans*. From one oral swab two colonies with different green coloration were isolated (Table 2). Isolation of the same species in sequential oral swabs was observed in 52 out of 59 patients (88.1%), *C. albicans* in 50 (84.7%), *C. albicans* together with *C. parapsilosis* in the other two (3.4%). In one case only *Candida* non-*albicans* were isolated in sequential oral swab (*C. glabrata* in the first

and *C. tropicalis* in the second visit). In the remaining six cases, different species or species associations were observed in sequential visits, totaling 15 *C. albicans*, three *C. tropicalis*, two *C. glabrata*, one *C. parapsilosis* and one *C. krusei* isolates.

The identification of 140 *C. albicans* isolates was confirmed by PCR using amplification of the primers CAL5/NL4CAL and CDU2/NL4CAL (Fig. 1).

3.1 RAPD Genotyping and Dendrogram Analysis of Uential Clinical *C. albicans* Isolates

The visual and gross examination of the fingerprint profiles of sequential *C. albicans* isolates indicated 61 different molecular profiles (Fig. 2). They were grouped into twenty main clusters, with 77.9% similarity (Fig. 3). S_{AB} ranged from 0.46 to 1.0 with mean plus standard deviation of 0.779 ± 0.178 . Clusters (I to XX) were derived at a threshold S_{AB} of 80.0%.

Seventeen samples isolated from six individuals with symptomatic oral candidiasis were grouped into seven clusters with 78.0% similarity among them; S_{AB} ranged from 0.54 to 1.0, with mean plus standard deviation of 0.78 ± 0.174 (Fig. 4).

Four of the seven clusters generated two to six subclusters with S_{AB} varying from 0.80 to 0.90 (Table 3). Clusters I and II included two strains with S_{AB} 0.70 between them, while cluster V included one strain with S_{AB} 0.76 to clusters III and IV, suggesting unrelated isolates. On the contrary, cluster VII grouped two isolates with S_{AB} 0.90, suggesting highly related strains (Table 3).

Table 2 Distribution of *Candida* spp. isolated from 59 HIV-infected individuals as the number of oral swabs per patient and phenotypic identification.

Oral swabs (no./patient)	Patients (no.)	Oral swabs (total)	Isolates (no.)	Phenotypic identification	
				<i>C. albicans</i> (no.)	Non- <i>Candida albicans</i> (no.)
2	41	82	88	79	09
3	13	39	43	40 ^a	03
4	04	16	16	16	00
5	01	05	06	05	01
Total	59	142	153	140	13

^a Two *C. albicans* isolates from the third oral swab of a patients, with tonality mildly different.

Fig. 1 Molecular differentiation between *Candida albicans* and *Candida dubliniensis* by amplification primer CAL5/NL4CAL and CDU2/NL4CAL by the PCR, product of 175 bp. Line I, 100-bp ladder; line II, *C. dubliniensis* (CBS 9768); line III, *C. albicans* (ATCC 76615); lanes IV to VI *Candida albicans* isolates from the oral cavity of human immunodeficiency virus infected individuals.

Fig. 2 RAPD profiles of *Candida albicans* obtained from 140 isolates of human immunodeficiency virus individuals.

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Fig. 3 Dendrograms generated for sequential *Candida albicans* from human immunodeficiency virus infected individuals. Vertical dashed line mark the positions of S_{AB} values of 0.46 and 0.80, clustering. Heavy vertical lines to the right of each dendrogram mark the position of clusters.

Fig. 4 Dendrograms generated by seventeen oral *Candida albicans* isolates from six human immunodeficiency virus patients with symptomatic oral candidiasis. Vertical dashed line mark the position of SAB 0.80, clustering. Heavy vertical lines to the right of each dendrogram mark the position of clusters.

Table 3 Distribution oral *Candida albicans* isolates obtained by sequential evaluations of 40 human HIV individuals colonized by *Candida albicans*, and six AIDS-patients with oral candidiasis according to clusters and coefficient of similarity (S_{AB}) of the subclusters.

Oral-colonization (41 HIV-infected individuals)			Oral candidiasis (06 AIDS-patients)		
Cluster number	Strains (no.)	S_{AB}	Cluster number	Strains (no.)	S_{AB}
I	11	0.84	I	1	-
II	5	0.84	II	1	-
III	6	0.81	III	2	0.81
IV	1	-	IV	6	0.83
V	4	0.88	V	1	-
VI	3	0.96	VI	4	0.80
VII	3	0.85	VII	2	0.90
VIII	9	0.87			
IX	2	1.0 ^a			
Total	44	-	Total	17	-

^a Identical strains.

