Oral colonization by *Candida* species in HIV-positive patients: association and antifungal susceptibility study

Colonização oral por espécies de *Candida* em pacientes HIV positivo: estudo de associação e suscetibilidade antifúngica

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**ABSTRACT**

**Objective:** To investigate antifungal susceptibility and factors associated with oral colonization by *Candida* species in HIV-positive patients. **Methods:** A prospective study based on convenience sampling of subjects recruited from a pool of confirmed HIV-positive individuals seen at a specialty outpatient service in Rondonópolis, Mato Grosso, Brazil. Oral swabs were collected from 197 patients. *Candida* species were identified by standard microbiological techniques (phenotypic and molecular methods). Antifungal susceptibility was investigated using the broth microdilution method. **Results:** A total of 101 (51.3%) patients were *Candida* spp carriers. *Candida albicans* was the most prevalent species (80%). Patients aged 45 to 59 years (Prevalence ratios: 1.90; 95%CI: 1.57-6.31) and 60 years or older (Prevalence ratios: 4.43; 95%CI: 1.57-34.18) were at higher risk of oral colonization by *Candida* species. Resistance to fluconazole and ketoconazole, or to itraconazole, corresponded to 1% and 4%, respectively. **Conclusion:** Age (45 years or older) was the only factor associated with oral colonization by *Candida*. Low rates of antifungal resistance to azoles were detected in yeast isolates obtained from HIV-positive patients. Findings of this study may contribute to proper therapeutic selection for oral candidiasis in HIV-positive patients.

**Keywords:** *Candida*; Candidiasis, oral; HIV; Microbial sensitivity tests; Antifungal agents

**RESUMO**

**Objetivo:** Investigar a suscetibilidade a antífungicos e os fatores associados à colonização oral por espécies de *Candida* isoladas de pacientes HIV positivo. **Métodos:** Estudo prospectivo realizado com amostragem por conveniência de indivíduos HIV positivo, acompanhados por um serviço de atendimento especializado da cidade de Rondonópolis, Mato Grosso, Brasil. Foram coletados swabs orais de 197 pacientes. As espécies de *Candida* foram identificadas por técnicas microbiológicas fenotípicas padrão e por método molecular. A sensibilidade antifúngica foi determinada pelo método de microdiluição em caldo. **Resultados:** Cento e um (51,3%) pacientes foram colonizados por *Candida* spp. *Candida albicans* foi a espécie mais prevalente (80%). Identificou-se um maior risco de colonização oral por espécies de *Candida* em pacientes com idade entre 45 e 59 anos (razão de prevalência: 1,90; IC95%: 1,57-6,31) e 60 anos ou mais (razão de prevalência: 4,43; IC95%: 1,57-34,18). A resistência ao fluconazol e ao cetoconazol foi de 1% cada e de 4% ao itraconazol. **Conclusão:** O único fator associado à colonização oral por espécies de *Candida* foi ter 45 anos ou mais. Identificamos baixa taxa de resistência antifúngica aos azóis entre as leveduras isoladas de pacientes HIV positivo. Estes achados podem contribuir para selecionar o tratamento da candidíase oral em pacientes HIV positivos.

**Descritores:** *Candida*; Candidíase bucal; HIV; Testes de sensibilidade microbiana; Antifúngicos
INTRODUCTION

Roughly 40.6 thousand cases of acquired immunodeficiency syndrome (AIDS) have been reported annually in Brazil over the last five years.\(^1\) AIDS is caused by the human immunodeficiency virus (HIV) and is characterized by reduced CD4 T-cell counts and increased patient susceptibility to opportunistic infections due to impaired immune response.\(^2\) Specific oral manifestations play a significant role in diagnosis and monitoring of disease progression.\(^3\)

