ORIGINAL ARTICLE

ACCURACY IN CLINICAL EXAMINATIONS FOR THE DIAGNOSIS OF VULVOVAGINITIS BY Candida SPP. AND \textit{in vitro} SUSCEPTIBILITY TO THE MAIN ANTIFUNGALS

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ABSTRACT

Vulvovaginal candidiasis (VVC) is a common infection. This work aims to determine the positive predictive value (PPV) of the clinical diagnosis of VVC and to characterize \textit{Candida} species isolated from the vaginal mucosa. This cross-sectional study was conducted from February 2016 to February 2017 at the Gynecology and Obstetrics Outpatient Clinic of the Hospital das Clínicas, in Goiânia, Goiás State, Brazil. The study included samples of vaginal secretion from 55 women who complained of vaginal discharge and itching as their main symptoms. The PPV of the clinical diagnosis of VVC was estimated in comparison to the laboratory culture method. The phenotypic methods and molecular tests were performed to identify \textit{Candida} spp. \textit{In vitro} susceptibility of \textit{Candida} spp. isolates to fluconazole, itraconazole, clotrimazole, nystatin, and amphotericin B was determined using the broth microdilution assay. Yeast growth using the enzymes protease, phospholipase, and hemolysin was carried out in media containing respectively bovine albumin, egg yolk, and sheep erythrocytes. A PPV of 61.8\% (34/55) was determined. Among the 55 vulvovaginal samples collected, we identified 36 isolates in which \textit{C. albicans} was the most common species. High resistance to fluconazole and low minimal inhibitory concentration (MIC) values for clotrimazole, nystatin and amphotericin B were observed. All isolates were proteinase and hemolysin producers, while seven strains were phospholipase negative. The clinical diagnosis of VVC presented a moderate PPV, which meant that cultures had to be conducted in the laboratory to confirm infection. The high resistance to fluconazole and itraconazole indicated the importance of the \textit{in vitro} susceptibility test.

KEY WORDS: Vulvovaginal candidiasis; antifungal susceptibility; enzymatic activity
INTRODUCTION

Vulvovaginal candidiasis (VVC) is a common fungal infection among women of childbearing age. VVC is a global public health issue since an estimated two-thirds of adult women experience at least one episode of VVC during their lifetime, and 50% of them experience VVC more than once (Ghaddar et al., 2019). Recurrent episodes have been observed in approximately 9% of women (Foxman et al., 2013). The disease occurs because of predisposing factors such as uncontrolled diabetes, use of oral contraceptives, sexual activity, genetic predisposition, deficiencies in the immune system, hormone replacement therapy, and microbiota alterations that favor yeast multiplication (Sobel, 2013).

Although symptoms such as itching, burning, dyspareunia, dysuria, and the presence of whitish plaques are associated with candidiasis (Wölber et al., 2020), these are insufficient for the clinician to reliably determine the cause of infection by Candida sp. Itching is present in approximately 90% of patients, but only 35% to 40% of women with this symptom have VVC (Mendling, 2015). According to a Ministry of Health guideline the differentiation of bacterial vaginosis and vulvovaginitis by Candida should be determined. Fresh examination and Gram staining of vaginal secretion allow this distinction. However, examination of vaginal secretion by adding 10% KOH or Gram-stained smears or Giemsa smears shows that 50% of women with Candida spp vulvovaginitis exhibit false-negative results. Unfortunately, due to the lack of a fast, reliable, and inexpensive test to confirm the diagnosis of VVC clinicians adopt an empiric approach in which antimycotic therapy is prescribed (Ministério da Saúde, 2015).

Laboratory culture is considered the gold standard method for diagnosis, yet it is not routinely performed as it is time consuming and more expensive. According to Aniebue et al. (2018), clinical evaluation and laboratory culture of vulvovaginal specimens should be the standard diagnostic method to eliminate the risk of misdiagnosis and eliminate potential long-term consequences of incomplete treatment, balanitis in the males, and vulvodynia in the affected females (Buyukbayrak et al., 2010; Sobel, 2013; Mendling, 2015; Aniebue et al., 2018).

