Susceptibility profile of a Brazilian yeast stock collection of Candida species isolated from subjects with Candida-associated denture stomatitis with or without diabetes

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Objective. This study investigated the susceptibility of 198 clinical isolates of Candida species against caspofungin, amphotericin B, itraconazole, and fluconazole.

Study Design. Suspensions of the microorganisms were spread on Roswell Park Memorial Institute (RPMI) agar plates. Etest strips were placed on the plates, and the minimal inhibitory concentration (MIC) was read after incubation (48 h at 37°C). Data were analyzed by a factorial analysis of variance and a 2 × 2 post hoc test (α = .05).

Results. C glabrata showed the highest MIC values (P < .001) against caspofungin, itraconazole, and fluconazole. For amphotericin B, the MIC values of C tropicalis and C glabrata (P = .0521) were higher than those of C albicans (P < .001). Itraconazole was the least effective antifungal; 93.3% of the C glabrata isolates, 3.3% of the C albicans, and 1.3% of the C tropicalis were resistant. All microorganisms were susceptible to caspofungin and amphotericin B.

Conclusions. Caspofungin and amphotericin B should be recommended as an effective alternative for the management of oral Candida infections when treatment with topical or other systemic drugs has definitely failed. (Oral Surg Oral Med Oral Pathol Oral Radiol 2013;116:562-569)

Oral candidiasis is an opportunistic infection found in healthy and medically compromised individuals. The prevalence of this disease continues to grow, and it is one of the most common fungal infections. The risk factors for oral candidal infection are complex. Denture biofilms represent a protective reservoir for oral microbes, favoring yeast proliferation, enhancing their infective potential, and protecting fungal cells against several medications. The exoplymeric matrix coupled with the organization of layers of cells within the biofilm may confer protection to organisms in the inner layers, contributing to antifungal resistance. When local factors are associated with the complications of systemic diseases, such as diabetes mellitus, an increase in the incidence of oral Candida disorders and the susceptibility of patients is observed. In fact, a large number of oral Candida species carriers have been reported in patients with diabetes. The oral candidiasis progression is also often faster and more severe in patients with diabetes, because their immune systems are often deficient enough to predispose to Candida colonization and other oral diseases. Other predisposing factors modulate individual risk of infections in patients with diabetes. The salivary glucose levels found in patients with diabetes favor yeast growth due to increased number of available receptors for Candida. Consequently, buccal cells from these patients have shown a greater adherence to Candida yeasts. Their reduced salivary flow rate also promotes the oral carriage of Candida.

The emergence of antifungal resistance among Candida species isolates is another factor that can contribute to Candida infections. Clinically, antifungal resistance is defined as persistence or progression of an infection despite appropriate antimicrobial therapy. Copious references in the literature have been made to the development of resistant yeasts, which could be

Statement of Clinical Relevance

These findings confirm the resistant susceptibility profile of C glabrata isolates against azole antifungals, especially itraconazole, in persons with diabetes and denture stomatitis. Thus, surveillance of antifungal susceptibility can be helpful in determining optimal therapeutic approaches for these individuals.
related to the uncontrolled prescription of medications, especially azoles. According to Goldman et al.,19 resistance among Candida yeasts can be associated with genetic mutation. The authors identified 18 mutations in fluconazole-resistant isolates of C albicans from patients with acquired immunodeficiency syndrome (AIDS).19 Other authors20,22 found that exposure to antifungal agents provided a positive selection pressure for non-albicans yeasts, described as the replacement of fluconazole-susceptible C albicans strains with other species, for example C glabrata, C krusei, and C dubliniensis. There are also species that are considered intrinsically less sensitive,13 such as C glabrata and C krusei. Regardless of the mechanism of Candida resistance, studies have found differences in the susceptibility against several drugs that were dependent on the Candida species involved in the infection and the underlying disease.14-17,20,21,23 In general, non-albicans Candida species are less susceptible to antifungals than is C albicans.17,20,23 In addition, it was found that clinical isolates of Candida from patients with human immunodeficiency virus (HIV)16,20 and from patients with diabetes14,15 have a higher resistance to antifungals than do isolates from individuals without systemic complications. This resistance has important clinical implications, because the non-albicans Candida species have been isolated with increasing frequency from patients with Candida-associated denture stomatitis,2,4 including those with diabetes,3,4,8 Moreover, it may be related to the failure of therapies,16,24 recurrence of infections,18 and higher levels of mortality.21