Oropharyngeal candidiasis is one of the first clinical signs of AIDS, affecting 50 to 95% of HIV-positive individuals.\(^4\) Candida species are commensal microorganisms of the oral mucosa; however, in the presence of predisposing factors, these may become pathogenic and cause infection.\(^5\) Several factors are thought to predispose to oral candidiasis, such as extremes of age, dental prosthesis, smoking and salivary, hormone, nutritional or immunological changes.\(^6\) Oral candidiasis may be pseudomembranous, erythematous, hyperplastic or mucocutaneous, or manifest as angular cheilitis.\(^7\) Candida albicans is responsible for most episodes of oral candidiasis, but other species, such as Candida glabrata, Candida krusei, Candida tropicalis, Candida parapsilosis and Candida dubliniensis, are often implicated.\(^8\)

Intrinsic and acquired (i.e., treatment-induced) antifungal resistance in Candida species have a negative impact on disease management.\(^9\) For this reason, standardized antifungal susceptibility testing methods were developed by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the Clinical and Laboratory Standards Institute (CLSI).\(^10\) These tests play an increasingly significant role in therapeutic decision making and drug development studies, and can be used to monitor antifungal resistance development in epidemiological investigations.\(^9,11\)

OBJECTIVE

To investigate antifungal susceptibility and factors associated with oral colonization by Candida species isolated from HIV-positive patients.

METHODS

A prospective study with HIV-positive individuals seen at the specialty service of the Secretaria Municipal de Saúde of Rondonópolis, Mato Grosso, Brazil. Participants were recruited via convenience sampling during routine medical appointments, between January and May 2015. All participants were informed about the study objectives, risks and benefits and signed an Informed Consent Form. This study was approved by the Research Ethics Committee of Hospital Universitário Júlio Muller, Universidade Federal de Mato Grosso (UFMT), committee opinion no. 749,382, CAAE: 31905114.6.0000.5541. Patients aged under 18 years were excluded. Data on age, sex, antiretroviral medication, previous opportunistic infections, concurrent sexually transmitted diseases, intravenous drug use and CD4 T-cell counts were extracted from medical records.

Yeast isolation and identification

Oral swabs collected from HIV-positive patients were seeded onto Sabouraud Dextrose agar (Difco, Detroit, USA) supplemented with chloramphenicol (100μg/mL) and chromogenic medium CHROMagar Candida (PROBAC, São Paulo, Brazil). Plates were incubated at 37°C for 48 to 72 hours and yeast species confirmed via species-specific polymerase chain reaction (PCR), as described by Liguori et al.\(^12\) DNA extraction was achieved using a DNA extraction kit (Mobio, Carlsbad, CA, USA), according to manufacturer’s instructions. Polymerase chain reaction was carried out in a total volume of 25μL; reactions contained 10mM Tris-HCl (pH 8.3), 50mM KCl, 1.5mM MgCl2, 0.38mM of deoxyribonucleotide triphosphate (0.2mM each), 3.2mM primers and 1.25U TaqDNA polymerase. Oligonucleotides CA (C. albicans, 5’-TCA ACT TGTCAC AGA TTA TT-3’), CGLA (C. glabrata, 5’-CAC GAC TCGACA CTT TCT AAT T-3’), CT (C. tropicalis, 5’-AAG AAT TTAACG TGG AAA CTT A-3’), CK (C. krusei, 5’-GAT TTA GAT CTACAC TGC GTC A-3’) and IT54 (5’-TCC TCCGCT TAT TGA TAT GC-3’) were used. Amplification reactions were carried out using the following parameters: initial denaturation (92°C for 2 minutes), 35 denaturation cycles (95°C for 1 minute), annealing (50°C for 1 minute), extension (72°C for 1 minute) and final extension (72°C for 10 minutes).

Antifungal susceptibility testing

Antifungal susceptibility of isolates was determined using broth microdilution, according to CLSI M27-A3 standards.\(^13\) Antifungal agents were diluted in RPMI-1640 medium with MOPS buffer (Sigma ChemicalCo., USA) at pH 7.0. Drugs were distributed into 96-well microplates at final 0.03 to 16μg/mL (itraconazole
and ketoconazole) or 0.125 to 64μg/mL (fluconazole) concentrations. Microdilution plates were incubated at 35°C and inspected within 24 to 48 hours to determine the minimum inhibitory concentrations (MIC), or the lowest concentration required to inhibit fungal growth by ≥50% compared to positive controls. Findings were expressed in terms of MIC variation (MIC50 or MIC90, growth inhibition in 50% and 90% of isolates, respectively).

