

ANTIFUNGAL SUSCEPTIBILITY PROFILE OF *CANDIDA* SPP. ORAL ISOLATES OBTAINED FROM DENTURE WEARERS

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ABSTRACT

Denture stomatitis is an inflammatory condition that occurs in denture wearers and is frequently associated with *Candida* yeasts. Antifungal susceptibility profiles have been extensively evaluated for candidiasis patients or immunosuppressed individuals, but not for healthy *Candida* carriers. In the present study, fluconazole, itraconazole, voriconazole, terbinafine and 5-flucytosin were tested against 109 oral *Candida* spp. isolates. All antifungal agents were effective against the samples tested except for terbinafine. This work might provide epidemiological information about *Candida* spp. drug susceptibility in oral healthy individuals.

Key words: Denture wearers, *Candida* carriage, normal oral mucosa, Antifungal agents

INTRODUCTION

Denture stomatitis is an inflammatory condition that frequently occurs in the palate of denture wearers. This pathology is usually associated with *Candida* yeasts (1,12,19). The most common treatment for the disturbance is the application of topical nystatin in multiple daily doses, what reflects in poor patient cooperation. Besides, the occurrence of recurrent disease is very common. With the discovery of triazolic antifungal drugs for systemic use, fluconazole and itraconazole emerged as alternative treatments. The use of fluconazole has demonstrated promising results; however, there are few therapeutic studies about the application of itraconazole for the treatment of denture stomatitis (6).

Other antifungal agents are also important for fungal infection treatment, however, not usually employed in the treatment of oral candidiasis. For instance, terbinafine is an antifungal agent, belonging to the allylamines group, which presents a potent in vitro activity against a number of filamentous and dimorphic fungi. Some authors have proposed the application of this agent against *Candida* yeasts (9), although, its ability in inhibit yeasts is still controversial. Other antifungal agents, such as amphotericin B, 5-flucytosine and

voriconazole have a proved action against *Candida* spp., and are used for the treatment of invasive candidiasis.

Several studies focusing on the susceptibility of *Candida* spp. have been developed using samples obtained from immunosuppressed individuals or individuals presenting candidiasis. However, the prevalence of *Candida* spp. resistant to antifungals has not been extensively studied among healthy *Candida* spp. carriers. The evaluation of the susceptibility profile of *Candida* spp. isolated from the oral cavity of healthy denture wearers and dentate individuals will provide additional information about this topic.

MATERIALS AND METHODS

A total of 112 subjects who had worn complete removable dentures for at least one year and 103 subjects with natural teeth were recruited from the Dental School of the Federal University of Minas Gerais (UFMG), Brazil, and from the Dental School of the Federal University of Alfenas (UNIFAL), Brazil. All the subjects were HIV negative, never presented malignant processes and were not under antimycotic therapy in the moment of the sampling. Samples were collected from the dorsum of the tongue with a sterilized swab. Before the

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collection, 109 samples were obtained, comprising 71 *C. albicans*, 15 *C. glabrata*, 12 *C. tropicalis*, eight *C. parapsilosis* and three *C. krusei*. Samples were identified through zymogram, auxanogram, gem tube formation test, growth at 45°C and microculture (Kurtzman and Fell, 1994).

Amphotericin B (Sigma Chemical Co., St. Louis, USA), 5-flucytosine (Hoffman La Roche, Bale, Switzerland), fluconazole (Pfizer São Paulo, Brazil), itraconazole (Janssen Pharmaceutica, Beerse, Belgium), voriconazole (Pfizer, São Paulo, Brazil), and terbinafine (Novartis Biociências S.A., São Paulo, Brazil) were obtained as reagent grade powders from their respective manufacturers. Stock solutions were prepared in dimethylsulphoxide (itraconazole, amphotericin B, terbinafine and voriconazole) or water (fluconazole and 5-flucytosine). Serial two fold dilutions were prepared exactly as outlined in CLSI document M 27-A (15). Final dilution were made in RPMI 1640 medium (Sigma, St Louis, Mo, USA) buffered to pH 7 with 0,165M morpholinepropane sulphonic acid (MOPS) buffer (Sigma). Aliquots (100ml) of each agent at a two fold final concentration were dispensed into the wells of plastic microdilution trays. The trays were sealed and frozen at -70°C until they were used, except for amphotericin B that was prepared in the moment of use.