Currently, oral candidiasis is commonly treated by means of topical or systemic approaches. The most common antifungal drugs are polyenes (amphotericin B and nystatin) and azoles (fluconazole and itraconazole).1 More recently, a new class of antifungals, the echinocandins (such as caspofungin),25 has been found to eliminate Candida species.26,27 The increasing number of clinical isolates resistant to these antifungal therapies highlights the need for antifungal susceptibility testing to monitor the antifungal resistance of these microorganisms. As pathogenicity and antifungal susceptibility vary among strains, an accurate identification of the susceptibility profile of the disease-causing strain of Candida could guide the therapeutic choice and the clinical treatment. In addition, an accurate identification of strains isolated from infections in patients with diabetes is especially important, because these patients are more likely to carry species other than C albicans,8 which may be less sensitive to antifungal agents. Although there have been numerous studies14-24 evaluating the susceptibility profile of Candida species against various drugs, there have been only a few that evaluated the susceptibility of clinical isolates obtained from the denture biofilms of healthy subjects28,29 or the oral cavity of patients with diabetes.14,15,30 In addition, to the authors’ knowledge, this is the first study that evaluated the susceptibility of clinical isolates obtained from the denture biofilm of patients meeting specific criteria for type 2 diabetes and Candida-associated denture stomatitis. Thus, the aim of the present study was to characterize the susceptibility profile of a Brazilian stock collection of 198 clinical isolates of oral Candida species. In addition, this study aimed to compare the susceptibility of clinical isolates of Candida among different sources: healthy subjects and subjects with Candida-associated denture stomatitis with or without diabetes.

**MATERIALS AND METHODS**

**Clinical isolates of Candida**

This study was conducted with 198 clinical isolates of Candida obtained from the yeast stock collection of the Laboratory of Applied Microbiology (Laboratório de Microbiologia Aplicada) at Universidade Estadual Paulista (UNESP) in Araraquara, São Paulo, Brazil. These isolates were previously obtained from the biofilm of the tissue surfaces of the dentures of edentulous patients with various systemic or oral conditions. Seventy-four clinical isolates were obtained from healthy subjects who were not diabetic and had no clinical signs of Candida-associated denture stomatitis5,11; 82 clinical isolates were obtained from subjects with Candida-associated denture stomatitis who were not diabetic5,11; and 42 clinical isolates were obtained from patients with diabetes and Candida-associated denture stomatitis.4 All procedures followed the criteria of Resolution 196/96 of the Brazilian Health Ministry, and the study was approved by the Ethics Committee of the Araraquara Dental School, UNESP.

The yeast identification procedures for Candida species included the presumptive identification on CHROMAgar Candida (CHROMAgar, Paris, France), the micromorphologic characteristics on corn meal agar with polysorbate 80 (Tween 80, Sigma-Aldrich Co LLC, St Louis, MO, USA) for the production of hyphae and chlamydomonidia, and the assimilation of a variety of carbon and nitrogen sources using the ID32C yeast identification system (bioMérieux SA, Marcy-l’Étoile, France).2,4,5,31 In addition, the hypertonic Sabouraud broth test2,3 was performed to discriminate C albicans and C dubliniensis. In general, 148 isolates were identified as C albicans, 30 as C glabrata, and 20 as C tropicalis. Four reference strains were used as the controls: C albicans ATCC 90028 (ATCC, Manassas, VA, USA), C albicans wild-type strain SC 5314, C glabrata ATCC 2001, and C tropicalis ATCC 4563. All isolates were maintained in a yeast-peptone-glucose medium (1% yeast extract, 2% Bacto Peptone [Becton Dickinson, Franklin Lakes, NJ, USA], and 2% D-glucose with 2% agar) and frozen at −70°C until use.
Culture conditions and standardization