Epidemiological cutoff values for antifungal susceptibility testing (CLSI M27-S4 guidelines) are as follows: fluconazole susceptibility of C. albicans and C. tropicalis - MIC ≤2μg/mL sensitive, ≥8μg/mL resistant, 4μg/mL dose-dependent susceptibility (DDS); fluconazole susceptibility of C. glabrata – MIC ≤32μg/mL DDS, MIC ≥64μg/mL resistant. C. krusei isolates and thought to be intrinsically resistant to fluconazole, therefore respective MICs should not be interpreted using this scale. Reference values of Candida species susceptibility to itraconazole correspond to MIC ≤0.125μg/mL (sensitive), ≥1μg/mL (resistant) and 0.25 to 0.5μg/mL DDS. Reference values for ketoconazole were not included in CLSI guidelines; therefore, parameters given by Mulu et al., were adopted (MIC ≥4μg/mL equals resistance).

Data analysis
Multivariate analysis was performed using a logistic regression model to investigate factors associated with oral colonization by Candida species. Prevalence ratios (PR), 95% confidence intervals (95%CI) and p values were calculated for different factors. The level of significance was set at 5%. Statistical analyses were performed using R software.

RESULTS
This study included 197 HIV-positive patients (99 men) aged between 19 and 78 years (mean age of 42.1 years). Sociodemographic and clinical features of participants are presented in table 1. Most (n=193, 98%) patients had received highly active antiretroviral therapy (HAART) for five years on average; the most common (52.8%) therapeutic regimen consisted of a combination of nucleoside and non-nucleoside reverse transcriptase inhibitors. Patient history analysis revealed 78 (39.6%) cases of opportunistic infections, with candidiasis accounting for most episodes, followed by herpes-zoster (11.2% and 10.6%, respectively). Concurrent sexually transmitted infections were diagnosed in 17.8% of patients. Intravenous drug use was reported by 14% of participants. CD4 T-cell counts ranged from 16 to 2,299 cells/mm³ (mean count, 663 cells/mm³).

Table 1. Demographic and clinical characteristics of HIV-positive patients

| Characteristics                                      | Positive (n=101) | Negative (n=96) |
|------------------------------------------------------|-----------------|-----------------|
| Sex                                                  |                 |                 |
| Male                                                 | 42 (41.6)       | 57 (59.3)       |
| Female                                               | 59 (58.4)       | 39 (40.7)       |
| Age, years                                           |                 |                 |
| 19-29                                                | 17 (16.8)       | 15 (15.6)       |
| 30-44                                                | 43 (42.6)       | 46 (46.9)       |
| 45-59                                                | 30 (29.7)       | 32 (33.4)       |
| ≥60                                                  | 11 (10.9)       | 3 (3.1)         |
| Antiretroviral regimen                               |                 |                 |
| PI+NRTI                                              | 47 (46.5)       | 41 (42.7)       |
| NRTI+NNRTI                                          | 49 (48.5)       | 55 (57.3)       |
| Duration of antiretroviral therapy, years            |                 |                 |
| 0-5                                                  | 57 (56.4)       | 47 (49)         |
| 6-11                                                 | 30 (29.7)       | 29 (30.2)       |
| ≥12                                                  | 14 (13.9)       | 20 (20.8)       |
| History of opportunistic infection                   | 40 (39.6)       | 38 (39.6)       |
| Other sexually transmitted infection                 | 40 (39.6)       | 38 (39.6)       |
| Use of intravenous drugs                             | 16 (15.9)       | 11 (11.5)       |
| CD4 T-lymphocytes (cells/mm³)                        |                 |                 |
| <200                                                 | 14 (13.9)       | 8 (8.3)         |
| 200-700                                              | 50 (49.5)       | 49 (51)         |
| >700                                                 | 37 (36.6)       | 39 (40.7)       |

PI: protease inhibitor; NRTI: nucleoside reverse transcriptase inhibitors; NNRTI: non-nucleoside reverse transcriptase inhibitors.