Although antifungal agents such as nystatin and azole derivatives are available to treat VVC, a reduction in the susceptibility of Candida species to these antifungal drugs has been observed, such as Candida spp resistance to azoles (Sobel et al., 2013; Sobel & Sobel, 2018; Bitew & Abebaw, 2018). This characteristic highlights the importance of in vitro susceptibility testing for this yeast. In this study, the objectives were to determine the positive predictive value (PPV) of the clinical diagnosis of VVC, characterize Candida species isolated from the vaginal mucosa, verify the in vitro susceptibility profile to antifungal agents, and determine the virulence factors considering the production of hydrolytic enzymes by Candida species.
MATERIALS AND METHODS

**Study design**

This cross-sectional study was conducted from February 2016 to February 2017 at the Gynecology and Obstetrics Outpatient Clinic of the Hospital das Clínicas, in Goiânia, Goiás State, Brazil. The study included samples of vaginal secretion from 55 women who complained of vaginal discharge and itching as their main symptoms. Previous use of antifungals was considered an exclusion criterion, except in cases of recurrent vulvovaginal candidiasis, when the patient’s clinical history presented more than three episodes during the previous twelve months.

Questionnaires were used to obtain data on age, marital status, and personal history regarding use of contraception, recent use of antibiotics, pregnancy, and diabetes. The study was reviewed and approved by the Ethics Committee of Hospital das Clínicas da Universidade Federal de Goiás (HC/UFG) under the number 1.374.728 on December 17, 2015.

**Collection, culture, and identification**

For each patient with clinical diagnosis of vulvovaginal candidiasis (VVC), vulvovaginal secretion was collected with sterile cotton swabs. The samples were stored in sterilized tubes containing 0.85% saline solution, at 8°C, until processing.

Vaginal swabs were streaked onto Sabouraud dextrose agar with chloramphenicol (ASD, Difco® Laboratories, USA) and incubated at 37°C for seven days.

**Phenotypic identification of Candida species**

Phenotypic identification of the recovered isolates was performed by germ tube test, corn meal tween-80 agar growth, subculture in CHROMagar Candida spp. medium (Difco® Laboratories, USA), auxanogram and zymogram assays (Kurtzman et al., 2011).

**Genotypic identification**

DNA from *Candida* species was extracted as described by Casali et al. (2003). Species identification were performed by amplifying an RPS0 gene fragment according to Martinez et al. (2010). The primers used in the study were described as follows: INT1(5’-AAGTATTT-GGGAGAAGGGAAAGGG-3’) and INT2 (5’-AAAATGGGCATTAAGGAAAAGAGC-3’) for *C.albicans*, CG1(5’-ACATATGTTTGCTGAAAAGGC-3’) and
CG2 (5′-ACTTTTTCTTAGTGTCAGGGACTTC-3′) for *C. glabrata*, CPF (5′-AGGGATTGCCAATATGCCTCCA-3′) and CPR (5′-GTGACATTGTGTAGATTCCTTG-3′) for *C. parapsilosis*, CTf (5′-TGATAGTTAGGAAGATCAGGTG-3′) and CTr (5′-AACATATCCCATGTGTGTTG-3′) for *C. tropicalis*, and CDf (5′-AGTATTGGGAGAGGGAAAGACC-3′) and CDr (5′-ACAGGGGAAGTCGATTTC-3′) for *C. dubliniensis*.

Amplification was performed in an automated thermocycler with a final volume of 25 µL containing 2.5 µL 10× buffer, 1 µl 50 mM of MgCl₂, 2.5 µl of Eco Taq polymerase (Ecogen®), 2.5 µl dNTPs (2.5 mM each; Sigma®, St. Louis, MO, USA), with each primer in 4-µM concentration. One microliter of DNA suspension (30-50 ng) was amplified in a PCR thermal cycler (Bio-Rad PTC-100®) with one cycle at 95°C for 5 min and then 35 cycles as follows: 30 s of denaturation at 95°C, 30 s of annealing at 56°C, and 90 s of primer extension at 72°C. PCR was completed by a final extension at 72°C for 10 min. The resulting fragments of amplified DNA were analyzed by electrophoresis through 1% agarose gel, stained with ethidium bromide, and visualized with UV light. A 100-bp ladder (Invitrogen® Life Technologies, USA) was used as a DNA Ladders.

