Species distribution and antifungal susceptibility to vulvovaginal Candida spp. in southern Mato Grosso State, Brazil

Distribuição de espécies e suscetibilidade a antifúngicos de Candida spp. vulvovaginais no sul de Mato Grosso, Brasil

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ABSTRACT

Introduction: Vulvovaginal candidiasis is a prevalent opportunistic mucosal infection, caused predominantly by Candida albicans. Candida species vary in their susceptibility to the antifungal agents, thus, the susceptibility tests have clinical significance in determining the appropriate therapeutic choice. Objective: To investigate the distribution of vulvovaginal yeasts and the susceptibility pattern to azoles antifungal isolated in southern Mato Grosso State, Brazil. Material and methods: Clinical samples from 166 patients were obtained regardless signs and symptoms of vulvovaginal candidiasis. Vaginal swabs were collected, seeded onto plates containing Sabouraud Dextrose agar and incubated at 35ºC, for five days. A pool of colonies that grown on each plate was subcultured in CHROMagar Candida medium. On the basis of a pure culture, the yeasts were identified using traditional phenotypic identification methods. Susceptibility tests for antifungal fluconazole and ketoconazole were performed using the broth microdilution method according to the reference protocol M27A3 of the Clinical Laboratory Standards Institute (CLSI).

Results: The frequency of Candida spp. in the study population was 30%, of which 28% were in the group of asymptomatic women and 35% among symptomatic. Among the isolated strains were C. albicans (50%), C. glabrata (33%) and C. tropicalis (17%). The minimum inhibitory concentration (MIC) for fluconazole ranged from 0.5 µg/ml to 16 µg/ml and for ketoconazole from 0.03 µg/ml to 4 µg/ml. The resistance rates were 1.7% for fluconazole and 3.4% for ketoconazole.

Conclusion: C. albicans was the predominant species. We observed a high susceptibility of Candida spp. to fluconazole and ketoconazole antifungal.

Key words: Candida; vulvovaginal candidiasis; microbial susceptibility tests.

INTRODUCTION

Vulvovaginal candidiasis (VVC) remains one of the most frequent diagnoses in gynecology daily practice. It is estimated that 70%-75% of adult women between 20 and 30 years, have at least one episode of fungal vulvovaginitis in their life; among these, 40%-50% will develop new infections and 5%-8% will have recurrent vulvovaginal candidiasis(5). About 85%-95% of the Candida species that colonize the vagina are C. albicans. Candida species and strains differ in their pathogenicity, so that the development of candidiasis depends on the species involved in the infection, as well as host defense mechanisms(2).

The presence of Candida in the vagina, in the absence of immunosuppression or damaged mucosa, is usually not associated with any disease signs, and is named colonization. In contrast with asymptomatic colonization, VVC is defined as inflammation of the vulvovaginal mucosa in the presence of Candida and absence of other infectious etiologies(3). The clinical signs and symptoms of VVC include intense itching, vaginal discharge, vulvar erythema, and dyspareunia(1). Use of antibiotic, pregnancy, diabetes, high levels of estrogen, immunosuppression and genetic predisposition have been identified as important risk factors for this infection(1, 5, 6).

VVC is usually treated by azole antifungals and nystatin; the medicinal presentation used and the route of administration vary.
according to the severity of clinical manifestation(7). Currently, cases of resistance to antifungal drugs emerge among Candida species, in particular, Candida non-albicans(2). Factors such as excessive or abusive use of antifungal and increasing population of immunocompromised patients may contribute to the increased antifungal resistance. Therefore, the first step to overcoming this problem is to track locally the resistance data(8).

The available methods for susceptibility testing include those standardized by the Clinical and Laboratory Standards Institute (CLSI) in the United States and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) in Europe. As a result, these tests play an important role in therapeutic decision-making. Epidemiological studies that seek to analyze local and regional data may be used to develop empirical treatment strategies and are essential for monitoring of resistance trends(9, 10). In this study, we sought to evaluate the frequency, distribution of species and the susceptibility profile to antifungals of vulvovaginal Candida spp. isolated in the south of Mato Grosso State, Brazil.