Broth microdilution testing was performed in accordance with the guidelines in the CLSI document M27-A (15). The inoculum suspension was prepared by the spectrophotometric method of inoculum preparation, with a final inoculum of $1.5 \pm 1.0 \times 10^3$ cells/ml. The final concentration of the antifungal agents was 0.3 to 64.0 mg/ml for amphotericin B, fluconazole, voriconazole and terbinafine, 0.12 to 256 µg/ml for 5-flucytosine and 0.02 to 25.0 µg/ml for itraconazole. The trays were incubated at 35°C and MIC (Minimum Inhibitory Concentration) endpoints were read after 48h of incubation. Drug free and yeast controls were included.

Following incubation, the MICs of fluconazole, terbinafine, voriconazole and itraconazole were read as the lowest concentration at which prominent decrease in turbidity relative to the growth control was observed (decrease of 80% in turbidity). For amphotericin B and 5-flucytosine, MIC was considered as the complete inhibition of growth. Quality control was ensured by testing the CLSI recommended strain *C. parapsilosis* ATCC 22019. All the tests were performed in triplicate. The interpretative breakpoints were the following:

- Fluconazole: Susceptible if MIC ≤ 8 µg/ml; Susceptible dose-dependent if MIC was between 16 to 32 µg/ml; Resistant if MIC ≥ 64 µg/ml (15,22).
- Itraconazole: Susceptible if MIC ≤ 0.125 µg/ml; Susceptible dose-dependent if MIC was between 0.25 to 0.5 µg/ml; Resistant if MIC ≥ 1 µg/ml (15,22).
- 5-flucytosine: Susceptible if MIC ≤ 4 µg/ml; Intermediary if MIC was between 8 to 16 µg/ml; Resistant if MIC ≥ 32 µg/ml (15,22)

Although no breakpoints have been established for amphotericin B, terbinafine and voriconazole, the following values were used as reference:

- Amphotericin B: Resistant if MIC > 1 µg/ml (15)
- Voriconazole: Resistant if MIC > 1 µg/ml (18)
- Terbinafine: Resistant if MIC > 1.4 µg/ml (24)

RESULTS

In the present study, 90% of *C. albicans*, *C. parapsilosis* and *C. tropicalis* isolates were inhibited by fluconazole at a concentration of 2.0 µg/ml. There was no difference between the samples obtained from denture wearers and from patients with natural teeth. Considering *C. glabrata* samples, MIC₉₀ (inhibition of 90% of the isolates) reached 8.0 µg/ml for samples obtained from denture wearers and in the totality of the samples. Regarding *C. krusei*, resistance was observed among the isolates.

Itraconazole demonstrated a good performance against *C. albicans* (MIC₉₀ = 0.06 µg/ml), *C. parapsilosis*, *C. krusei* and *C. tropicalis* (MIC₉₀ = 0.03 µg/ml). Among the isolates obtained from denture wearers, MIC₉₀ for *C. glabrata* was 0.05 µg/ml, being considered susceptible dose-dependents. However, MIC₉₀ for itraconazole was 0.12 µg/ml for the isolates obtained from individuals with natural teeth and for the totality of the samples.

Voriconazole was highly efficient against all the isolates tested, with MIC₉₀ of 1.0 µg/ml. Similarly, amphotericin B presented MIC₉₀ of 1.0 µg/ml for all species studied, considering both samples obtained from denture wearers and from individuals with natural teeth. All of the isolates were also considered susceptible to 5-flucytosine. On the other hand, terbinafine showed high MIC values for all species evaluated (MIC₉₀ ≥ 64.0 µg/ml), except for *C. parapsilosis* isolated from individuals with natural teeth, with MIC₉₀ of 2.0 µg/ml. Results are expressed on Table 1.

DISCUSSION

The present study provides the susceptibility profile of *Candida* spp. samples obtained from healthy individuals, with no signs of oral candidiasis. Several studies have reported the prevalence and antifungal susceptibility testing in individuals with oral candidiasis manifestations (2,4,10,14), but only a few studies have focused on samples obtained from *Candida* carriers without signs of infection (21).

The prevalence of *Candida* spp in the oral cavity of denture wearers is considerably greater than in individuals with natural teeth (13), even considering that the presence of the yeast does not implicates in the manifestation of disease. Both groups were compared regarding the susceptible profile and no significant differences were recorded for the antifungal agents tested,

Table 1. Minimum Inhibitory concentration (MIC) in µg/ml obtained for *Candida* species obtained from denture wearers and from individuals with natural teeth.