**Antifungal susceptibility testing**

Antifungal susceptibility tests of the recovered isolates were performed according to Clinical and Laboratory Standards Institute (CLSI) documents M27-A3(2008) and M-59(2016). The drugs fluconazole (Pfizer®, United States), itraconazole (Janssen Pharmaceuticals®, Beerse, Belgium), clotrimazole (Medley®, Campinas, São Paulo), nystatin (Medley®, Campinas, São Paulo), and amphotericin B (National Chemical Pharmaceutical Union®, India) were evaluated. Each isolate was tested in duplicate and the visual readings were obtained after 48 h of incubation and compared with the growth control. The minimal inhibitory concentration (MIC) of azole derivatives was defined as the lowest concentration that inhibited 50% of the yeast growth and for the polyene derivatives as the lowest concentration that inhibited growth of the microorganisms. Based on the MIC results for fluconazole, itraconazole, and amphotericin B, the isolates were classified as susceptible or resistant according to the CLSI (2008, 2016). For clotrimazole, there are no established breakpoints; therefore, we considered MIC value thresholds, using a value 0.5 µg/mL for resistance (Pelletier et al., 2000). The MIC₉₀, which inhibited 90% of the isolates, was determined for nystatin. Quality control was performed using standard strain *C. parapsilosis* ATCC 22019.
Enzymatic activity

The evaluation of proteinase activity was performed according to Ruchel et al. (1982), using yeast carbon base (YCB) medium (1.7%) with 0.2% bovine serum albumin (BSA). The YCB-BSA was adjusted to pH 5.0, sterilized by filtration using Millipore membrane 0.22 μm, and added to previously autoclaved agar (1.8%). The medium was distributed in Petri dishes with surface inoculation of 10 μL yeast suspension containing $10^7$ cells/mL, and incubated at 37°C for 7 days. The proteolytic activity results in a zone around the colony, which corresponds to the hydrolysis of the BSA present in the medium.

The Price et al. (1982) method was used to verify phospholipase activity. The Sabouraud dextrose agar medium (1.2%; SDA, Difco Laboratories®, USA) containing 5 mM CaCl$_2$, 1 M NaCl was autoclaved and added with 8% egg yolk emulsion. The medium was distributed in Petri dishes, with surface inoculation of 10 μL yeast suspension containing $10^7$ cells/mL and incubated at 37°C for 7 days. The precipitation zone around the colony results by formation of a calcium complex with fatty acids. The activity of hemolysin was determined according to Manns et al. (1994). The medium containing Sabouraud dextrose agar supplemented with 3% glucose and 7% defibrillated sheep’s erythrocytes (Newprov®, Brazil) was distributed in Petri dishes. Subsequently, 10 μL yeast suspension containing $10^8$ cells/mL was inoculated in this medium. The plates were incubated for 24 h in 5% CO$_2$ atmosphere. Hemolytic activity was defined as the formation of a translucent halo around the colonies.

The enzyme activity tests were performed in triplicates and measured in terms of the ratio between the diameter of the colony and the total diameter of the colony, plus a precipitation zone around the colony, according to Price et al. (1982), and classified into four categories, according to Ramos et al. (2015). Pz value of 1 meant no enzymatic activity; between 0.99 and 0.70 low enzymatic activity; between 0.69 and 0.40 moderate activity and between 0.39 and 0.10 high activity.

Data analysis

The PPV was calculated by dividing the number of positive cases diagnosed clinically by the number of positive cases by laboratory culture, multiplied by 100.

The socio demographic and clinical information of the participants were inserted into a database and analyzed using SPSS 22.0 Statistical software (SPSS Inc.®, Chicago, IL, USA). The categorical variables were compared using the chi-square test or Fisher’s exact test. Statistical differences were considered significant when p < 0.05.
RESULTS

Vulvovaginal secretions totaled 55 samples collected from women with clinical suspicion of VVC, aged 14 to 64 years, with a mean age of 32.0 years (standard deviation ± 12.0 years). Among the 55 women, 34 had diagnostic confirmation by laboratory culture, presenting a positive predictive value (PPV) of 61.8% (Table 1). Of the women with positive cultures, 16.4% had had more than four episodes of the disease, characterized as recurrent. Among women with VVC, 94.4% were infected with one species of Candida, and 5.6% were infected with two species (C. albicans and C. glabrata in one case and C. albicans and C. tropicalis in the other cases). Candida albicans was the predominant species (75%) followed by C. glabrata (14%) and C. tropicalis (11%) (Figure).