Oral colonization by Candida species was detected in 51.3% of patients, C. albicans being the most common species (80%), followed by C. glabrata (14%), C. tropicalis (4%) and C. krusei (2%).

Logistic regression results are given in table 2. Colonized and non-colonized patients did not differ significantly with regard to sex (p=0.3760), duration of antiviral therapy (p=0.6820), antiviral regimen (p=0.405), history of opportunistic infections (p=0.392), concurrent sexually transmitted infections (p=0.718) or intravenous drug use (p=0.413). CD4 T-cell counts were not correlated with the presence of Candida species in the oral cavity of HIV-positive patients. Age was the only risk factor for oral colonization by Candida spp.; colonization was associated to patients aged 45 to 59 years (PR: 1.90; 95%CI: 1.57-6.31) and 60 years or
more (PR: 4.43; 95%CI: 1.57-34.18), and colonization risks increased with age.

Testing of Candida spp. isolates revealed 84% sensitivity, 15% DDS and 1% resistance to fluconazole; 99% sensitivity and 1% resistance to ketoconazole; and 73% sensitivity, 23% DDS and 4% resistance to itraconazole. One isolate (C. albicans) was resistant to fluconazole, one (C. tropicalis) to ketoconazole and four (two C. glabrata, one C. albicans and one C. tropicalis) to itraconazole. MIC50 and MIC90 values for fluconazole, ketoconazole and itraconazole corresponded to 0.5, 0.03 and 0.125 μg/mL, and 0.5, 0.03 and 0.5 μg/mL, respectively (Table 3).

**DISCUSSION**

Oral colonization by Candida species is common in HIV-positive individuals (16) and affected 51.3% of patients in this sample. Similar findings have been reported in studies carried out in China (49.5%), (17) Brazil (50.4%), (18) Taiwan (51.4%) (19) and Nigeria (52.5%). (20) Identification of asymptomatic carriers of Candida spp. is important for identification of prevalent species in epidemiological studies, and may assist therapeutic decision making. However, oral colonization should not be investigated in routine medical practice, given the lack of clinical significance and potential generation of unnecessary costs.

**Table 2. Prevalence ratios of oral colonization by Candida species**

| Factors                                  | PR    | 95%CI      | P value* |
|------------------------------------------|-------|------------|----------|
| Sex                                      |       |            |          |
| Male                                     | 1 (reference) | 0.3760    |          |
| Female                                   | 1.43  | 0.65-3.13  |          |
| Age, years                               |       |            |          |
| 19-29                                    | 1 (reference) | 0.0273    |          |
| 30-44                                    | 1.04  | 0.36-2.97  |          |
| 45-69                                    | 1.90  | 1.57-6.31  |          |
| ≥60                                      | 4.43  | 1.57-34.18 |          |
| Antiretroviral regimen                   |       |            |          |
| PI + NRTI                                | 1 (reference) | 0.405     |          |
| NRTI + NNRTI                             | 0.73  | 0.35-1.53  |          |
| Duration of antiretroviral therapy, years|       |            |          |
| 0-5                                      | 1 (reference) | 0.6820    |          |
| 6-11                                     | 0.89  | 0.37-2.12  |          |
| ≥12                                      | 0.61  | 0.21-8.4   |          |
| History of opportunistic infection       | 0.72  | 0.34-1.53  | 0.392    |
| Other sexually transmitted infection     | 1.19  | 0.47-2.98  | 0.718    |
| Use of intravenous drugs                 | 0.60  | 0.18-2.05  | 0.413    |
| CD4 T-lymphocytes (cells/mm³)            |       |            |          |
| <200                                     | 1 (reference) | 0.718     |          |
| 200-700                                  | 0.6   | 0.17-2.09  |          |
| >700                                     | 0.68  | 0.18-2.48  |          |

* Logistic regression. 95%CI: 95% confidence interval; PR: prevalence ratios; PI: protease inhibitor; NRTI: nucleoside reverse transcriptase inhibitors; NNRTI: non-nucleoside reverse transcriptase inhibitors.