MATERIAL AND METHODS

Population
The participants were women attended at family basic health units in the city of Rondonópolis (MT), regardless symptoms for VVC. The subjects were divided into two groups: symptomatic characterized by the presence of vaginal lumpy discharge, itching, edema and erythema of the vulva and vagina, and asymptomatic patients, who did not have the above-mentioned characteristics. Patients under 18 years of age, women using oral antifungal, or vaginal creams, ointments, antiseptics in the 15 days were excluded from the study. The sampling was not population, it was from a gynecology outpatient clinic, that is, by spontaneous demand.

Isolation and identification of Candida spp.
Swabs were collected from the vaginal mucosa of each patient in the period from September 2012 to June 2013; these were sowed in Sabouraud Agar (Himedia Laboratories, Mumbai, India) and incubated at 37°C for up to five days. A sample of colonies that grown on each plate was subcultured in CHROMagar Candida® medium (Difco, Le Point de Claix, France) in order to determine the species of the isolates, classical phenotypic tests germ tube and microculture were also performed.

Antifungal susceptibility tests
To determine whether the susceptibility profile to antifungal, the broth microdilution technique was performed according to CLSI M-27A3 protocol(11). The inoculum was used at a final concentration of $1.5 \times 10^7$ cells/ml in Roswell Park Memorial Institute (RPMI)-1640 medium (Gibco, Grand Island, New York, USA) incubated at 35°C for 48 hours. The antifungal tested were fluconazole in concentrations from 0.125 to 64 µg/ml and ketoconazole in concentrations ranging from 0.03 to 16 µg/ml. The minimum inhibitory concentration (MIC) was defined as the lowest concentration able to inhibit ≥ 50% of fungal growth, compared to the positive control. The results were expressed in terms of MIC, MIC50 and MIC90 variation, where MIC50 and MIC90 were considered the concentration capable of inhibiting 50% and 90% of the isolates, respectively. Assays were performed in duplicate.

According to the CLSI for fluconazole in C. albicans and C. tropicalis, it is considered susceptible (S) MIC ≤ 2 µg/ml, susceptible-dose dependent (SDD) MIC of 4 µg/ml and, resistant (R) ≥ 8 µg/ml; in C. glabrata, it is considered SDD ≤ 32 and resistant ≥ 64(12). The reference values for ketoconazole are not described by the CLSI, so we adopted the parameters described by Mulu et al. (2013)(13) that considered ≥ 4 µg/ml resistant.

Ethical aspects
The patients were informed about the protocol and after the voluntary agreement on their participation in the study they signed a free and informed consent. The study was approved by the Research Ethics Committee of the Hospital Júlio Muller, under the registration number CAE 01582312.2.0000.5541.

RESULTS
The participants of the study were 166 women, of which 118 (71%) were asymptomatic and 48 (29%) symptomatic for VVC. The frequency of Candida spp. in the study population was 30% (50/166), of which 28% (33/118) in the group of asymptomatic women and 35% (17/48) among the symptomatic patients.

After microbiological analyzes we obtained 58 isolates as eight women harbored two species of Candida, of which we identified four cases of C. tropicalis in association with C. glabrata and four of C. albicans with C. glabrata. C. albicans was the most prevalent species (50%), followed by C. glabrata (35%) and C. tropicalis (17%).
The susceptibility testing revealed that the MIC values for fluconazole ranged from 0.5 to 16 µg/ml, with MIC50 of 1 µg/ml and MIC90 of 4 µg/ml. MIC for ketoconazole ranged from 0.03 to 4 µg/ml, MIC50 was 0.06 µg/ml and MIC90 was 0.25 µg/ml (Table 1). The assays with fluconazole showed 60.3% (35/58) of isolated S, 38% (22/58) SDD and 1.7% (1/58) R. A percentage of 96.6% (56/58) of the strains were susceptive to ketoconazole and 3.4% (2/58), resistant. The resistance to fluconazole was observed in one C. tropicalis isolate, and to ketoconazole, in a sample of C. albicans and in another of C. glabrata (Table 2).