Table 1. Distribution of age group, marital status and specialty of care for women with clinical suspicion of vulvovaginal candidiasis coming from Hospital das Clínicas, in Goiânia-GO, 2016-2017.

| Characteristics          | Total (n=55) | Culture                  |
|--------------------------|-------------|--------------------------|
|                          |             | positive (n=34)          | negative (n=21) | p-value |
| Age (in years)           |             |                          |                |         |
| Mean ± standard deviation| 32.0 ±12.0  | 29.7 ±9.7                | 35.6 ±14.5     | 0.074*  |
| Median (IIQ 25-75)       | 32 (21-38)  | 29 (21-35)               | 36 (22-43)     |         |
| Minimum/maximum          | 14/64       | 14/60                    | 15/64          |         |
| Marital status           |             |                          |                |         |
| Married                  | 36 (65.5)   | 23 (67.3)                | 13 (61.9)      | 0.430** |
| Single                   | 18 (32.7)   | 11 (32.4)                | 7 (33.3)       |         |
| Divorced                 | 1 (1.8)     | 1 (4.8)                  |               |         |
| Clinical specialty       |             |                          |                |         |
| General gynecology       | 22 (40.0)   | 11 (32.4)                | 11 (52.4)      | 0.514** |
| Family planning          | 18 (32.7)   | 13 (38.2)                | 5 (23.8)       |         |
| Pregnant                 | 10 (18.2)   | 7 (20.6)                 | 3 (14.3)       |         |
| Adolescent               | 4 (7.3)     | 2 (5.9)                  | 2 (9.5)        |         |
| Pelvic pain              | 1 (1.8)     | 1 (2.9)                  |               |         |
| Positive Predictive Value| 34/55 (61.8)|                         |               |         |

* T test (p<0,05). ** Chi-Square Test (p<0,05).
Figure. Agarose gel electrophoresis (1.2%), showing the amplification of the DNA of the species of C. albicans (1 and 3), C. glabrata (17R and 46) and C. tropicalis (7 and 12). M: 100 bp molecular marker (Invitrogen).

The main signs and symptoms observed were itching, burning, leukorrhea, dysuria, and dyspareunia. A higher proportion of women with pruritus was found among those diagnosed with candidiasis than with other diagnoses (p=0.020) (Table 2).

Table 2. Signals and symptoms associated to vulvovaginal candidiasis in Women from Hospital das Clínicas, in Goiânia-GO, 2016-2017.

| Signals and symptoms     | Total n (%) | Culture                      |
|--------------------------|-------------|------------------------------|
|                          |             | Positive (34) n (%)          | Negative (21) n (%) | p-value |
| Itching                  | 41 (74.5)   | 29 (70.7)                    | 12 (29.3)           | 0.020*  |
| Burning                  | 29 (52.7)   | 20 (69.0)                    | 9 (31.0)            | 0.249*  |
| Like-curd discharge      | 54 (98.2)   | 33 (61.1)                    | 21 (38.9)           | 1.000** |
| Dysuria                  | 27 (49.1)   | 18 (66.7)                    | 9 (33.3)            | 0.467*  |
| Dyspareunia              | 26 (47.3)   | 18 (69.2)                    | 80 (30.8)           | 0.284*  |

*Chi-Square Test (p<0.05). ** Fisher’s Exact Test (p<0.05). Significant differences in bold.

All 55 women received empirical treatment. Of the antifungal agents, 44 patients received fluconazole and 5 were treated with clotrimazole. Only 26 (59.1%) of the women treated with fluconazole and eight (30.9%) who used other antifungals were culture positive for Candida spp. (Table 3).
Table 3. Empirical treatment used for women with clinical suspicion of VVC and positive culture for Candida sp.

| Antifungal agents                  | Total n (%) | Culture               |
|-----------------------------------|-------------|-----------------------|
|                                   |             | positive n (%)        | negative n (%)       |
| Fluconazole                       | 41 (74.5)   | 23 (56.1)             | 18 (43.9)            |
| Clotrimazole                      | 5 (9.1)     | 3 (60.0)              | 2 (40.0)             |
| Miconazole                        | 2 (3.6)     | 2 (100)               | -                    |
| Cetoconazole                      | 1 (1.8)     | 1 (100)               | -                    |
| Secnidazole                       | 1 (1.8)     | 1 (100)               | -                    |
| Nystatin                          | 1 (1.8)     | -                     | 1 (100)              |
| Fluconazole/Cetoconazole          | 1 (1.8)     | 1 (100)               | -                    |
| Fluconazole/Miconazole            | 1 (1.8)     | 1 (100)               | -                    |
| Fluconazole/Secnidazole           | 1 (1.8)     | 1 (100)               | -                    |
| Itraconazole                      | 1 (1.8)     | 1 (100)               | -                    |