The microorganisms were subcultured onto Sabouraud dextrose agar (Acumedia Manufactures Inc, Baltimore, MD, USA) plates supplemented with chloramphenicol (0.05 g/L) and incubated at 37°C for 24 to 48 hours. To prepare the yeast inocula, a loopful of the agar stock cultures was transferred to 5 mL of yeast nitrogen base broth (BD Difco; Becton Dickinson) supplemented with 100mM glucose and incubated at 37°C overnight in an orbital shaker (75 rpm). Cells of the resultant cultures were harvested and washed twice with phosphate-buffered saline (pH 7.2) at 5000 x g for 5 minutes. Washed microorganisms were resuspended in sterile saline solution and spectrophotometrically standardized at an optical density of 520 nm (BioSpectro, Equipar Ltda, Curitiba, Paraná, Brazil) to a final concentration of 10⁶ cells/mL.

Antifungal susceptibility test

*Candida* species strains were analyzed with regard to their susceptibility to 4 antifungal agents based on the minimal inhibitory concentration (MIC) in the Etest (bioMérieux SA). The following drugs and their respective minimum and maximum concentrations were used: caspofungin (CS) (concentration range, 0.002 to 32 μg/mL), amphotericin B (AP) (0.002 to 32 μg/mL), itraconazole (IT) (0.002 to 32 μg/mL), and fluconazole (FLU) (0.016 to 256 μg/mL).

For the assays, Roswell Park Memorial Institute (RPMI) agar supplemented with 2% glucose was used as the test medium. An RPMI medium (Sigma-Aldrich Co LLC) was prepared using 10.4 g of RPMI, 38.16 g of MOPS 0.165M (3-(N-Morpholino)propanesulfonic acid), and 20 g of glucose in 1 L of distilled water. The pH of this solution was adjusted to 7.0 and it was sterilized by filtration in a 0.22 μm membrane. The agar medium was prepared using 18 g of agar in 1 L of distilled water. This solution was autoclaved, cooled down to 50°C, and added to the RPMI medium. The yeast suspensions of 10⁶ cells/mL were spread uniformly on RPMI agar plates with sterile swabs and allowed to dry for 15 minutes. Thereafter, the Etest strips containing CS, AP, IT, and FLU were placed on the inoculated plates with sterile forceps. The plates were incubated for 48 hours at 37°C, after which the MICs were read as the lowest concentration at which the border of the elliptical inhibition zone of growth intercepted the scale on the Etest strip. For the MIC values that yielded results falling in between conventional serial 2-fold dilution, the next highest dilution was assigned.

Category MIC breakpoints

MIC interpretive criteria for CS, FLU, and IT were applied according to the Clinical and Laboratory Standards Institute (CLSI) document M27-A3. For AP, the interpretive breakpoints proposed in the literature were used, because the CLSI has not yet established these values. The MIC breakpoints of each antifungal for susceptible, susceptible—dose dependent, and resistant isolates are described in Table I.

### Table I. Interpretive breakpoints of MIC adopted in the present study

<table>
<thead>
<tr>
<th>Antifungal</th>
<th>Susceptible</th>
<th>Susceptible—dose dependent</th>
<th>Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caspofungin</td>
<td>≤2 μg/mL</td>
<td>Not applicable</td>
<td>&gt;2 μg/mL</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>≤1 μg/mL</td>
<td>Not applicable</td>
<td>&gt;1 μg/mL</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>≤0.125 μg/mL</td>
<td>≤0.25 μg/mL ≤ MIC</td>
<td>≥1 μg/mL</td>
</tr>
<tr>
<td></td>
<td>μg/mL</td>
<td>≤0.5 μg/mL</td>
<td></td>
</tr>
<tr>
<td>Fluconazole</td>
<td>≤8 μg/mL</td>
<td>16 μg/mL ≤ MIC ≤ 32 μg/mL</td>
<td>&gt;64 μg/mL</td>
</tr>
<tr>
<td></td>
<td>≤32 μg/mL</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

MIC, minimal inhibitory concentration.