### TABLE 1 – Variation of the MIC to azole antifungals in clinical vaginal isolates of Candida spp. from women of the southern Mato Grosso State, Brazil

<table>
<thead>
<tr>
<th>Species (n)</th>
<th>Fluconazole (µg/ml)</th>
<th>Ketoconazole (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC variation</td>
<td>MIC50</td>
</tr>
<tr>
<td>C. albicans (29)</td>
<td>0.25-4</td>
<td>1</td>
</tr>
<tr>
<td>C. glabrata (19)</td>
<td>0.25-16</td>
<td>1</td>
</tr>
<tr>
<td>C. tropicalis (10)</td>
<td>0.25-16</td>
<td>1</td>
</tr>
</tbody>
</table>

MIC minimum inhibitory concentration.

### TABLE 2 – Susceptibility profile to azole antifungals in clinical vaginal isolates of Candida spp. from women of the southern Mato Grosso State, Brazil

<table>
<thead>
<tr>
<th>Species (n)</th>
<th>Fluconazole n (%)</th>
<th>Ketoconazole n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S</td>
<td>SDD</td>
</tr>
<tr>
<td>C. albicans (29)</td>
<td>27 (93.1)</td>
<td>2 (6.9)</td>
</tr>
<tr>
<td>C. glabrata (19)</td>
<td>0 (0)</td>
<td>19 (100)</td>
</tr>
<tr>
<td>C. tropicalis (10)</td>
<td>8 (80)</td>
<td>1 (10)</td>
</tr>
<tr>
<td>Total (58)</td>
<td>35 (60.3)</td>
<td>22 (38)</td>
</tr>
</tbody>
</table>

S: susceptible; SDD: susceptible-dose dependent; R: resistant.

**DISCUSSION**

VVC shows broad spectrum of clinical manifestations varying from colonization in asymptomatic patients to severe acute symptomatic infections, and its frequency is estimated by 30% in the first group and 39% in the second[13]. In this research, *Candida* spp. was present in 30% of women studied, with a prevalence of 28% as a comensal, and 35% as a pathogen. *C. albicans* is the predominant species in the vaginal mucosa with values ranging from 42.5% to 91.4%(14-18). In our research, *C. albicans* corresponded to 50% of the isolates, similar values were reported by a research conducted in Iran[19] (47.2%), in the Turkey[20] (50%), and in the United States[21] (50%).

*C. glabrata* was the second species most commonly found in the study population, matching with the results of other authors[14-17]. This microorganism has arise in recent years as an emerging pathogen, and is related to the VVC recurrent[21]. The increase of incidence of VVC caused by non-*albicans* species is associated with factors such as single-dose treatment, regimens based on low-dose antifungal and use of antifungal agents without prescription[19].

Vulvovaginal infections by *C. krusei* are relatively rare[21, 22], with frequencies of this yeast in vaginal specimens of 1.6%(20), 1.9%(21) and 3.35%(24). In our study, this species was not isolated, but our results are in agreement with previous studies, which used molecular biology techniques to determine the *Candida* species present in the vaginal mucosa and that, similarly, did not identify *C. krusei*(21, 24, 25). The distribution of Candida species found in women with VVC varies greatly depending on the location as well as the study population[2, 20].

Usually, a single species of *Candida* is identified in vaginal discharge, but two or more species may be isolated from the same culture in a minority of women (2% to 5%)[25]. The detection of mixed infection in VVC is enabled by the use of chromogenic culture media, or facilitated by the isolation of more than one microbial colony sampling in each culture plate, when using non-traditional chromogenic culture media, such as Sabouraud Dextrose agar supplemented with chloramphenicol[20]. In our study, eight women (6.7%) harbored two species of yeast, other researchers also observed this phenomenon[20, 24, 25].

Due to the fact that *Candida* species present a varied response to antifungal agents commonly used in the clinic, and the event that some species have intrinsic resistance to antifungal therapy, represent an important impact on the management of infections caused by these yeasts[27]. Accordingly, the results of local susceptibility testing can provide effective guidelines for prophylaxis, empirical therapy and candidiasis management[28]. In this research, the M-27A3 methodology proposed by the CLSI proved to be reproducible and allowed to determine the epidemiological profile of susceptibility to fluconazole in vaginal clinical isolates of *Candida* species.

