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Profile Of Sensitivity Of Candida Spp. Isolated From The Oral Mucosa Of Denture Wearers

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Abstract:
Background: *Candida species are part of the normal microbiota of human anatomical sites, especially the oral cavity. In balance with the host, they do not cause disease; however, immunosuppressed patients may develop clinical manifestations, leading to fatal outcomes such as systemic candidemia. Denture wearers frequently report discomfort caused by oral candidiasis, often precipitated by ill-fitting or poorly sanitized prostheses. Sensitivity tests are essential for determining in vitro resistance to antifungals, guiding proper treatment selection, and predicting the sensitivity profile of circulating microorganisms and emerging resistance patterns. Despite this, antifungal treatment is often not conditioned by sensitivity tests, leading to increasingly common resistance, especially with azole class drugs like fluconazole.*
Objective: *This study aimed to determine the sensitivity pattern of Candida spp. strains isolated from the oral mucosa of denture wearers to antifungals fluconazole and nystatin.*
Methods: *Patients seeking care at a Dental Clinic and a college in Araguaína-TO were included if they had predisposing factors such as denture use and clinical manifestations of fungal infection. Oral mucosa lesions were sampled using swabs, cultured on Sabouraud agar, and identified using chromogenic medium. Sensitivity tests were performed using the microdilution technique in accordance with CLSI guidelines. The minimum inhibitory concentration (MIC) was determined using RPMI-1640 medium supplemented with glucose and assessed by colorimetric changes induced by resazurin.*
Results: *Thirty-eight Candida spp. isolates were obtained, with C. albicans being the most prevalent (26), followed by C. tropicalis (11) and one C. krusei. Sensitivity to fluconazole varied, with 11 strains being sensitive,*

6 showing dose-dependent sensitivity, and 9 resistant. Nystatin showed better efficacy, with 24 strains sensitive and only 2 resistant among C. albicans isolates. Similar results were observed for C. tropicalis and C. krusei.
Conclusion: *Nystatin demonstrated better efficacy than fluconazole, especially against C. tropicalis and C. krusei isolated from oral mucosa. These findings emphasize the importance of sensitivity testing in guiding antifungal therapy, especially considering the increasing resistance to commonly used antifungals. Further studies are needed to assess the resistance profiles of different Candida species and to explore the therapeutic potential of combination therapies.*
Key Word*: Antifungal resistance; Candida Species; Oral Candidiasis*

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I. Introduction

Fungi of the *Candida* genus are part of the normal microbiota in human anatomical sites, especially the oral cavity. In balance with the host, they do not cause diseases. However, immunosuppressed patients can develop clinical manifestations, which, in cases like systemic candidemia, can be fatal. Denture wearers, both partial and complete, often report discomfort caused by oral candidiasis, particularly when dentures are poorly fitted, poorly cleaned, or old[1].

In vitro sensitivity tests are crucial for determining resistance to specific antifungal agents, guiding appropriate treatment choices, predicting the sensitivity profile of circulating microorganisms in a community, and identifying emerging resistance patterns[2]. Typically, antifungal treatment is not conditioned on sensitivity tests as it is with antibiotics, leading to increasing resistance to common antifungals such as azoles, with fluconazole being the most well-known[2,3,4].

This study aims to determine the sensitivity pattern of *Candida* spp. strains isolated from the oral mucosa of denture wearers to the antifungal agents fluconazole and nystatin.

II. Material And Methods

Study Design and Population

This study was conducted with patients seeking care at a dental clinic affiliated with a college in Araguaína, TO, Brazil. The inclusion criteria for the study were the use of dental prostheses and the presence of clinical manifestations of fungal infection. The research was approved by the institution's Ethics Committee under protocol CAAE: 00516511.9.0000.0014.

Sample Collection and Identification

Oral mucosal lesions were sampled using a sterile swab. The swabs were stored in tubes containing sterile saline solution. The swabs were then streaked onto Sabouraud dextrose agar plates. After the characteristic growth of yeasts on this medium, the Candida spp. were identified by culturing on chromogenic medium (BD™ CHROMagar™ Candida Medium).