Antifungal agent	Species	Denture wearers without signs of candidiasis		Healthy individuals with natural teeth		Total of individuals	
		MIC ₉₀ (µg/ml)	MIC Range	MIC ₉₀ (µg/ml)	MIC Range	MIC ₉₀ (µg/ml)	MIC Range
Fluconazole	<i>C. albicans</i>	2.0 ^a	0.25-64.0	2.0 ^a	0.25-4.0	2.0 ^a	0.25-64.0
	<i>C. glabrata</i>	8.0 ^d	1.0-16.0	4.0 ^a	0.5-8.0	8.0 ^d	0.5-16.0
	<i>C. parapsilosis</i>	1.0 ^a	1.0-64.0	4.0 ^a	1.0-4.0	4.0 ^a	0.25-4.0
	<i>C. krusei</i>	64.0 ^d	32.0-64.0	64.0 ^d	64.0	64.0 ^d	32.0-64.0
	<i>C. tropicalis</i>	2.0 ^a	2.0	1.0 ^a	1.0	2.0 ^a	1.0-2.0
Itraconazole	<i>C. albicans</i>	0.06 ^a	0.03-1.0	0.06 ^a	0.03-0.5	0.06 ^a	0.03-1.0
	<i>C. glabrata</i>	0.5 ^a	0.06-0.5	0.12 ^a	0.03-0.5	0.12 ^a	0.03-0.5
	<i>C. parapsilosis</i>	0.03 ^a	0.03-0.06	0.03 ^a	0.03-0.06	0.03 ^a	0.03-0.06
	<i>C. krusei</i>	0.03 ^a	0.03-0.06	0.03 ^a	0.03-0.12	0.03 ^a	0.03-0.12
	<i>C. tropicalis</i>	0.03 ^a	0.03-0.12	0.03 ^a	0.03-0.25	0.03 ^a	0.03-0.25
Voriconazole	<i>C. albicans</i>	0.25 ^a	0.12-64.0	0.5 ^a	0.12-2.0	0.25 ^a	0.12-64.0
	<i>C. glabrata</i>	1.0 ^a	0.12-16.0	0.12 ^a	0.12	1.0 ^a	0.12-16.0
	<i>C. parapsilosis</i>	0.5 ^a	0.12-0.5	0.5 ^a	0.25-1.0	0.5 ^a	0.12-1.0
	<i>C. krusei</i>	0.12 ^a	0.5-1.0	1.0 ^a	1.0	1.0 ^a	0.5-1.0
	<i>C. tropicalis</i>	0.12 ^a	0.12	0.12 ^a	0.12	0.12 ^a	0.12
Amphotericin B	<i>C. albicans</i>	1.0 ^a	0.25-2.0	1.0 ^a	0.25-1.0	1.0 ^a	0.25-2.0
	<i>C. glabrata</i>	1.0 ^a	0.5-32.0	0.5 ^a	0.12-8.0	1.0 ^a	0.5-32.0
	<i>C. parapsilosis</i>	0.25 ^a	0.25-0.5	0.5 ^a	0.25-2.0	0.5 ^a	0.25-2.0
	<i>C. krusei</i>	0.5 ^a	0.5	1.0 ^a	1.0	1.0 ^a	0.5-1.0
	<i>C. tropicalis</i>	0.25 ^a	0.25-1.0	0.25 ^a	0.25-0.5	0.25 ^a	0.25-1.0
5-Flucytosine	<i>C. albicans</i>	1.0 ^a	0.5-264	1.0 ^a	0.5-32	1.0 ^a	0.5-264
	<i>C. glabrata</i>	1.0 ^a	0.5-1.0	1.0 ^a	0.5-8.0	1.0 ^a	0.5-8.0
	<i>C. parapsilosis</i>	0.5 ^a	0.5-264	0.5 ^a	0.5	0.5 ^a	0.5-264
	<i>C. krusei</i>	0.5 ^a	0.5-4.0	0.5 ^a	0.5	0.5 ^a	0.5-4.0
	<i>C. tropicalis</i>	0.5 ^a	0.5	0.5 ^a	0.5-4.0	0.5 ^a	0.5-4.0
Terbinafine	<i>C. albicans</i>	64.0	2.0-64.0	64.0	2.0-64.0	64.0	2.0-64.0
	<i>C. glabrata</i>	64.0	1.0-64.0	64.0	1.0-64.0	64.0	1.0-64.0
	<i>C. parapsilosis</i>	64.0	4.0-64.0	64.0	4.0-64.0	64.0	4.0-64.0
	<i>C. krusei</i>	64.0	64.0	64.0	64.0	64.0	64.0
	<i>C. tropicalis</i>	64.0	64.0	2.0	2.0	64.0	64.0

^a Susceptible, ^b Susceptible dose-dependent, ^c Intermediate, ^d Resistant; MIC₉₀ = Inhibition of 90% of the isolates.

except for itraconazole and for fluconazole against *C. glabrata*. The continued exposure of *Candida* yeasts to antifungals in certain patient groups has already been shown to alter the susceptibility of strains (10,20). Besides, the treatment with antifungal agents might lead to the selection of non-*albicans*

Candida species. The development of resistance mechanisms is common, especially with *C. glabrata*, and *C. krusei* is intrinsically resistant to fluconazole.