**Table 3. Antifungal susceptibility to Candida species in HIV-positive patients**

| Species (n)                | Antifungal | MIC range (µg/mL) | MIC50 (µg/mL) | MIC90 (µg/mL) | Sensitive n (%) | DDS n (%) | Resistant n (%) |
|----------------------------|------------|-------------------|---------------|---------------|-----------------|-----------|-----------------|
| Candida albicans (n=81)    | Fluconazole| 0.125-8           | 0.125         | 0.125         | 79 (98)         | 1 (1)     | 1 (1)           |
|                            | Cetoconazole| 0.03-2            | 0.03          | 0.03          | 81 (100)        | 0 (0)     | 0 (0)           |
|                            | Itraconazole| 0.03-4            | 0.125         | 0.5           | 65 (81)         | 15 (18)   | 1 (1)           |
| Candida glabrata (n=14)    | Fluconazole| 0.25-4            | 0.5           | 2             | 0 (0)           | 14 (100)  | 0 (0)           |
|                            | Cetoconazole| 0.03-0.5          | 0.03          | 0.5           | 14 (100)        | 0 (0)     | 0 (0)           |
|                            | Itraconazole| 0.06-2            | 0.125         | 2             | 7 (50)          | 5 (36)    | 2 (14)          |
| Candida tropicalis (n=4)   | Fluconazole| 0.125-0.5         | 0.125         | 0.125         | 4 (100)         | 0 (0)     | 0 (0)           |
|                            | Cetoconazole| 0.03-16           | 0.06          | 0.125         | 3 (75)          | 0 (0)     | 1 (25)          |
|                            | Itraconazole| 0.03-16           | 0.25          | 0.5           | 1 (25)          | 2 (50)    | 1 (25)          |
| Candida krusei (n=2)       | Fluconazole| 0.25              | 0.25          | 0.25          | -               | -         | -               |
|                            | Cetoconazole| 0.03              | 0.03          | 0.03          | 2 (100)         | 0 (0)     | 0 (0)           |
|                            | Itraconazole| 0.125             | 0.125         | 0.125         | 1 (100)         | 1 (100)   | 0 (0)           |
| Total (n=101)              | Fluconazole| 0.125-8           | 0.125         | 0.5           | 83 (84)         | 15 (15)   | 1 (1)           |
|                            | Cetoconazole| 0.03-16           | 0.03          | 0.03          | 100 (99)        | 0 (0)     | 1 (1)           |
|                            | Itraconazole| 0.02-16           | 0.125         | 0.5           | 74 (73)         | 23 (23)   | 4 (4)           |

MIC: minimum inhibitory concentration; DDS: dose-dependent sensitivity.
C. albicans was the prevailing species (80%) in this group of patients, while C. glabrata was the most common non-albicans species. Prevalence of these microorganisms in the oral mucosa of patients with HIV/AIDS has been reported elsewhere.\(^{18,21,22}\) C. albicans is the most common species isolated from the oral mucosa of HIV-positive individuals, with prevalence ranging from 70 to 82.1%.\(^{23-26}\) C. glabrata has emerged as a significant pathogen, particularly in the oral mucosa, either as a co-infecting agent associated with C. albicans or a sole species isolated from oral lesions. Oropharyngeal infections associated with C. glabrata tend to be more severe and refractory to treatment compared to candidiasis caused by C. albicans alone.\(^{17,21,22,27}\)

Factors potentially associated with oral colonization by Candida spp. in HIV-positive patients were analyzed in this study. Patients aged 45 years or over were at increased risk of yeast colonization, and risks increased with age. Positive associations between increased risks of oral colonization by Candida spp. and age in HIV-positive patients undergoing HAART were described by Esebelahie et al.,\(^{29}\) with higher prevalence rates between 61 and 70 years. Correlations between the presence of Candida in the oral cavity of HIV-positive individuals and age were also demonstrated by Kantheti et al.\(^{28}\) In that study,\(^{28}\) risks were identified in non-HAART treated patients aged 41 to 50 years and HAART treated patients aged 51 to 60 years. More frequent use of dental prosthesis in middle-aged and elderly patients may explain the increased risk of Candida spp. colonization and infection in these age groups.\(^{17}\)

