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Prevalence of vulvovaginal candidiasis in Amazonas, Brazil

Prevalência de casos de candidíase vulvovaginal no Amazonas, Brasil

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ABSTRACT

Vulvovaginal candidiasis (VVC) is caused by abnormal growth of yeast on the female genital mucosa. We aimed to analyze the prevalence of VVC in Manaus, its association with pre-exposure factors and antifungal resistance. Study conducted in the public healthcare, involved 1226 women aged 13-84 years. The yeasts were isolated and identified using Candifast test. Symptomatic VVC patients were grouped in symptomatic with one episode in year (SVVC) and symptomatic with recurrent VVC (RVVC), had two or more episodes in year. The total vaginal yeast isolation occurred in 231 women (18.86%). Mixed resistance was observed in 39% of patients. The RVVC group (21.6%) had a higher percentage of positive test for *Candida* spp. than the symptomatic non-recurrent group (13%). The prevalence of non-*albicans* spp. demostrated the importance of identify the *Candida* spp. present in infected patients.

Keywords: *Candida*. Vulvovaginal. Candidiasis. Symptomatic. Fungal. Mycosis.

RESUMO

A candidíase vulvovaginal (CVV) é causada pelo crescimento anormal de leveduras na mucosa genital feminina. O objetivo do trabalho foi analisar a prevalência da CVV na cidade de Manaus, associação com fatores pre-exposição e a resistência a antifúngicos. O estudo foi realizado na rede pública de saúde, com participação de 1.226 mulheres na faixa etária de 13 a 84 anos. As leveduras foram isoladas e identificadas pelo teste comercial Candifast. Os pacientes sintomáticos com CVV foram agrupados em sintomáticos com um episódio no ano (SVVC) e sintomáticos com CVV recorrente (RVVC), sendo as que apresentaram dois ou mais episódios no ano. O isolamento das leveduras ocorreu em 231 mulheres (18,86%). A resistência mista foi observada nos isolados de 39% das pacientes. O grupo RVVC (21,6%) apresentou maior porcentagem de teste positivo para *Candida* spp. do que o grupo sintomático não recorrente (13%). A prevalência de não-albicans spp. demonstrou a importância de identificar a *Candida* spp. presente nas pacientes infectadas..

Palavras-chave: *Candida*. Vulvovaginal. Candidiase. Sintomático. Fungo. Micose.

1. INTRODUCTION

Vulvovaginal candidiasis (VVC) is caused by opportunist growth of polymorphic fungus, which usually presents in the yeast state on the mucosa of the female genital tract. It is clinically characterized by occurrences of intense vulvar itching, leucorrhea, dyspareunia, dysuria, edema and vulvovaginal erythema [1].

VVC is classified by the World Health Organization (WHO) as a pathological condition that affects millions of women annually, thereby causing great discomfort, interfering with sexual and affective relations, impairing work performance, and considered to be an important worldwide public health problem [2].

Almost 75% of women experience at least one episode of VVC at some point during their lifetime and 5% experience recurrent VVC [1]. Nearly one-third of patients diagnosed with vulvovaginitis are infected with different *Candida* species [3]. *Candida* species are among the normal vaginal microbiota of 20–50% of women without any clinical presentation [1]. Several studies have shown that the distribution of the various species of *Candida* is region and culture specific [4]. Although *C. albicans* is still the most common *Candida* sp. found in the vaginal microbiota (80-90%), both under normal conditions and during opportunistic infections, the proportion of *non-albicans* spp. (*C. tropicalis*, *C. glabrata*, *C. krusei*, *C. parapsilosis*) in the vaginal microbiota has been increasing (10-20%). The widespread use of antifungal agents has caused an increase in cases of pathogens related to *non-albicans* species of the *Candida* genus [1,5]. Thus, to determine therapeutic approach and prognosis, and to guide epidemiological investigations, it is important to identify the *Candida* spp. present in infected patients [6].

In the north region of Brazil, Manaus (AM), the annual mean temperature is 26.7ºC and relative humidity is 76.85%, hot and humid. These factors aggravate the incidence of this pathology in women [7]. This study aimed to analyze the prevalence of VVC in Manaus city, its association with certain pre-exposure factors and the patterns of antifungal resistance associated to the commonly used drugs in VVC therapy.