Patients were inquired about the use of antifungal agents and only those who reported not to be under antifungal therapy

were included in the study. However, due to the high incidence of denture stomatitis among denture wearers, this variable is difficult to control. Patients might have a history of antifungal use or even unknown the name or function of a medicine prescribed.

Despite most antifungal drugs tested in the present article are not available for oral administration, data about the susceptibility profile against oral isolates provides epidemiological evidences and might be helpful in guidelines clinical practice. Besides, formulations can be developed for these agents due to the great seek for new forms of antifungal therapy. On the other hand, oral microorganisms can be responsible for spread infection or severe oral candidiasis if an individual became susceptible, In this case, the profile of susceptibility could be extrapolated.

As it could be expected, fluconazole demonstrated great efficacy against *C. albicans*, *C. parapsilosis* and *C. tropicalis* isolates. On the other hand, *C. krusei* and *C. glabrata* were resistant to this drug. These results are consistent to those reported by several authors (8,23,26). On the other hand, itraconazole demonstrated a surprisingly low efficacy against *C. albicans* isolates. *C. glabrata* were also resistant to this drug, what had been already reported by Pfaler *et al.* (18) and Swoboda-Kopec (27). Considering the higher prevalence of *C. albicans* among denture stomatitis patients, these results disagree with Cross *et al.* (6) affirmative regarding the efficacy of itraconazole for the treatment of this pathologic condition. However, it is important to notice that, unlike the referred work, clinical trials were not developed in the present study.

Results obtained in the present study were consistent with those obtained by Wingeter *et al.* (28) regarding the susceptibility of oral isolates to amphotericin B. According to Ghannoum and Rice (7), resistance to polyenic antifungal agents is rare. The most common treatment of denture stomatitis is the use of nystatin, a polyene antifungal agent.

Burn *et al.* (3) found elevate MIC values for voriconazole against *C. glabrata* isolated from head irradiated patients. However, in the present study, this drug demonstrated high efficacy against *C. glabrata* strains. About this discrepancy, we can argue that endpoints are not well established for this drug. Besides, the group of patients was different and only a small number of strains were evaluated in the present work. Other researchers found susceptibility profiles to voriconazole similar to those represented in this article.

Unlike results obtained by Jessup *et al.* (9) and Ryder *et al.* (26), who obtained good results for terbinafine against *Candida* spp. isolates, several authors have reported that this drug does not present significant in vitro activity against *Candida* yeasts (17,25). These finds are corroborated by the present study. Furthermore, good activity against *C. parapsilosis* and poor efficacy against other *Candida* species is a common report (16). According to Ryder *et al.* (26), poor results obtained with

terbinafine against *Candida* yeasts in vitro are not corroborate by clinical trials where this drug has proven to be effective. These authors suggest that a buffer (MOPS) should be added to the culture medium since terbinafine is less effective under low pH conditions.

The present study reported the susceptibility profile of *Candida* yeasts isolated from the oral cavity of healthy individuals. We can conclude that the overall outcome was very satisfactory for the antifungal drugs tested, with exception of terbinafine. This work might provide epidemiological information about *Candida* spp. drug susceptibility in oral healthy individuals.

RESUMO

Perfil de sensibilidade antifungica de isolados de candida sp obtidos de usuários de prótese total

A estomatite protética é uma condição inflamatória que ocorre em usuários de prótese total e está frequentemente associada a leveduras do gênero *Candida*. Os perfis de suscetibilidade a antifúngicos têm sido extensivamente estudados em pacientes com candidíase ou em indivíduos imunossuprimidos, mas não em portadores sadios de *Candida*. No presente estudo, fluconazol, itraconazol, voriconazol, terbinafina e 5-flucitosina foram testados contra 109 isolados orais de *Candida* spp. Todos os agentes antifúngicos mostraram-se eficazes contra as amostras avaliadas, exceto a Terbinafina. O presente trabalho pode fornecer dados epidemiológicos com relação à susceptibilidade a antifúngicos de *Candida* spp em indivíduos com saúde oral.

Palavras-chave: Usuários de prótese total, Portadores de *Candida*, Mucosa oral normal, agentes antifúngicos

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