Protease inhibiting antivirals revolutionized AIDS treatment, with significant reduction in opportunistic infection rates, particularly candidiasis.\(^{29}\) Infection attenuation may reflect not only improved immunological status but also direct inhibition of aspartic proteases in Candida spp.\(^{30}\) Protease inhibitors block aspartic protease expression in vivo and promote fungal biotype selection, affecting Candida spp. prevalence and susceptibility to antifungal agents.\(^{31}\) Similar to other trials,\(^{17,26,32,33}\) Candida spp. carrier state was not significantly associated with protease inhibitor-based antiretroviral therapy in this study.

CD4 T-cell counts were not associated with the presence of yeasts in the oral mucosa of patients. Similar findings have been described in previous studies reporting equivalent CD4 T-cell counts in HIV-positive patients with and without oral colonization by yeasts.\(^{16,20,32,34}\) However, CD4 T-cell counts below 200 cells/mL are thought to be a risk factor for Candida spp. colonization.\(^{19,26,27}\)

Antifungal susceptibility testing permits accurate treatment selection and provides significant contributions to the understanding of local and global fungal resistance epidemiology.\(^{12}\) In this trial, tests performed using the broth microdilution method revealed low prevalence of oral Candida spp. resistance to fluconazole, ketoconazole and itraconazole (1%,1% and 4% respectively). Low rates of yeasts resistance to fluconazole (0.7%), ketoconazole (1.5%)\(^{35}\) and itraconazole (4.7%)\(^{15}\) have been reported. Higher resistance to itraconazole compared to the other azoles tested in this analysis supports findings of previous studies.\(^{17,26,36}\) Resistance to azolic compounds in Candida is often attributed to selection pressures exerted by antifungal agents in response to exposure of oral candidiasis patients to repeated, short- or long-term suppressive therapy.\(^{15}\) Treatment of candidiasis remains challenging to date. Antifungal susceptibility testing should precede antifungal therapy whenever possible.\(^{7}\)

## CONCLUSION

Candida albicans was the most prevalent Candida species in the oral mucosa of HIV-positive patients in this sample. Individuals aged 45 years or older were at greater risk of oral colonization by Candida species. Most isolates were susceptible to azole antifungal agents. Findings of this study emphasize the relevance of accurate molecular identification of Candida species for proper therapeutic agent selection in patients with oral candidiasis.

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## REFERENCES

1. Brasil. Ministério da Saúde. Secretaria de Vigilância em Saúde. Departamento de DST, AIDS e Hepatites Virais. Boletim Epidemiológico: HIV, AIDS. Ano v, n° 01. 27a a 53ª semanas epidemiológicas - julho a dezembro de 2015. 01a a 26a - semanas epidemiológicas - janeiro a junho de 2016 [Internet]. Brasília (DF): Ministério da Saúde; 2016 [citado 2018 Jan 22]. Disponível em: http://www.far.fiocruz.br/wp-content/uploads/2017/12/boletim_2016_1_ pdf_16375-1.pdf.
2. Robbins MR. Recent Recommendations for Management of Human Immunodeficiency Virus-Positive Patients. Dent Clin North Am. 2017;61(2):365-87. Review.
3. Gonçalves LS, Gonçalves BM, Fontes TV. Periodontal disease in HIV-infected adults in the HAART era: Clinical, immunological, and microbiological aspects. Arch Oral Biol. 2013;58(10):1385-96. Review.
4. Fidel PL Jr. Candida-host interactions in HIV disease: implications for oropharyngeal candidiasis. Adv Dent Res. 2011;23(1):45-9. Review.

5. Li X, Lei L, Tan D, Jiang L, Zeng X, Dan H, et al. Oropharyngeal Candida colonization in human immunodeficiency virus infected patients. APMIS. 2013;121(5):375-402. Review.