2. MATERIALS AND METHODS

The observational cross-sectional study involved the analysis of 1226 samples of vaginal secretions collected from December 2005 to December 2006. These samples originated from women aged between 13 and 84 years, who visited the four public

healthcare units from the gynecologic attendance system of Manaus, Amazonas, Brazil, regardless of signs and symptoms of VVC. This study was approved by the research ethics committee for projects involving patient's participation (CONEP 0050101100006 - at that time, the processes related to ethical analysis did not occur through the Plataforma Brasil). All the patients signed a consent form after reading the information sheets and before participating in the study. The women answered a standardized questionnaire that sought information regarding VVC symptoms. The exclusion criteria were refusal to answer the questionnaire, and the research ethics consent form. A vaginal sample was collected using a sterile swab, inoculated in 1.5% peptone sterile solution and maintained for up to three days. The sample was later sent to the laboratory at Fiocruz/ILMD, where it was immediately seeded onto plates containing Sabouraud dextrose agar (SDA) (Difco, United States) with 100 mg/ml of chloramphenicol, and incubated at 30°C for 48 hours. Four colonies grown on each plate were analyzed by the Candifast identification test (International Microbio), which combined the yeast identification and antifungal resistance evaluation.

Profiles of patients with specific positive culture for *Candida* spp. were grouped into three categories: colonized but without symptoms of VVC (WVVC); symptomatic with VVC (SVVC), reporting having had an episode of VVC with at least two of the following symptoms: discharge, itching, dysuria and dyspareunia, and was at that time with VVC, with the first episode being within the past 12 months; and the group of symptomatic with recurrent VVC (RVVC), presenting with two or more of these symptoms and reporting at least 2 or more episodes per year [8]. The different species of *Candida* spp. found in SVVC and RVVC patients were evaluated. Data analysis were carried out by means one-way analysis the chi-square test, descriptive statistics using SPSS 23.0 computer statistical analysis software. All variables were expressed as absolute and relative frequencies, differences were considered as statistically significant at $p < 0.05$.

3. RESULTS AND DISCUSSION

A total of 995 (81.16%) women had negative culture for *Candida* spp. (mean age: 30.7 years). The total vaginal yeast isolation occurred in 231 women (18.86%), distributed as C. albicans (n = 169; 73.2%) as predominantly and non-albicans species (n = 62; 26.8%) (odds ratio [OR] = 2.75; 2.2-11.5; P = 0.05), of which prevalence was the follow: *C. tropicalis* (35%), *C. glabrata* (34%), *C. parapsilosis* (27%) and *C. krusei* (3%), constituted 26.8% (231

isolates) of the total isolates (Table 1). In none of the patients was isolated more than one yeast species.

Among women with SVVC, 8% (5/21) were infected with *C. glabrata*. Of those with RVVC, 13% (8/22) were infected with *C. tropicalis*. With regard to clinical profiles, in the positive culture group 151/231 women (65.4%) were colonized or had SVVC or RVVC (80/231; 34.6%) (P = 0.3), with significant difference between SVVC (30/231; 13%) and RVVC (50/231; 21.6%) (P = 0.2) (Table 1). In the negative culture group had 9.95% (99/995) of women with symptoms for VVC and RVVC groups and in 90% (896/995) had none of the symptons.

Table 1. Clinical condition of colonization (WVVC), vulvovaginal candidiasis (SVVC) and recurrent VVC (RVVC) and frequency of total isolation of vaginal *Candida* spp.

***Candida* species**	**Total isolation**		**WVVC**		**SVVC**		**RVVC**	
	n	**(%)**	**n**	**(%)**	**n**	**(%)**	**n**	**(%)**
C. albicans	169	(73.2)	108	(71.5)	23	(76.7)	38	(76.0)
Non-*albicans*	62	(26.8)	43	(28.5)	7	(23.3)	12	(24.0)
Total	231	(21.2)	151	(65.4)	30	(13.0)	50	(21.6)
Isolated non-albicans species								
C. tropicalis	22	(35.0)	13	(21.0)	1	(2.0)	8	(13.0)
C. glabrata	21	(34.0)	13	(21.0)	5	(8.0)	1	(2.0)
C. parapsilosis	17	(27.0)	15	(24.0)	1	(2.0)	3	(5.0)
C. krusei	2	(3,0)	2	(3.0)	0	(0.0)	0	(0.0)

WVVC, without symptoms of vulvovaginal candidiasis.
SVVC, non-recurrent vulvovaginal candidiasis.
RVVC, recurrent vulvovaginal candidiasis.