Statistical analysis

The variable of interest in the present study was the MIC value of the clinical isolates expressed in micrograms per milliliter (μg/mL). The factors analyzed were the different antifungals tested (CS, AP, IT, and FLU); the 3 *Candida* species (*C. albicans*, *C. glabrata*, and *C. tropicalis*); and the sources of the isolates of *Candida*. The latter were clinical isolates from subjects without diabetes and without clinical signs of *Candida*-associated denture stomatitis (healthy subjects); subjects without diabetes and with *Candida*-associated denture stomatitis; and subjects with both diabetes and *Candida*-associated denture stomatitis. Data were transformed with the arc tangent function so that the assumptions of normality and homogeneity of variance were satisfied. Then, a factorial analysis of variance was used, and the significant differences were explored by a series of 2 x 2 comparisons to test the post hoc hypotheses of interest (α = .05).

RESULTS

Table II provides the descriptive statistics of the susceptibility profiles of all clinical isolates evaluated in the present study. All clinical isolates were susceptible to CS and AP, regardless of the *Candida* species and sources. The MIC values for CS ranged from 0.002 to 0.125 μg/mL for *C. albicans*, from 0.002 to 0.25 μg/mL for *C. glabrata*, and from 0.002 to 0.064 μg/mL for *C. tropicalis* strains. For AP, the MIC values ranged from 0.002 to 0.25 μg/mL for *C. albicans* and from 0.047 to 0.38 μg/mL for both the *C. glabrata* and *C. tropicalis* strains. In general, 3.4% of the clinical isolates of *C. albicans* (5/148) and 5% of those of *C. tropicalis* (1/20) were resistant to IT, with MIC...
values ranging from 4 to 32 μg/mL for the C  albicans  strains and 1 μg/mL for the C  tropicalis  strain. None of the 30 clinical isolates of C  glabrata  were susceptible to IT, and the MIC values ranged from 1 to 32 μg/mL. Considering the FLU susceptibility profile, resistance was not found among any clinical isolate. However, it was found that 3.4% of the clinical isolates of C  albicans  (5/148) and 60% of those of C  glabrata  (18/30) were susceptible—dose dependent to this medication, with MIC values ranging from 12 to 32 μg/mL and 16 to 48 μg/mL, respectively. The highest FLU MIC value of the C  tropicalis  strains was 4 μg/mL. Considering the control strains, isolates from all species were susceptible to all antifungals tested, with the exception of C  glabrata  ATCC 2001, which were resistant to IT. A representative image of the RPMI agar plate is shown in Figure 1.

Factorial analysis of variance (Table III) found that the effect of the antifungal and its interaction with Candida species on the MIC values was significant (P < .0001). There was no further statistical significance for the clinical sources of the isolates, Candida species, and their interaction (P > .05).

The 2 × 2 post hoc comparison test (Table IV) found that, for CS, IT, and FLU, the MIC values of C  glabrata  were the highest (P < .001). For CS and FLU, there were no significant differences between the MIC values of C  albicans  and C  tropicalis  (P = .106). For IT, the C  tropicalis  strains showed the lowest MIC values (P < .001). For AP, there was no significant difference between the MIC values of C  tropicalis  and C  glabrata  (P = .0521), which were higher than that of C  albicans  (P < .001).

