

CANDIDA SPECIES IN THE GENITAL TRACT OF WOMEN ATTENDING A UNIVERSITY HOSPITAL FOR GYNECOLOGICAL INTERVENTIONS

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ABSTRACT | Introduction: Vulvovaginal candidiasis occurs in about 75% of all women during reproductive age, and around half of those will have at least one recurrent episode. **Objectives:** To evaluate the occurrence of *Candida* species in the genital tract of women attending a clinic of Gynecology and Obstetrics at the University Hospital for gynecological interventions, related to signs and symptoms of infection and also to evaluate the susceptibility to antifungal agents of the *Candida* isolates. **Methods and materials:** Samples of vaginal secretions were taken of 128 women during gynecological and colposcopic examination, through sterile swab, to carry out culture for fungi. Susceptibility tests were performed for fluconazole, itraconazole, voriconazole, nystatin and amphotericin B. **Results:** Twenty (15.6%) patients had positive culture for yeasts identified as: *C. albicans* (57.1%), *C. glabrata* (19%), *C. parapsilosis complex* (4.8%), *C. lipolytica* (4.8%), *Trichosporon sp* (9.5%), and *Rhodotorula sp* (4.8%). Ninety women (70.3%) reported no symptoms and 38 (29.6%) were symptomatic. The most frequent complaints were: discharge, vulval itching, and dyspareunia. All isolates were susceptible to the antifungal agents tested, except for some isolates of *C. parapsilosis complex*, which showed in vitro resistance to itraconazole. **Conclusions:** Although *Candida* species were isolated in only some of the women, *C. albicans* was the most common species. The more frequent complaints were discharge and vulvar itching. Most of the isolates were susceptible to the antifungals tested.

Keywords: candidiasis; Women's health; Signs and symptoms.

INTRODUCTION

Diseases that affect the vulva and vagina can have different causes. Infectious vaginitis is mainly caused by bacteria, yeasts and *Trichomonas vaginalis*^{1,2}.

Among these diseases, vulvovaginal candidiasis (VVC) is the second most common infection, representing 20 to 30% of gynecologic diseases occurring in women aged 20 to 30 years³. VVC occurs in about 75% of all women during reproductive age, and around half of those will have at least one recurrent episode. It is seen more frequently in women in sexual activity, possibly due to colonizing microorganisms penetrating the epithelium⁴.

Candida genus comprises several species, of which only a few are able to cause human infections, and the species responsible for vulvovaginitis are mainly *C. albicans*, but also others like *C. glabrata*, *C. tropicalis*, *C. krusei* and *C. parapsilosis*¹.

Clinically, VVC is characterized by the occurrence of intense vulvar itching, vaginal discharge, dyspareunia, dysuria, edema and vulvovaginal erythema, as well as signs of inflammation accompanied by burning when urinating. The misdiagnosis of infectious agent may lead to incorrect treatment, resulting in permanent symptoms or a recurrence of infection^{5,6}.

Multiple factors contribute to the onset and development of VVC: genetic, hormonal, advanced age, diseases such as diabetes, stress, poor local hygiene, sharing underwear, tight clothing, use of antibiotics and factors related to the state of immunosuppression of the individual⁷. The transmission of infectious agents can occur through contact with mucous membranes and skin secretions of patients with *Candida* spp., or sickness, sexual contact, contaminated water and vertical transmission during childbirth^{2,8}.

The diagnosis of VVC is performed clinically, associating history with pelvic examination and the assistance of laboratory analysis of clinical specimens. Laboratory tests may be use secretions which are immediately analyzed microscopically. Fungal culture mainly contributes to determine the species causing vulvovaginitis and is the gold standard^{9,10}.

In women undergoing gynecologic intervention procedures, such as hysterectomy or perineoplasty, there are few studies that refer to infection or fungal colonization before or after those procedures. Then, this study aimed to evaluate the occurrence of *Candida* species in the genital tract of women attending a clinic of Gynecology and Obstetrics at the University Hospital for gynecological interventions, in order to relate signs and symptoms of infection and assess the susceptibility of the isolates to antifungal agents.