The in vitro activity of the antifungals showed that Candida species presented high resistance to fluconazole (13 C. albicans and 3 C. tropicalis) and to itraconazole (17 C. albicans and 2 C. tropicalis). For clotrimazole, 63.8% of the isolates presented a very low MIC (>0.03 µg/mL), and the MIC90 was 0.5µg/mL. Furthermore, five strains showed clotrimazole MIC values equal to 16 µg/mL (four C. albicans and one C. glabrata). Among the polyenic drugs, all isolates were susceptible to amphotericin B, and MIC with values 8 µg/mL were verified for nystatin for 94.4% strains (Table 4).

Incubation of Candida spp. colonies for 7 days at 37°C on BSA agar showed that out of 36 Candida spp. strains obtained from women suffering from different vulvovaginal infections, 11 strains had very high protease activity (Pz < 0.39), 24 presented moderate activity (0.69 > Pz < 0.40), and 1 had low activity (Pz > 0.69) as shown in Table 5.

For phospholipase activity, out of the 36 tested strains, 9 (7 strains of C. albicans and 2 strains of C. glabrata) were moderately positive, 20 (19 strains of C. albicans and 1 of C. glabrata), had low activity, while 7 strains (one C. albicans, two C. glabrata and four C. tropicalis) were negative (Table 5).
Table 4. Antifungal susceptibility profile (µg/mL) of Candida species isolated from vulvovaginal candidiasis.

| Species (No. de isolates) | Antifungal agent | MIC (µg/mL) | Susceptible | Resistant |
|--------------------------|------------------|-------------|-------------|-----------|
|                          |                  | Range       | GM          | n (%)     | n (%)     |
| All species (36)         | Fluconazole      | 0.12-64     | 4.75        | 22 (61.1) | 14 (38.9) |
|                          | Itraconazole     | 0.12-16     | 1.88        | 17 (47.2) | 19 (52.8) |
|                          | Clotrimazole     | 0.03-1.0    | 0.06        | 31 (86.1) | 5 (13.9)  |
|                          | Amphotericin B   | 0.12-2.0    | 0.64        | 36 (100)  | -         |
|                          | Nystatin         | 0.06-16     | 2.11        | -         | -         |
| C. albicans (27)         | Fluconazole      | 0.12-64     | 6.03        | 14 (51.9) | 13 (48.1) |
|                          | Itraconazole     | 0.12-16     | 2.27        | 10 (37.0) | 17 (63.0) |
|                          | Clotrimazole     | 0.03-1.0    | 0.06        | 23 (85.2) | 4 (14.8)  |
|                          | Amphotericin B   | 0.25-1.0    | 0.54        | 27 (100)  | -         |
|                          | Nystatin         | 0.06-8.0    | 1.70        | -         | -         |
| C. glabrata (5)          | Fluconazole      | 0.5-4.0     | 1.74        | 5 (100)   | -         |
|                          | Itraconazole     | 1.0-4.0     | 1.51        | 5 (100)   | -         |
|                          | Clotrimazole     | 0.03-0.5    | 0.07        | 4 (80.0)  | 1 (20.0)  |
|                          | Amphotericin B   | 0.5-1.0     | 0.25        | 5 (100)   | -         |
|                          | Nystatin         | 1.0-16      | 2.29        | -         | -         |
| C. tropicalis (4)        | Fluconazole      | 1.0-32      | 3.36        | 3 (75.0)  | 1 (25.0)  |
|                          | Itraconazole     | 0.5-1.0     | 0.70        | 2 (50.0)  | 2 (50.0)  |
|                          | Clotrimazole     | 0.03-0.5    | 0.07        | 4(100)    | -         |
|                          | Amphotericin B   | 1.0-2.0     | 1.4         | 4 (100)   | -         |
|                          | Nystatin         | 4.0-16      | 8           | -         | -         |