A similar dendrogram, based on the S_{AB} values of genetically related isolates, was generated for the other 44 samples from individuals without clinical manifestations of candidiasis. Due to the large number of strains, their RAPD profiles were carried out only in samples with different appearance by visual analysis (Fig. 2). The Dendron database formed nine clusters with 87.0% similarity among them and S_{AB} ranging from 0.46 to 1.0, with mean plus standard deviation of 0.87 ± 0.123 . Eight of the nine clusters showed two to 11 subclusters with S_{AB} varying from 0.81 to 1.0 (Table 3). Cluster II contained two identical strains (S_{AB} equal 1.0), and three other genetically related strains, with S_{AB} 0.93. Two identical strains were found in cluster III, together with four other strains connecting at an S_{AB} 0.81. Cluster IV was composed of only one isolate, with S_{AB} 0.70 to cluster III. Cluster VIII had nine isolates with S_{AB} 0.87, and isolates connected to each other with high similarity ($S_{AB} > 0.93$); finally, cluster IX with S_{AB} 0.10 showed a S_{AB} 0.46 to all other clusters, suggesting unrelated isolates.

3.2 Comparison of the Similarity in Sequential Evaluation of *C. albicans* Isolates as to Host-Parasite Relationship

The degree of similarity among *C. albicans* was higher ($p = 0.048$) for isolates from colonization [$S_{AB} < 0.80$ for 13 (25.0%) and $S_{AB} \geq 0.80$ for 29 (75.0%) patients] than those from infection [$S_{AB} < 0.80$ for four (67.0%) and $S_{AB} \geq 0.80$ for two (33.0%) patients].

Antifungal susceptibility testing, all tested isolates grew after 24 h incubation and were susceptible to the different antifungal agents evaluated. Median and ranges of the MICs were 0.25 [0.125-1.0] for FLC, 0.015 [0.015-0.06] for ITC, 0.015 [0.015-0.06] for KTC, and 0.25 [0.03-1.0] for AMB. In addition, no differences in MICs of any given antifungal compound were calculated for sequential *C. albicans* isolates ($p > 0.05$).

4. Discussion

The pathogenicity of *Candida* species is a result of the characteristics of the strains, immunological status of the host, and conditions of infection sites. Oropharyngeal candidiasis (OPC) has been considered the most common opportunistic infection among HIV-patients [19]. Due to recurrent episodes of candidiasis, patients are frequently subjected to repeated courses of FLC therapy [5, 6, 13, 20]. After the introduction of HAART (highly active antiretroviral therapy), the occurrence of OPC has receded [19, 21, 22]. However, OPC is frequent among patients with low compliance or poor response to HAART [22]. Longitudinal studies on the oral colonization and infection patterns of the human pathogen *C. albicans* in HIV-infected individuals are scarce. Using RFLP as the method of analysis, Barchiesi et al. [23], carried out a sequential evaluation of five AIDS-patients with recurrent candidiasis during a 5-year period and observed that four of them maintained the similarity of isolates along time. Samaranayake et al. [9] selected five *C. albicans* colonies from each sample obtained in the visits, during a 1-year, totalizing 443 isolates from AIDS-patients, 10 of them with symptomatic candidiasis in one or more visits, and analyzed them by RAPD. All patients presented *C. albicans* isolates with high degree of similarity ($S_{AB} \geq 0.80$) to isolates from other visits. From 11 of the 16 patients, genetically identical isolates were taken in two to four sequential visits, three of which colonized and eight infected. These authors observed a higher degree of similarity among OPC patients. Several isolates presented a similarity degree between S_{AB} 0.70 and 0.80, suggesting a genetic shuffling during HIV disease progression. Similar genetic shuffling in *C. albicans*, leading to evolution of subtypes during HIV disease progression, has been reported by other authors [24-29]. Our study showed higher prevalence of similarity for colonizing (75.0%) than infecting (33.3%) *C. albicans*

isolates from HIV-positive individuals, with degrees of similarity of S_{AB} 0.87 and 0.78, respectively. In our study, we compared isolates above and under S_{AB} 0.80 of similarity, from 59 HIV-infected patients, a higher number than those reported by Barchiesi et al. (1997) and Samaranayake, et al. (2003) [9, 23].