METHODS

This is an exploratory and quantitative study carried out at the clinic of Gynecology and Obstetrics at the Clinical Hospital of the Federal University of Uberlandia, Minas Gerais, Brazil. The sample included 128 non-pregnant women aged 18 years or more, who were sent to the service for gynecological interventions (hysterectomy or perineoplasty with sling) in the period from July to November 2013.

All study subjects agreed to participate and signed the free and informed consent form. The approach to the patients included interview and collection of vaginal secretion specimens to conduct culture for fungi, and after susceptibility testing to antifungal drugs. The study was approved by the Ethics Committee on Human Research of the Federal University of Uberlandia (number 307.615/2013, CAAE 14189513.5.0000.5152).

The collection of clinical specimens for research into fungal structures was done after gynecologic inspection. A sterilized swab with a long stem was used, which was introduced directly into the vaginal canal, in the vaginal fornix and endocervical region, gently rolled and placed in Stuart transport medium for further processing in the laboratory.

The samples contained in the swabs were plated sequentially on Petri dishes (90x15 mm) containing Sabouraud dextrose agar added by chloramphenicol, and in another dish containing chromogenic agar for

Candida (Pronadisa, Madrid, Spain). The plates were incubated at 30°C for up to 5 days, and growth of colonies was monitored daily. The green colored colonies on chromogenic agar for *Candida* were identified presumptively as *C. albicans*. Other species were identified using the classical methodology: germ tube, micromorphological analysis cornmeal agar-Tween 80, and auxanogram¹¹. If there was need, the identification was also made in Auxacolor™ commercial kit (Bio-Rad, Rio de Janeiro, RJ, Brazil) and/or FungiFast®. *C. albicans* and *C. dubliniensis* were differentiated by PCR utilizing specific primers, according to Estrada-Barraza et al.¹².

The susceptibility testing to antifungal agents was made by disk diffusion method in agar, according to the M44-A2 document of the Clinical Laboratory Standards Institute¹³. The disks used contained the following antifungals: fluconazole (25 µg, Cecon, São Paulo, SP, Brazil), itraconazole (10 µg, Cecon, São Paulo, SP, Brazil), voriconazole (1 µg, Bio-Rad, Marnes-la-Coquette, France), nystatin (100 U, Bio-Rad, Marnes-la-Coquette, France) and amphotericin B (1 µg Bio-Rad, Marnes-la-Coquette, France). The cutoff points for the halos were those indicated by the CLSI for fluconazole¹³, and when not available, the cutoffs listed in the manufacturer's instructions were used. The standard strains *C. parapsilosis* ATCC 22019 and *C. albicans* ATCC 90028 were used as controls.

Data were related in absolute and relative frequency distribution tables. The G and Fisher's exact test were used for the association between the colonization variables and predisposing factors for the development of vulvovaginal candidiasis. The statistical significance was defined at 95% ($P \leq 0.05$).

RESULTS

The women's ages ranged from 18 to 83 years, with an average and median of 48 and 46 years, respectively. Clinical specimens of twenty women (15.6%) showed positive culture. The isolates are

shown in Table 1. *C. albicans* was the most frequent species (57.1%), and in one patient there was association between *C. albicans* and *Trichosporon* sp.

Table 1. Frequency of yeasts isolated from vaginal secretions of women attending at Clinic of Obstetrics Gynecology of the Clinical Hospital of Uberlandia, Minas Gerais, Brazil, July to November 2013.