MIC: Minimal inhibitory concentration; GM: Geometric mean

The study of the hemolytic activity of vaginal Candida spp. isolates showed that after 48h of incubation in sheep blood agar, all strains degraded hemoglobin by erythrocyte lysis. Two strains presented a very high hemolysin activity and 34 showed moderate activity (Table 5).
Table 5. Enzymatic activity of *Candida* species isolated from the vaginal mucosa of women attended at the Hospital das Clínicas in Goiânia-GO.

| Enzymatic activity | *C. albicans* (n=27) | *C. glabrata* (n=5) | *C. tropicalis* (n=4) | Total (n=36) |
|--------------------|----------------------|---------------------|-----------------------|--------------|
|                    | n (%)                | n (%)               | n (%)                 | n (%)        |
| Proteinase         |                      |                     |                       |              |
| High               | 6 (22.2)             | 4 (80.0)            | 1 (25.0)              | 11 (30.6)    |
| Moderate           | 21 (77.8)            | -                   | 3 (75.0)              | 24 (66.7)    |
| Low                | -                    | 1 (20.0)            | -                     | 1 (2.8)      |
| Negative           | -                    | -                   | -                     | -            |
| Phospholipase      |                      |                     |                       |              |
| High               | -                    | -                   | -                     | -            |
| Moderate           | 7 (25.9)             | 2 (40.0)            | -                     | 9 (25.0)     |
| Low                | 19 (70.4)            | 1 (20.0)            | -                     | 20 (55.6)    |
| Negative           | 1 (3.7)              | 2 (40.0)            | 4 (100.0)             | 7 (19.4)     |
| Hemolysin          |                      |                     |                       |              |
| High               | 1 (3.7)              | 1 (20.0)            | -                     | 2 (5.6)      |
| Moderate           | 26 (96.3)            | 4 (80.0)            | 4 (100.0)             | 34 (94.4)    |
| Low                | -                    | -                   | -                     | -            |
| Negative           | -                    | -                   | -                     | -            |

Pz < 0.39 = high activity; Pz between 0.40 and 0.69 = moderate activity; Pz between 0.70 and 0.99 = low activity; Pz=1.0 = negative.

**DISCUSSION**

Candidiasis is a major vulvovaginal infection that mainly affects women of reproductive age at least once in their life span. The present study revealed vaginal disorders in women in their second and third decades. This group is within the reproductive age, which is considered a risk factor for VVC due to sexual activity.

In the present work, we verified a PPV of 61.8% when observing such signs and symptoms as vaginal pruritus, leucorrhea, dysuria, dyspareunia, and burning presented by women in comparison with laboratory cultures, the diagnostic gold standard for VVC. Some authors have verified different PPV
values. Farhan et al. (2017), found a PPV of 76.2% in women with vaginal disorders seen in Egypt, Vijayalakshmi et al. (2016), in the city of Pune, India, verified a PPV of 88.9%, however in Brazil, Rosa and Rumel (2004), verified a PPV of 43%. The World Health Organization recommends empirical treatment for women with abnormal vaginal discharge. However, signs and symptoms noted for clinical diagnosis are nonspecific and may be related to other vaginal infections, therefore microbiological diagnosis is the best method and avoids many women receiving antifungal therapy unnecessarily. Although all 55 women included in this work received antifungal therapy, 21 (38.2%) were negative culture for *Candida* spp. Antifungal therapy is associated with renal and hepatic complications and hypersensitivity reactions, in addition to increasing rates of microbial resistance (Sobel, 2013).

Although azole derivatives have been used to treat and prevent VVC, *in vitro* studies have shown *Candida* spp. resistance to these drugs. In our work, high resistance to fluconazole and itraconazole was detected. Brandolt et al. (2017) in the State of Rio Grande do Sul, Brazil, in 2013, reported resistance in 42% of the isolates to fluconazole, and in 48% to itraconazole. Kandeel and Elmitwalli (2017), reported resistance to fluconazole in 17.3% of *Candida* spp. isolates in Saudi Arabia. The *in vitro* susceptibility of an infecting organism to the antifungal agent plays an important part in determining successful therapy, once high MIC values may be associated with resistance, indicating the need for different treatment (Pfaller & Diekema, 2012). In this study, many participants (32.7%) received fluconazole treatment despite having negative cultures. Thus, the high rates of resistance to these azole derivatives demonstrated in the present study are worrying. Unfortunately, we did not follow these patients.