Antifungal Susceptibility Testing: Microdilution Method

The susceptibility profile was evaluated using the broth microdilution technique in 96-well plates, following the guidelines of the Clinical Laboratory Standards Institute (CLSI) document M27-A2. The minimum inhibitory concentration (MIC) was determined, which is defined as the lowest concentration of an antifungal drug that inhibits the visible growth of the microorganism.

The medium used was RPMI-1640 with MOPS buffer, supplemented with 2% glucose, as recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST). The antifungal agents tested were fluconazole (Galena) and nystatin (Sigma). Due to the difficulty of visually observing fungal growth, resazurin dye was used as an indicator of metabolic activity. This method relies on a redox reaction where growing cells maintain a reduced environment (color change from blue to pink), while growth inhibition maintains an oxidized environment (color remains blue).

Standardization Criteria and Result Interpretation

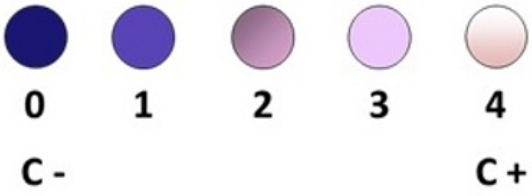
The microdilution test used MIC as an evaluation parameter for the antifungal agents tested, with concentrations ranging from 0.125 µg/mL to 64 µg/mL. Positive controls (C+) consisted of RPMI medium and the inoculum of the evaluated strain, and negative controls (C-) contained only RPMI medium and the antifungal solution. ATCC® (American Type Culture Collection) strains were used as controls, and the C. parapsilosis 22019 strain was used for quality control in the sensitivity tests with fluconazole.

For result interpretation, each well of the microdilution plate was assigned a score based on the extent of fungal growth compared to the C+ well. The scoring scale was as follows: 0 = optically clear; 1 = indeterminate

growth; 2 = prominent growth reduction; 3 = slight growth reduction; 4 = no growth reduction. The results were correlated with the color change observed with resazurin dye, providing a standardized visualization scale, as can be seen in Figure 1.

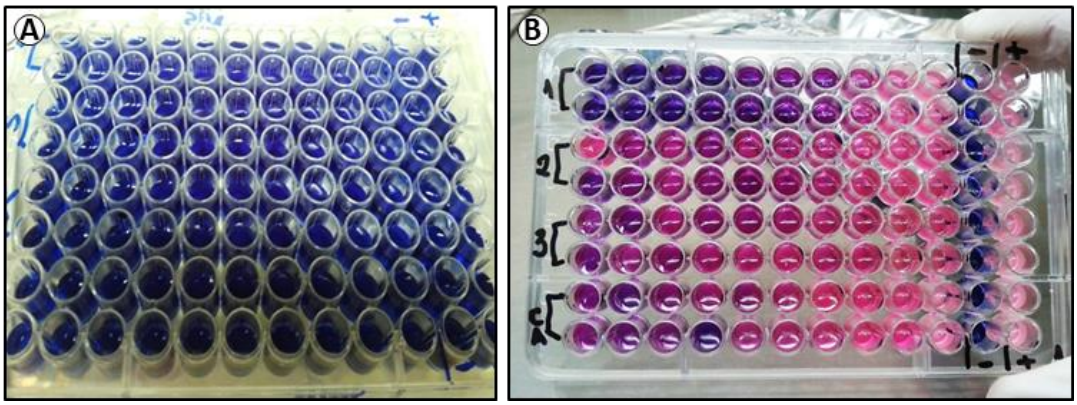
Figure no 1: Standardization of the visualization scale for correlation with the colorimetric method.