To switch from saprophytic (colonization) to pathogenic (infection) state, *Candida* needs to increase both its adhesive properties to attach to host components and its production of lytic enzymes to penetrate the tissues [8, 13]. The main hydrolytic enzyme involved in its virulence is the Sap (secretory aspartyl proteinase), belonging to the same protease class as HIV protease, the target for the PIs (proteinase inhibitors) used in AIDS therapy. In contrast to the very small and structurally simplified HIV proteinase, Saps are larger and more complex. Thus, the inhibition of these proteinases by PIs would reduce the virulence of *C. albicans*, as demonstrated when the inhibitory activities of PIs were comparable to those exerted by pepstatin A, a prototypal inhibitor of Saps [30].

When colonization occurs, *Candida* species may be balanced and more stable, a behavior that changes to unbalanced in a pathogenic state, under the pressure of several factors, among them, intense production of no less than nine Saps (SAP1 to SAP9) encoded by a single gene family [30, 31], the cell-mediated immune response of the host, and the antifungal and antiretroviral substances in use for therapeutical reasons. This may explain the higher similarity among colonizing than infecting *C. albicans* isolates, found in our experiments, as it was observed by the failure of oral host defenses in AIDS patients, indicated by the increase in *Candida* spp. Carriage [3, 22] may simply provide highly adapted strains to the oral cavity with an opportunity to compete with and subsequently replace the original oral commensal strains, thus causing OPC [29] as a consequence, *Candida* species may be unbalanced and less stable. Our study indicates that the sequential evaluation of *Candida* species produced a few *Candida* non-*albicans* species, a result not shared

by Barchiesi et al. and Samaranayake, et al., who did not report the isolation of such species [9, 23].

The finding of resistant *C. albicans* strains from AIDS- patients with OPC suggests that fungal strains may become unresponsive to certain drug treatments [2]. Thus, determining the sensitivity of sequential *C. albicans* strains isolates from HIV-positive individuals becomes imperative to establish an effective drug therapy. Our results demonstrated that *Candida* isolates did not change their pattern of susceptibility of any of the antifungals used throughout the 5 years period of the study. The fact that only 12.7% patients had been exposed to previous antifungal treatments, could explain our results. These findings are very important from a clinical point of view, constituting a safe guide for therapy. While Barchiesi et al. (1997) and Samaranayake et al. (2003) [9, 23] did not evaluate the antifungal susceptibility of sequentially isolated *C. albicans* strains, Korting et al. (1999) evaluated the antifungal susceptibilities and the biotype profiles of *C. albicans* isolates from 62 AIDS-patients. They found a predominant biotype among these isolates and a new biotype in 27.3% of patients. Antifungal susceptibility testing revealed higher MICs for amphotericin B, flucytosine, ketoconazole, and itraconazole for *C. albicans* isolates from patients with advanced AIDS stages and prolonged exposure to antifungal agents [30]. These results disagree with our findings and suggested that long-term antifungal therapy for OPC in AIDS-patients may result in decreased susceptibility to commonly used antifungal. In contrast to the studies of Pfaller et al. (1994), we found evidence of low strain diversity among *C. albicans* isolates from AIDS-patients with OPC; 38% of their patients were infected or colonized with the same DNA subtype on sequential cultures, and 62% of the patients experienced episodes of infection with two or more different *C. albicans* DNA subtypes. Differences in patient populations, frequency of cultures, and duration of follow-up, intensity of exposure to specific antifungal agents and methods of DNA subtyping may

all have contributed to the different conclusions of the studies described above Ref. [26].

The evidence that *C. albicans* reproduces in a clonal way, Pujol et al. (1993) and Tibayrenc, (1997) suggests that it can adapt to its environments if such niches are stable [27, 28]. Alternatively, when exposed to frequent environmental changes, these microorganisms respond through micro-evolutionary selection based on their capacity to adapt to varied niches [28]. Oral mucous membranes of HIV-infected individuals with reduced cell-mediated immune response, associated with the pressure of antifungal and antiretroviral compounds, can behave as a dynamic environment subjected to frequent changes.

5. Conclusions

Our findings showed that in our region *C. albicans* remains the major species colonizing or infecting the oral cavity of HIV-positive individuals, with a high degree of similarity, mainly among the colonized individuals; all isolates were susceptible to AMB, KTC, FLC and ITC and maintained its susceptibility along the studied period.

Conflicts of Interest

None.

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