Resistance to antifungals, especially azole derivatives, is associated with indiscriminate use. Antifungals are widely applied in agriculture, in antifouling coatings, in wood preservation, in addition to being routinely used in empirical therapy and human prophylaxis (Sanguinetti et al., 2015; Fisher et al., 2018).

*C. albicans* resistance to clotrimazole has not been previously well documented. In our study, the isolates presented high susceptibility to this drug, with five resistant isolates. In cases of fluconazole-resistant strains, topical clotrimazole is an effective therapeutic option. According to Kasper et al. (2015), clotrimazole ovule application may lead to a rise in vaginal lactate levels and may affect growth and membrane integrity of the infecting *C. albicans* cells.

Taking values of MIC > 2 μg/ml as resistant, no isolates were resistant to amphotericin B. This high susceptibility to Amphotericin B may be explained by the fact that this drug is not a choice in the empirical treatment for VVC. Resistance to polyenic agents such as amphotericin B is not seen as a clinical difficulty, few cases of species resistant to this antifungal are reported (Sanguinetti et al., 2015).
The susceptibility parameters of *Candida* species associated with VVC to nystatin are not established. According to Consolaro et al. (2012), nystatin has excellent *in vivo* activity against *C. albicans*, but low success rates against non-albicans species. In this study, we found low MIC values for nystatin for all *C. albicans* strains, but values of 16 µg/mL for two non-albicans species. This drug has been used as one of the main topical treatments of VVC in Brazil; it is available in the Brazilian Unified Health System (SUS) and is also a relatively safe option for treating pregnant or lactating patients (Consolaro et al., 2012; Brandolt et al., 2017).

The identification of *Candida* species is relevant not only to compose epidemiological data, but also to aid therapeutic response. Phenotypic methods are not always accurate, in addition to requiring a long time for species identification, while genotypic tests have high specificity and are less time consuming (ElFeky et al., 2016; Bonyadpour et al., 2016; Nurat et al, 2016; Devadas, 2017).

The production of hydrolytic enzymes is an important virulence factor for establishing infection. Proteinases assist in the invasion of host tissues and the protection of yeasts against antifungal action and the host’s immune system (Rapala-Kozik et al., 2018), whereas phospholipases are related to membrane rupture at the time of host cell invasion, allowing the hyphal tip to enter the cytoplasm (Kantarciog & Yücel, 2002). The findings obtained in the present study demonstrated that all *Candida* spp. exhibited considerable proteinase activity. Similarly, Shirkhani et al. (2016) verified that the *Candida* spp. isolates from VVC exhibited high proteinase activity. In contrast, we found that 25% of *C. albicans* isolates had moderate phospholipase activity and no *C. tropicalis* isolates produced this enzyme. Shirkhani et al. (2016) demonstrated low phospholipase activities in *Candida* spp. isolates from VVC.

Although the hemolytic activity of *Candida* species has been poorly reported, the ability of these yeasts to use iron derived from hemoglobin from the production of erythrocyte lysis is associated with *Candida* spp. resistance (Figueiredo-Carvalho et al., 2017). In addition, iron depletion in *Candida albicans* enhances its sensitivity to several drugs, such as fluconazole, facilitating the drug’s fluidity and diffusion, rendering fungal cells susceptible (Figueiredo-Carvalho et al., 2017). The knowledge of the role of these enzymes in establishing the infection is relevant as they present a promising target for treating fungal infections caused mainly by resistant species of *Candida* (Figueiredo-Carvalho et al., 2017; Rapala-Kozik et al., 2018).

This study presents limitations as the samples of vaginal secretion from women who complained of vaginal discharge and itching as their main symptoms were insufficient to establish the prevalence of candidiasis in the entire female patient population of the Gynecology and Obstetrics Outpatient Clinic. Although strains resistant to fluconazole were detected in the present study and this was the main antifungal agent used in treatment, we did not...
perform the second collection to evaluate treatment progression. Each individual has peculiarities that interfere with therapy.

In conclusion, the results of this study showed \textit{C. albicans} to be the most common species as the cause of VVC. The clinical diagnosis of VVC presented a moderate PPV, which highlights the need for laboratory confirmation by culture of \textit{Candida} spp. isolates, which are important for the reliable diagnosis of VVC. The high resistance observed to azole derivative fluconazole and itraconazole indicates that the \textit{in vitro} antifungal susceptibility test must be performed before treatment is prescribed.

CONFLICTS OF INTEREST

The authors have no conflicts of interest relative to this article.

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