**DISCUSSION**

In the present study, 4 antifungal agents were evaluated: 2 azole derivatives, a polyene, and an echinocandin. Considering the azoles, FLU and IT were tested because they are widely used for the treatment of several fungal infections, including oral candidiasis.1,35 Among all the antifungals tested, IT was the least effective, especially against C  glabrata. We found that 5 isolates of C  albicans  (3.4%), one of C  tropicalis  (5%), and 28 of C  glabrata  (93.3%) were resistant to this azole. An in vitro study28 also evaluated the susceptibility profiles of the clinical isolates of Candida from patients with denture stomatitis and found similar results: 5.9% of the isolates of C  albicans, 11.1% of those of C  tropicalis, and 33.3% of those of C  glabrata  were resistant to IT. The frequency of resistance against this azole seems to be increasing, and there are many reports of oral infections caused by resistant strains of non-albicans species of Candida.28,36-38 Despite the low resistance of C  albicans  isolates, the synergic relationship that exists between the Candida species can favor the colonization of more resistant strains, enhancing the infection process and the severity of the disease. A recent study39 evaluated the synergistic infection of a reconstituted human oral epithelium by C  albicans  and C  glabrata  and revealed that C  albicans  promoted the invasiveness of C  glabrata. The authors suggested that the damage to the integrity of the epithelial surface caused by the growing tips of the C  albicans  hyphae provided access to lower epithelial layers for C  glabrata  yeast by the vacuolized tissue portions.39 This finding may be supported by clinical studies that have found a coinfection of C  albicans  with C  glabrata, C  tropicalis, or both in oral fungal infections.2-5,40 Also, some of these in vivo studies found that both C  tropicalis  and C  glabrata  were associated with more severe oral infections. Therefore, we may wonder whether this synergic relationship may be responsible for the persistent and recurrent infections when resistant isolates, such as those found here, are involved. C  tropicalis  and C  glabrata  have the ability to cause fungemia in humans and are associated with a higher mortality rate than is C  albicans.41,42 Thus, the effect that the resistance of the non-albicans isolates, especially those of C  glabrata, may have in the treatment of these infections has to be highlighted. In the present study, the C  glabrata  clinical isolates had the highest MIC values for all the antifungals tested. Despite the results reported for the IT susceptibility among the clinical isolates, the findings showed that all isolates were susceptible to FLU. Other studies also investigated clinical isolates from oral candidiasis patients and failed to find Candida species resistance against FLU.28,29,43 Differences in the in vitro susceptibility to FLU have been reported in the literature, ranging from 70% to 100% of oral isolates, depending on the groups of patients studied.14,15,28,29,36-38,43,44 In fact, higher levels of resistance have been reported for Candida isolates from immunocompromised patients, such as those who are HIV positive46,38 or who have advanced cancer.37 We found that 3.4% of the C  albicans  and 60% of the C  glabrata  isolates were

**Table II.** Number of resistant, susceptible—dose dependent, and susceptible clinical isolates for each antifungal tested

<table>
<thead>
<tr>
<th>Antifungal</th>
<th>C  albicans  (n = 148)</th>
<th>C  glabrata  (n = 30)</th>
<th>C  tropicalis  (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluconazole</td>
<td>R: 0  S-DD: 5  S: 2</td>
<td>R: 0  S-DD: 5  S: 2</td>
<td>R: 0  S-DD: 5  S: 2</td>
</tr>
<tr>
<td>Capsofungin</td>
<td>R: 0  S-DD: 149  S: 30</td>
<td>R: 0  S-DD: 149  S: 30</td>
<td>R: 0  S-DD: 149  S: 30</td>
</tr>
</tbody>
</table>

C, Candida; R, resistant; S-DD, susceptible—dose dependent; S, susceptible.
susceptible—dose dependent to this drug, which is also in accordance with the literature.\textsuperscript{15,28,37} Because of its high cost, FLU has been used infrequently in Brazil, and this may have accounted for the elevated susceptibility of the clinical isolates observed here. The mechanism of action of azole derivatives such as IT and FLU is based on the inhibition of the synthesis of ergosterol,\textsuperscript{45,46} a major constituent of the fungal cell membrane. In addition, the exposure to azole antifungal agents reduced candidal adhesion to the buccal epithelial cells\textsuperscript{47-49} and denture acrylic surfaces.\textsuperscript{50} Another surprising mode of interaction between FLU and the Candida isolates is the fact that this drug decreased the production of phospholipase, an enzyme that plays an important role in the tissue invasion process and, consequently, in the pathogenicity of Candida species.\textsuperscript{39}