Proposed by CLSI, 2002	**Proposed by the authors**
0 = optically clear; 1 = indeterminate growth; 2 = prominent growth reduction; 3 = slight growth reduction; 4 = no growth reduction;	0 = inhibited growth (color similar to C-); 1 = indeterminate growth; 2 = prominent growth reduction; 3 = slight growth reduction; 4 = no growth inhibition (color similar to C+);



Different criteria were established for each antifungal agent. For fluconazole, the MIC was defined as the lowest concentration showing a prominent growth reduction (score 2, approximately 50% growth inhibition compared to C+). The M27-A2 standard does not specify MIC values for nystatin; thus, values from other studies were adopted. As a polyene antifungal, the MIC for nystatin was defined as the lowest concentration showing a score of 0, indicating 100% growth inhibition. The dye in contact with the solution contained in the wells exhibits the characteristics shown in Figure 2.

Figure no 2: [A] Microdilution plate after incubation (37°C ± 1°C) for 24 hours and addition of 10µL of resazurin solution. In [B] Microdilution plate after re-incubation for 4 hours. From right to left, it is possible to observe the inhibition of fungal growth according to the increase in antifungal concentration in the same direction.



The determination of the MIC (scores associated with the colorimetric scale) allowed for the definition of the sensitivity pattern of the isolated *Candida* spp. strains, classified as: Sensitive (S); Dose-Dependent Sensitivity (S-DD) or Resistant (R), as shown in Table 1.

Table no 1: Interpretation of Minimum Inhibitory Concentration (MIC) Values for Tested Antifungals

	Minimum Inhibitory Concentration (µg/ml)		
Antifungal	Sensitive (S)	Dose-Dependent Sensitivity (S-DD)	Resistant (R)
Fluconazole*	≤ 8	16 - 32	≥ 64
Nystatin**	≤ 4	8 - 32	≥ 64

* CLSI (2002); **Adaptado de Wingeter et al. (2007)

Statistical Analysis

Data were analyzed using chi-square tests to compare the proportions of sensitivity, dose-dependent sensitivity, and resistance among different Candida species and antifungal agents. A p-value of less than 0.05 was considered statistically significant. This robust methodological approach ensures the reliability and reproducibility of the findings, providing a solid foundation for the analysis of antifungal resistance in Candida spp. isolated from denture wearers.

III. Result

Isolation and Identification of Candida spp.

The cultivation and identification of samples from oral mucosal lesions resulted in 38 isolates of *Candida spp.*, comprising 26 strains of *C. albicans*, 11 of *C. tropicalis*, and 1 of *C. krusei*. These clinical strains, along with standard strains (ATCC), were evaluated for their sensitivity patterns to fluconazole and nystatin.

Fluconazole Sensitivity Tests

The results of the fluconazole sensitivity tests are presented in Table 2. Of the 26 C. albicans strains tested against fluconazole, 11 were sensitive (S), 6 exhibited dose-dependent sensitivity (S-DD), and 9 were resistant (R), with MIC ≥ 64 μg/mL. The MIC ranges for fluconazole varied from 1 μg/mL to ≥ 64 μg/mL.

Table no 2 : Sensitivity to fluconazole of 38 *Candida* spp. strains isolated from oral mucosal lesions of denture wearers. The strains were classified as Sensitive (S), Dose-Dependent Sensitivity (S-DD), or Resistant (R) based on Minimum Inhibitory Concentration (MIC) values.

Species	Sensitive (S)	S-DD	Resistant (R)	Total
C. Albicans	11	6	9	26
C. Tropicalis	1	1	9	11
C. Krusei	0	0	1	1

Nystatin Sensitivity Tests

The results of the nystatin sensitivity tests are presented in Table 3. Of the 26 *C. albicans* strains tested against nystatin, 24 were sensitive (S), 0 exhibited dose-dependent sensitivity (S-DD), and 2 were resistant (R), with MIC ≥ 64 μg/mL.

Table no 3: Sensitivity to nystatin of 38 *Candida* spp. strains isolated from oral mucosal lesions of denture wearers. The strains were classified as Sensitive (S), Dose-Dependent Sensitivity (S-DD), or Resistant (R) based on Minimum Inhibitory Concentration (MIC) values.