6. Barbudo LS, Sgarbi DB. Candidiase. J Bras Doencas Sex Transm. 2010;22(1):22-38.

7. López-Martínez R. Candidiasis, a new challenge. Clin Dermatol. 2010;28(2):178-84. Review.

8. Hebecker B, Naglik JR, Hube B, Jacobsen ID. Pathogenicity mechanisms and host response during oral Candida albicans infections. Expert Rev Anti Infect Ther. 2014;12(7):867-9. Review.

9. Sanguinetti M, Postero B, Lass-Fiörl C. Antifungal drug resistance among Candida species: mechanisms and clinical impact. Mycoses. 2015;58(Suppl 2):2-13. Review.

10. Alastreuy-Izquierdo A, Melhem MS, Bonfietti UX, Rodriguez-Tudela JL. Susceptibility test for fungi: clinical and laboratory correlations in medical mycology. Rev Inst Med Trop Sao Paulo. 2015;57(Suppl 19):57-64. Review.

11. Pfaller MA, Diekema DJ. Progress in antifungal susceptibility testing of Candida spp. by use of Clinical and Laboratory Standards Institute broth microdilution methods; 2010 to 2012. J Clin Microbiol. 2012;50(3):2846-56. Review.

12. Liguori G, Di Onofrio V, Gallé F, Lucariello A, Albano L, Catania MR, et al. Clinical and microbiological assessment of patients with a long-term antiretroviral therapy. Rev Inst Med Trop Sao Paulo. 2010;52(3):121-4.

13. Clinical and Laboratory Standard Institute (CLSI). Reference method for broth dilution antifungal susceptibility testing of yeasts; approved standard. CLSI document M27-A3 [Internet]. 3rd ed. Pennsylvania (USA): CLSI; 2008 [cited 2018 Jan 22]. Available from: https://clsi.org/media/1461/m27a3_sample.pdf

14. Clinical Laboratory and Standards Institute (CLSI). Reference method for broth dilution antifungal susceptibility testing yeasts. CLSI document M27-S4 [Internet]. Pennsylvania (USA): CLSI; 2017 [cited 2018 Jan 22]. Available from: https://clsi.org/media/1897/m27ed4_sample.pdf

15. Mulu A, Kassu A, Anagaw B, Moges B, Gelaw A, Alemayahu M, et al. Frequent detection of ‘azole’ resistant Candida species among late presenting AIDS patients in northwest Ethiopia. BMC Infect Dis. 2013;13:82. doi.org/10.1186/1471-2334-13-82.

16. Costa CR, Cohen AJ, Fernandes OF, Miranda KC, Passos XS, Souza LK, et al. Asymptomatic oral carriage of Candida species in HIV-infected patients in the highly active antiretroviral therapy era. Rev Inst Med Trop Sao Paulo. 2006;48(5):257-21.

17. Li YY, Chen WY, Li X, He L, et al. Asymptomatic oral yeast carriage and antifungal susceptibility profile of Candida albicans isolated from HIV-infected patients in Kunming, Yunnan Province of China. BMC Infect Dis. 2013;13:46. doi:10.1186/1471-2334-13-46.

18. Paula SB, Morey AT, Santos JP, Santos PM, Gameiro DG, Kerbauy G, et al. Oral Candida colonization in HIV-infected patients in Londrina-PR, Brazil: antifungal susceptibility and virulence factors. J Infect Dev Ctries. 2015;9(12):1350-9.

19. Lin JN, Lin CC, Lai CH, Yang YL, Chen HT, Weng HC, et al. Predisposing factors for oropharyngeal colonization of yeasts in human immunodeficiency virus-infected patients: a prospective cross-sectional study. J Microbiol Immunol Infect. 2013;46(2):129-35.

20. Essebelahie NO, Eweamni IB, Omoroge R. Candida colonization in asymptomatic HIV patients attending a tertiary hospital in Benin City, Nigeria. Libyan J Med. 2013;8:20322. doi: 10.3402/lij.v8i6.20322.

21. Moges B, Bitew A, Shewaamare A. Spectrum and the In Vitro Antifungal Susceptibility Pattern of Yeast Isolates in Ethiopian HIV Patients with Oropharyngeal Candidiasis. Inter J Microbiol. 2016;2016:3037817.