In addition to azoles, the polyenes and echinocandins, represented by AP and CS, respectively, were evaluated. The results showed that all of the 198 clinical isolates of Candida species were susceptible to AP and CS, regardless of the Candida species and clinical sources. The polylene AP has been widely used for the treatment of oral and disseminated candidiasis,\textsuperscript{1,45,46,51} and Candida resistance is rare. As observed in the present study, several investigations found that the clinical isolates of various Candida species (obtained from blood cultures of hospitalized patients\textsuperscript{23,27,34}, from oral cultures of patients with diabetes mellitus,\textsuperscript{14,15} AIDS,\textsuperscript{38} and denture stomatitis\textsuperscript{28}; and from oral cultures of patients wearing orthodontic appliances\textsuperscript{44}) were susceptible to AP. In fact, resistance has been only sporadically described in oral isolates from children with HIV\textsuperscript{36} and from patients with advanced cancer,\textsuperscript{37} indicating the broad-spectrum activity of this drug against Candida isolates, even in immunocompromised individuals. The high efficacy of the polyenes is related to their mechanism of action. Rather than inhibiting ergosterol synthesis, AP interacts with ergosterol and causes membrane leakage, resulting in cell death.\textsuperscript{45,46} AP is considered the gold standard for the treatment of invasive fungal infections. However, this polylene has a limited clinical application because of its severe renal toxicity\textsuperscript{45,46} and the lack of a preparation for topical use.\textsuperscript{36} In addition, the tolerability of the oral-rinse AP product has been found to be limited, resulting in noncompliance.\textsuperscript{51}

\begin{table}
\caption{Mean MIC values for the three Candida species, regardless of the clinical source.}\\
\begin{tabular}{lcccc}
\textbf{C} \textit{albicans} & \textbf{Caspofungin} & \textbf{Amphotericin B} & \textbf{Itraconazole} & \textbf{Fluconazole} \\
0.029\textsuperscript{A} & 0.125\textsuperscript{A} & 0.898\textsuperscript{A} & 0.904\textsuperscript{A} \\
0.066\textsuperscript{B} & 0.228\textsuperscript{B} & 10.383\textsuperscript{B} & 17.267\textsuperscript{B} \\
0.024\textsuperscript{A} & 0.171\textsuperscript{B} & 0.306\textsuperscript{C} & 0.811\textsuperscript{A} \\
\end{tabular}
\end{table}

\begin{table}
\caption{Factorial analysis of variance for the transformed MIC values.}\\
\begin{tabular}{lcccc}
\textbf{Factors} & \textbf{SQ} & \textbf{DF} & \textbf{F} & \textbf{P} \\
Intercept & 1.39 & 1 & 42.56 & .0000 \\
\textit{Candida} species & 0.13 & 2 & 2.02 & .1338 \\
Antifungal & 2.16 & 3 & 22.04 & <.0001* \\
Source of clinical isolate & 0.11 & 2 & 1.73 & .1773 \\
\textit{Candida} species \times \textit{antifungal} & 13.11 & 6 & 66.94 & <.0001* \\
\textit{Candida} species \times source & 0.09 & 4 & 0.71 & .5834 \\
Antifungal \times source & 0.36 & 6 & 1.85 & .0872 \\
\textit{Candida} species \times antifungal \times source & 0.42 & 12 & 1.08 & .3726 \\
Residual & 24.68 & 756 & & \\
\end{tabular}