Species	Sensitive (S)	S-DD	Resistant (R)	Total
C. albicans	24	0	2	26
C. tropicalis	7	4	0	11
C. krusei	1	0	0	1

Statistical Analysis

To compare the efficacy of the antifungal agents and the distribution of sensitivity/resistance among different *Candida* species, a chi-square (χ^2) test was performed. The chi-square value for fluconazole was 7.99 with a p-value of 0.092, indicating no statistically significant difference in the distribution of sensitivity, S-DD, and resistance to fluconazole among the different *Candida* species.

In contrast, the chi-square value for nystatin was 11.53 with a p-value of 0.021, indicating a statistically significant difference in the distribution of sensitivity and resistance to nystatin among the different *Candida* species. These results highlight the superior efficacy of nystatin compared to fluconazole, especially against *C. tropicalis* and *C. krusei*.

IV. Discussion

A study conducted by Dalazen et al. (2011) in the state of Santa Catarina evaluated the sensitivity of *Candida spp*. strains to antifungals: amphotericin B, fluconazole, and miconazole, using the broth microdilution technique. Thirty samples isolated during episodes of recurrent oral candidiasis were tested. Among the oral isolates (elderly patients with clinical signs of erythematous candidiasis), the resistance rate to fluconazole was 93%, indicating that the vast majority of oral isolates were resistant to the drug. Conversely, Lyon et al. (2008) investigated the sensitivity profile of 109 Candida spp. strains isolated from denture wearers and non-denture

wearers; in their study, 90% of the C. albicans, *C. parapsilosis*, and *C. tropicalis* strains were inhibited by the drug at a concentration of 2.0 µg/mL.

According to Crocco et al. (2004) and Pfaller et al. (2006), non-albicans species tend to be more resistant to azole treatment. This characteristic was corroborated in this study, where 83% (n=10) of non-albicans species were resistant to fluconazole.

The sensitivity profile observed for C. albicans with respect to nystatin was quite different: 24 strains were sensitive to nystatin (MIC ≤ 4 µg/mL), and only 2 exhibited a resistance pattern to the antifungal (MIC ≤ 64 µg/mL). These results are consistent with those obtained by Brito et al. (2010), where 86 isolates of *Candida spp*. from the oral mucosa of HIV-positive patients were evaluated for nystatin sensitivity, and only 1 strain was resistant (MIC = 8 µg/mL).

Wingeter et al. (2007) isolated *Candida spp*. strains from the oral rinse of HIV-positive patients and evaluated the resistance pattern to fluconazole, itraconazole, nystatin, and amphotericin B. Of the 58 strains tested, 50 were sensitive to fluconazole and 55 to nystatin.

Despite its frequent prescription for the treatment of candidiasis, there is a notable lack of publications addressing the sensitivity pattern of yeast-like fungi to nystatin. Therefore, we chose to include studies addressing the sensitivity of fungi isolated from vaginal mucosa infections, due to some similarities (presence *of Candida spp.* as a component of the normal microbiota and innate or adaptive immunological factors such as IgA and lysozyme) found between these two anatomical sites.

A study conducted by Alves et al. (2010) evaluated the sensitivity pattern, using the macrodilution technique, of 28 *C. albicans* strains from patients with vulvovaginal candidiasis. The results indicated sensitivity of all evaluated strains to fluconazole.