22. Shanizadeh A, Shokri H. Oropharyngeal Candidiasis and antifungal assessment of Candida glabrata in patients with HIV infection. Trakia J Sciences. 2016;14(1):60-6.

23. Castro LA, Álvarez MI, Martínez E. Candida en la cavidad oral de pacientes con VIH en Cali, Colombia: determinación de especies y sensibilidad al fluconazol. Iatreia. 2015;28(4):368-77.

24. Owotade FJ, Patel M, Ralephanyra TR, Vergotine G. Oral Candida colonization in HIV positive women: associated factors and changes with antiretroviral therapy. J Med Microbiol. 2013;62(Pt 1):126-32.

25. Owotade FJ, Patel M. Virulence of oral Candida isolated from HIV-positive women with oral candidiasis and asymptomatic carriers. Oral Surg Oral Pathol Oral Radiol. 2014;118(4):455-60.

26. Wu CJ, Lee HC, Yang YL, Chang CM, Chen HT, Lin CC, et al. Oropharyngeal yeast colonization in HIV-infected outpatients in southern Taiwan: CD4 count, efavirenz therapy and intravenous drug use matter. Clin Microbiol Infect. 2012;18(5):485-90.

27. Junqueira JC, Vilela SF, Rossoni RD, Barbosa JO, Costa AC, Rasteiro VM, et al. Oral colonization by yeasts in HIV-positive patients in Brazil. Rev Inst Med Trop Sao Paulo. 2012;54(1):17-24.

28. Kantheti LP, Reddy B, Ravikumar S, Anuradha CH, Chandrasakhar P, Rajeswari MS. Isolation, identification, and carriage of candidal species in PHLAs and their correlation with immunological status in cases with and without HAART. J Oral Maxillofac Pathol. 2012;16(1):38-44.

29. Mastrolorenzo A, Rusconi S, Scozzafava A, Barbaro G, Supuran CT. Inhibitors of HIV-1 Protease: current state of the art 10 years after their introduction. From antiretroviral drugs to antifungal, antibacterial and antitumor agents based on aspartic protease inhibitors. Curr Med Chem. 2007;14(26):2734-48. Review.

30. Dos Santos AL. HIV aspartyl protease inhibitors as promising compounds against Candida albicans André Luis Souza dos Santos. World J Biol Chem. 2010;1(2):21-30.

31. De Bernardis F, Tacconelli E, Mondello F, Cataldo A, Arancia S, Cauda R, et al. Anti-retroviral therapy with protease inhibitors decreases virulence enzyme expression in vivo by Candida albicans without selection of avirulent fungus strains or decreasing their anti-mycotic susceptibility. FEMS Immunol Med Microbiol. 2004;41(1):27-34.

32. Ho MW, Yang YL, Lin CC, Chi CY, Chen HT, Lin PC, et al. Yeast Oropharyngeal colonization in human Immunodeficiency virus-infected patients in central Taiwan. Mycopathology. 2014;177(S-6):309-17.

33. Delgado AC, de Jesus Pedro R, Aoki FH, Resende MR, Tabespo P, Colombo AL, et al. Clinical and microbiological assessment of patients with a long-term diagnosis of human immunodeficiency virus infection and Candida oral colonization. Clin Microbiol Infect. 2009;15(4):364-71.

34. Ribeiro Ribeiro AL, de Alencar Menezes TO, de Melo Alves-Junior S, de Menezes AL, et al. Clinical and microbiological assessment of patients with a long-term antiretroviral therapy. J Med Microbiol. 2013;62(Pt 1):126-32.

35. Zomorodian K, Bandegani A, Mirhendi H, Pakshir K, Alinejhad N, Poostforoush D. Asymptomatic oral carriage of Candida albicans in patients infected with HIV-1 in Yazd, Iran. Jundishapur J Microbiol. 2016;9(2):e28666.

36. Song YB, Suh MS, Ha GY, Kim H. Antifungal Susceptibility Testing with Etest for Candida Species Isolated from Patients with Oral Candidiasis. Ann Dermatol. 2015;27(6):715-20.