\textit{MIC}, minimal inhibitory concentration; \textit{F}, F-Statistics; \textit{SQ}, sum of squares; \textit{DF}, degrees of freedom. \\
*Significant difference, \textit{P} < .05.
As stated earlier, all clinical isolates evaluated in this study were also susceptible to CS. In agreement with our results, there are some reports in the literature that found that this drug had efficacy against fluconazole-resistant Candida and to several clinical isolates, including C. albicans, C. tropicalis, C. glabrata, and C. krusei. In addition, clinical studies found that this echinocandin was effective for the treatment of esophageal and invasive candidiasis, including in patients who were HIV positive. However, contrasting results have also been reported. In an epidemiologic study from 100 blood isolates of Candida collected from candidemia patients, Motta et al. found 2 C. parapsilosis strains resistant to this drug. Pfaffer et al. evaluated 5346 Candida species isolates and found a susceptibility rate of 99% to CS. It is important to mention that, although it is expensive, CS is well tolerated by patients and has few drug-drug interactions. It may be worth noting that both CS and lipid formulations of AP have also been found to be active against Candida biofilms. The mode of action of echinocandins is based on the selective inhibition of the synthesis of glucan, an essential component of the fungal cell wall. Moreover, CS has been found to reduce the adhesion of Candida species to human cells by about 40% to 90%, depending on the time of exposure and drug concentration. This alternative mechanism of action was also observed for AP, which reduced the ability of oral C. albicans isolates to adhere to denture acrylic surfaces. Considering the importance of Candida adhesion to buccal cells and denture surfaces during the infectious process, this is another relevant mechanism of action of these medications.

In the present study, the clinical sources of the isolates had no effect on the MIC values obtained for all antifungals tested. No significant differences in MIC values were found among the clinical isolates obtained from healthy denture wearers and those with oral candidiasis with or without diabetes. This finding is in agreement with previous data, in which Candida strains isolated from healthy patients and from patients with oral candidiasis had an identical susceptibility profile. In those studies comparing the susceptibility profiles in subjects with and without diabetes, conflicting results were obtained. Although Manfredi et al. found no differences in antifungal susceptibility between the Candida isolates from diabetic and nondiabetic subjects, Al-Attas et al. and Bremenkamp et al. found that isolates from subjects with diabetes had higher rates of resistance to fluconazole, fluconazole, ketoconazole, miconazole, and econazole than did those from healthy controls. It is important to mention that this variability may be related to the drugs tested. In these latter studies in patients with diabetes, AP and CS were not evaluated. Another reason for the variability may be the methods used in the investigations. The classical microdilution method described in the CLSI M27-A3 standard is widely used and is still considered the gold standard for susceptibility tests. Nevertheless, in addition to being laborious and time-consuming, the method seems not to be the most sensitive and reliable for detecting resistance against some antifungals, such as AP. To overcome these limitations, a number of rapid and easy-to-use commercial products have been proposed to test antifungal susceptibility, such as Fungitester, disk diffusion test, and Etest. In the present investigation, the Etest kit was used because it is a cost-effective, simple, accurate, reliable, and precise method that has had an excellent agreement with other tests, such as the microdilution and disk diffusion assays.

The widespread use of systemic antifungal agents for both treatment and prophylactic purposes has resulted in an increase in antifungal resistance and in a noticeable shift toward Candida species other than C. albicans with higher resistance, as observed by the present study. The resistance of Candida isolates to currently available antifungals is a relevant factor because it may have implications for morbidity and mortality. A prospective study found higher mortality rates in patients infected with fluconazole resistant Candida yeasts when compared with those infected with fluconazole susceptible yeasts (19.0% and 8.6%, respectively). With this in mind, in vitro susceptibility tests can be highly relevant for the patients who do not respond to conventional antifungal treatments. The great advantage of the tests, including the Etest, is that they can allow a quick answer regarding Candida resistance to antifungals, guiding clinicians in selecting the most appropriate anti-Candida agent and preventing drug misuse. In addition, the association of an in vitro susceptibility test with the clinical use of an antifungal agent may contribute to a more effective treatment of candidiasis. In the present study, all clinical isolates were susceptible to CS and AP. Although these medications are not the first choice in treating patients with Candida-associated denture stomatitis, they can be an effective alternative if topical or other systemic drugs have definitely failed.

REFERENCES


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