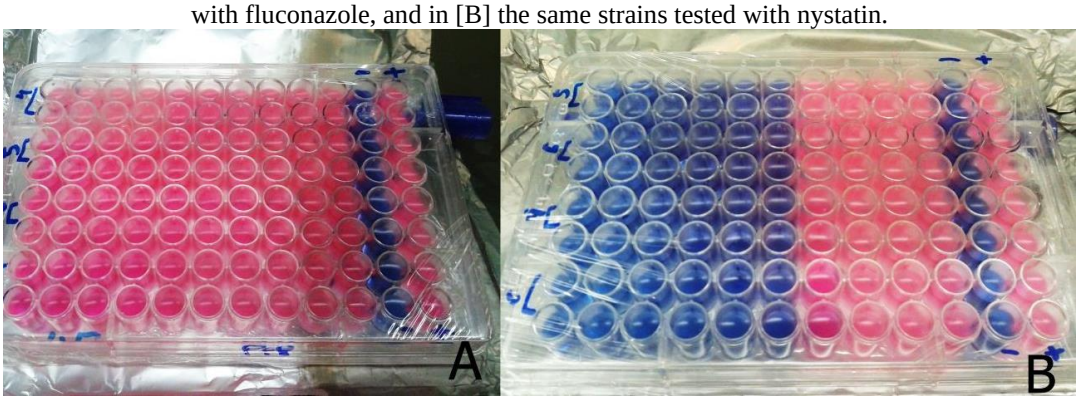
A study conducted by Pádua et al. (2003) with isolates from vaginal mucosa evaluated 83 *Candida spp.* strains; broth microdilution was used to predict the sensitivity pattern to nystatin, fluconazole, and amphotericin B. The results of this study indicated no resistance associated with nystatin.

Although the number of isolates of the species *Candida krusei* was limited to only one (1) strain, the results obtained are consistent with those found in the literature. The strain was sensitive to nystatin (MIC = 2 µg/mL) and resistant to fluconazole.

According to Loeffler and Stevens (2003) and Dalazen et al. (2011), *C. krusei* isolates frequently exhibit a resistance pattern to fluconazole in vitro, indicating that the resistance of this species to the drug should be considered an intrinsic resistance pattern of the species.

The sensitivity pattern of the *C. tropicalis* strains showed distinct results for fluconazole and nystatin. Of the 11 strains tested, 7 were considered sensitive to nystatin and 4 showed S-DD. Regarding fluconazole, 9 strains were resistant, only 1 was sensitive to the drug, and 1 showed an S-DD pattern. Figure 3 illustrates the described result for *C. tropicalis.*

Figure no 3. Microdilution plate ready for reading, in [A] strains 5, 6, 7, and ATCC of *Candida tropicalis* tested with fluconazole, and in [B] the same strains tested with nystatin.



Overall, nystatin proved to be more effective compared to fluconazole, especially in the treatment of oral mucosal infections caused by *C. tropicalis* and *C. krusei*. Due to the pharmacokinetic and pharmacodynamic differences between the two tested antifungals (fluconazole is administered orally and nystatin topically), and the nature of the lesion associated with the increased resistance profile to fluconazole, nystatin can be used in the treatment of denture stomatitis, as only 2 *C. albicans* samples showed a resistance pattern.

According to Pedroso et al. (2014), microdilution tests are not widely used in Brazil, and it is still necessary to invest in professional training so that the obtained results can be utilized in clinical practice.

V. Conclusion

This study demonstrated that nystatin is more effective than fluconazole in treating oral mucosal infections caused by *Candida tropicalis* and *Candida krusei* in denture wearers. Statistical analysis reinforced the superiority of nystatin, particularly due to the pharmacokinetic and pharmacodynamic differences between the two antifungals. However, resistance to fluconazole remains a significant challenge, highlighting the need for continuous monitoring and effective therapeutic strategies. The relatively small sample size may limit the generalizability of the results. Additionally, data collection was conducted at a single institution, which may not reflect the diversity of patients from other regions. The use of only two antifungal agents does not allow for a comprehensive evaluation of the antifungal sensitivity profile. This study underscores the need for further research to explore the efficacy of a broader range of antifungal agents, including new molecules and combination therapies. Multicenter studies with larger sample sizes and more comprehensive methodologies are crucial to validate and expand upon the findings presented. Moreover, investigations into the molecular mechanisms of resistance can provide valuable insights for the development of new antifungal treatments. Overall, the findings contribute to a better understanding of the antifungal sensitivity of *Candida* species and highlight the importance of ongoing and innovative research in this field.

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