Yeast	Positive Samples (n=21)	
	n	%
<i>C. albicans</i>	12	57.1
<i>C. glabrata</i>	4	19.0
<i>C. parapsilosis</i> complex	1	4.8
<i>C. lipolytica</i>	1	4.8
<i>Rhodotorula</i> sp	1	4.8
<i>Trichosporon</i> sp	2	9.5

All isolates of *Candida* species¹⁸ were susceptible to fluconazole, voriconazole, nystatin and amphotericin B. For itraconazole, one (1/1) isolate of *C. parapsilosis* complex showed in vitro resistance; eight (8/12) isolates of *C. albicans*, three (3/4) *C. glabrata* and one (1/1) of *C. lipolytica* showed intermediate susceptibility to itraconazole.

Ninety (70.3%) women reported being asymptomatic and 38 (29.6%) had non-specific symptoms, which were compatible with VVC. The main complaints were discharge, vulval itching, dyspareunia, external dysuria and vaginal burning (Table 2). In relation to symptoms reported by patients with positive culture, nine had one or more signs and symptoms: seven reported discharge, two had vaginal burning, six had vulvar itching, six had dyspareunia and three had external dysuria. *Candida* species related to these symptoms were *C. albicans* (six women), *C. albicans* associated with *Trichosporon* sp (one woman), *C. glabrata* (one) and *C. lipolytica* (one).

Table 2 also shows that the discharge occurrences and dyspareunia were associated with the culture results ($P \leq 0.05$). Six women with a positive culture (30%) reported having simultaneously vaginal discharge, vulvar itching and dyspareunia.

The socio-demographic profile of the sample of the study population is shown in Table 3. Most women

lived in urban areas (96.8%) and were 40 years of age or older. Caucasian women who self-reported corresponded to 67.9% of the total, while black women accounted for 19.5%. Of the participants who had a positive culture for fungi, 80% reported having an active sex life.

DISCUSSION

Infections of the reproductive tract, including sexually transmitted infections, deserve special attention of public health¹⁴. Vulvovaginal candidiasis affects millions of women of all ages each year, causing great discomfort, negatively impacting sexual and emotional relationships^{6,14}.

In present study, 20 (15.6%) women had a positive vaginal culture for yeast. Compared to the literature, it is observed that this frequency is considerably lower than those reported in other studies (usually at a frequency greater than 30%)^{9,15,16}. This was probably because the sample population included in this study was of women attending the outpatient clinic of General Gynecology, which were forwarded by Health Services of the municipality, after screening by the primary care network gynecologist, requiring gynecological interventions (hysterectomy, perineoplasty with sling). Thus, it becomes important for this study to highlight the need for research and subsequent treatment of the infection, before any intervention of another kind, such as surgical.

Candida species were isolated in 90% (18/20) of samples which were positive for yeast. Some studies conducted in Brazil reported the incidence of *C. albicans* ranging from 53.2% to 81.25% of all the isolates, followed by *C. glabrata*^{2,15,16}. Uncommon species such as *Rhodotorula* sp and *Trichosporon* sp can be found associated with other species or genera, including *Candida*. Among *Candida* species, *C. lipolytica* was isolated in one patient; however, there is no confirmation in the literature about the pathogenicity of this species. Mardh et al.⁵ showed that the incidence is variable for different species, and that *Candida*-non-*C. albicans* species contribute to the high resistance to treatment. In addition, recent studies have showed that new species *C. glabrata* related, *C. nivariensis* and *C. bracarensis*, exist in the vaginal samples of patients with VVC¹⁷.

The complaints reported by women were vaginal discharge, vulval itching, dyspareunia, external dysuria, and vaginal burning, and were mainly referred by women who had positive culture for fungi. Regarding the symptoms, discharge and dyspareunia were statistically significant. Vaginal discharge was the most frequently reported by

Table 2. Frequency distribution of signs and symptoms reported by patients attending at Clinic of Obstetrics Gynecology of the Clinical Hospital of Uberlandia, Minas Gerais, Brazil, July to November 2013.