Cases of antifungal azole resistance in vaginal candidiasis are rare, differently than in oral candidiasis[13]. In our study, fluconazole resistance rate was 1.7% (one isolate of *C. tropicalis*), which is consistent with other research indicating high susceptibility of vaginal *Candida* isolates to this antifungal[17, 24, 25, 20]. In a study of women in southern China presenting VVC, the resistance to fluconazole in *C. albicans* was 1.1% and in *Candida non-albicans* was 11.9%(20). Zhang et al. (2014)[20] studied the susceptibility profile to the antifungal in the *Candida* species that colonize women with and without infection.
by the human immunodeficiency virus (HIV). The authors found that in the group of women without the virus, 100% of the isolates were susceptible to fluconazole, while among women with HIV, the susceptibility was 68%. The lower susceptibility to the drug in the HIV group was correlated with the frequent use of antifungal as therapeutic routine. Although fluconazole is active for most vaginal isolates of C. albicans, there are reports in the literature of resistance frequencies with values 12.3% to 21% (8, 18, 31).

In this study, ketoconazole was active against 96.6% of the samples. In another epidemiological survey conducted in the State of Mato Grosso, with vaginal strains obtained from pregnant and non-pregnant women, the susceptibility for ketoconazole was 100% in C. albicans, C. tropicalis and C. glabrata (28). Badiee et al. (2010) (32) found that all of the Candida strains isolated from the oral and vaginal mucous of HIV positive patients from Iran were suscepive to this antifungal. The analysis of 54 isolates of C. albicans and 19 of C. glabrata from Japanese women with de VVC showed 90% susceptibility to ketoconazole (33). The profile of susceptibility of yeast varies according to the population studied, previous therapy, the intrinsic characteristics of patient and the predisposing factors (8).

**CONCLUSION**

Our research allowed us to know the epidemiological profile of VVC in the southern Mato Grosso State, Brazil, in which Candida albicans was the predominant species. The high susceptibility of the Candida spp. strains to the antifungal fluconazole and ketoconazole became clear.

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**RESUMO**

*Introdução:* A candidíase vulvovaginal é uma prevalente infecção mucosa oportunista, causada predominantemente por Candida albicans. As espécies de Candida variam de acordo com a suscetibilidade aos agentes antifúngicos, assim os testes de sensibilidade têm importância clínica para uma adequada escolha terapêutica. **Objetivo:** Investigar a distribuição de leveduras vulvovaginais e o padrão de suscetibilidade a antifúngicos azólicos em isolados do sul de Mato Grosso. **Material e métodos:** Foram obtidas amostras clínicas de 166 pacientes, independentemente de sinais e sintomas para candidíase vulvovaginal. Swabs vaginais foram coletados, semeados em placas contendo agar Sabouraud e incubados a 35°C. Uma amostra das colônias que cresceram em cada placa foi subcultivada em meio CHROMagar Candida. Partindo de uma cultura pura, as leveduras foram identificadas por métodos fenotípicos clássicos. Os testes de suscetibilidade aos antifúngicos cetoconazol e fluconazol foram realizados, usando o método de microdiluição de acordo com o protocolo de referência M27A3 do Clinical Laboratory Standards Institute (CLSI).

**Resultados:** A frequência de Candida spp. na população em estudo foi de 30%, sendo 28% no grupo de mulheres assintomáticas e 35% entre as sintomáticas. As espécies isoladas foram C. albicans (50%), C. glabrata (33%) e C. tropicalis (17%). A concentração inibitória mínima (CIM) para o fluconazol variou de 0,5 µg/ml a 16 µg/ml e para o cetoconazol, de 0,03 µg/ml a 4 µg/ml. A frequência de resistência ao fluconazol foi de 1,7% e ao cetoconazol, de 3,4%. **Conclusão:** C. albicans foi a espécie predominante. Observamos elevada sensibilidade de Candida spp. aos antifúngicos fluconazol e cetoconazol.

**Unitermos:** Candida; candidíase vulvovaginal; testes de sensibilidade microbiana.

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**REFERENCES**