Signs and symptoms		Culture				Total	P	
		Positive (n=20)		Negative (n=108)				
		n	%	n	%			
Discharge	Yes	7	5.5	16	12.5	23	18.0	0.05
	No	13	10.1	92	71.9	105	82.0	
Vulvar itching	Yes	6	4.7	15	11.7	21	16.4	0.09
	No	14	10.9	93	72.7	107	83.6	
Dyspareunia	Yes	6	4.7	9	7.0	15	11.7	0.01
	No	14	10.9	99	77.4	113	88.3	
Disuria externa	Yes	3	2.3	8	6.3	11	8.6	0.37
	No	17	13.3	100	78.1	117	91.4	
Burning	Yes	2	1.6	7	5.5	9	7.1	0.62
	No	18	14.0	101	78.9	119	92.9	

Table 3. Sociodemographic conditions of 128 women attending the Clinic of Obstetrics Gynecology at the Clinical Hospital of Uberlandia, Minas Gerais, Brazil, July to November 2013.

Data		Culture				Total (n=128)	
		Positive (n=20)		Negative (n=108)		n	%
		n	%	n	%		
Address	Countryside	0	0	4	3.1	4	3.1
	Urban area	20	15.6	104	81.3	124	96.8
Age (years)	18 – 29	3	2.3	13	10.2	16	12.5
	30 – 29	5	3.9	23	18.0	28	21.9
	40 – 49	4	3.1	30	23.4	34	26.5
	50 or more	8	6.3	42	32.8	50	39.1
Ethnicity	White	13	10.2	74	57.8	87	68.0
	Black	5	3.9	20	15.6	25	19.5
	Mestizo	2	1.6	13	10.1	15	11.7
	Indigenous	0	0	1	0.8	1	0.8
Sexually active	Yes	16	12.5	82	64.1	98	76.6
	No	4	3.1	26	20.3	30	23.4

patients (see Table 2). This sign is one of the most common concerns among women, especially those of reproductive age¹⁸.

It is noteworthy that the clinical symptoms of VVC are non-specific and can be associated with a variety of other diseases and vaginal infections such as bacterial vaginosis, trichomoniasis, gonorrhea and chlamydia. It is also noted that many genital infections are asymptomatic; therefore, laboratory tests are important in the diagnosis and identification of the causative organism^{1,19}. Some species, such as *C. glabrata* and *C. krusei* may not respond to treatment when fluconazole is chosen as the antifungal. *C. glabrata* can develop resistance during treatment, and *C. krusei* is intrinsically resistant to this antifungal²⁰.

It is also worth mentioning that sexual partners may be important reservoirs of *Candida* species and be related to the maintenance of vulvovaginal candidiasis¹⁹. However, sexual partners of women with VVC do not need to be treated unless they have symptoms¹⁴.

Preventing VVC can be achieved with the right treatment, followed by changes to behavior and habits. The health education actions for guidance include proper genital hygiene, use of appropriate clothing for the weather, vaginal douches, use of intimate deodorants and soaps, and even safe sex practices, all of which enable the prevention or the exacerbation of symptoms⁷.

With this study, we concluded that the occurrence of VVC in the population is lower than that reported in the literature and *C. albicans* was the most common fungus isolated in cultures. The signs and symptoms most frequently reported among patients with VVC were vaginal discharge and dyspareunia. Regarding antifungals, all isolates showed in vitro susceptibility for most of the drugs tested.

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AUTHOR CONTRIBUTIONS

Felix TC contributed to the preparation and elaboration of the project, the execution of the methodology, tabulation of the data and in writing the article. Menezes RP contributed in the execution of the methodology, in the writing of the article and also in the preparation of the final version. Berardi MC contributed in the execution of the methodology and has contributed in the preparation of the final version. Röder DVDB contributed in the design of the research, in the discussion of the results and also in the critical analysis of the final version of the text. Pedroso RS contributed in the design of the research, in the discussion of the results, in the writing of the article and also in the critical analysis of the final version of the text.

COMPETING INTERESTS

No financial, legal or political competing interests with third parties (government, commercial, private foundation, etc.) were disclosed for any aspect of the submitted work (including but not limited to grants, data monitoring board, study design, manuscript preparation, statistical analysis, etc.).

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