Among those infected with non-albicans, 27% (17/61) were using contraceptive. And 17% (29/168) infected with C. albicans and 10% (6/61) with non-albicans were immunocompromised, and 6% (10/168) infected with *C. albicans* used vaginal douching. 51% of patients with *C. albicans* related had other episodes of VVC, and 70% (118/168) had leucorrhea (Table 2). The results of the chi-square test showed no significant association between these groups (p < 0.6).

Table 2. Predisposing risk factors investigated in patients with vulvovaginal candidiasis

	R (+) N= 231		R (-) N=995		C.a N=168		C.n.a N=61	
	n	(%)	n	(%)	n	(%)	n	(%)
Behavioral factors								
Used vaginal douching	11	(5.0)	53	(5.0)	10	(6.0)	1	(2.0)
Used contraceptive	45	(20.0)	222	(22.0	29	(17.0)	17	(27.0)
Antifungal	24	(10.0)	95	(10.0	20	(12.0)	4	(6.0)
Immunocompromised	17	(7.0)	93	(9.0)	11	(6.0)	6	(10.0)
Other episods	108	(47.0)	412	(41.0)	85	(51.0)	15	(25.0)
Physical aspects								
Dysuria	48	(21.0)	216	(22.0)	25	(5.0)	5	(8.0)
Dyspareunia	41	(18.0)	129	(13.0)	19	(9.0)	9	(14.0)
Leucorrhea	79	(34.0)	601	(60.0)	70	(33.0)	33	(53.0)
Ulcer	2	(1.0)	3	(0.0)	1	(1.0)	1	(2.0)

R (+), positive culture group . R (-),negative culture group. C.a, with *C. albicans*. C.n.a, with non-albicans *Candida*.

The resistance of *Candida* spp. isolates for the antifungals in the Candifast test kit indicates that 24% (56/168) of *C. albicans* were multiresistant to antifungal drugs, and 15% (34/62) of non-*albicans* were also multiresistant strains. The predominance was for the antifungals of the azole group, FCZ and MCZ. Among the non-*albicans*, *C. glabrata* and *C. tropicalis* were the ones that presented higher resistance. By comparison of non-resistance to seven antifungals tested, revealing 49% (112/168) of *C. albicans* and 12% (28/62) of non-*albicans* (Table 3).

Table 3. Number of isolates resistentes for seven antifungical agent for diferents *Candida* spp.

	Antifungical agents in Candifast test							Number episodes of resistance				
Species	AB	NY	FCT	ECZ	KTZ	MCZ	FCZ	Zero		1-2	3-7	
								n	(%)	n	n	(%)
C. albicans	3	2	14	19	5	34	51	112	(49.0)	33	23	(24.0)
Non-*albicans*	4	0	5	24	21	27	29	28	(12.0)	10	24	(15.0)
Non-*candida*												
C. tropicalis	1	0	1	12	13	13	13	8	(3.0)	0	13	(6.0)
C. glabrata	1	0	1	1	0	2	2	14	(6.0)	1	2	(1.0)
C. parapsilosis	2	0	3	9	8	13	13	6	(3.0)	7	9	(7.0)
C. krusei	0	0	0	2	0	1	1	0	(0.0)	2	0	(1.0)

AB - amphotericin B, FCZ - fluconazole, KTZ -ketoconazole, NY - nystatin, FCT - flucytosine, ECZ -econazole, MCZ - miconazole.

Studies have shown that the distribution of the various species of *Candida* is region- and culture-specific. Although *C. albicans* is still the most common *Candida* sp. found in the

vaginal microbiota, both under normal conditions and during opportunistic infections, the proportion of non-*albicans* spp. in the vaginal microbiota has been increasing [2,4].

The prevalence of non-*albicans* species, especially *C. glabrata*, seems to increase steadily and this raises the concern regarding the antifungal drug resistance [9]. The findings of this study showed that 70% of VVC patients had at least one symptom of the disease (discharge, itching, ulceration, dysuria, dyspareunia), or one pre-exposure factors for the disease (antibiotic and antifungal use, immunodeficiency, vaginal douching), the data were consistent with the results of others studies [4, 9-10].

Although the predominance of *C. glabrata* and *C. tropicalis* species in VVC Manaus´s patients, the malignancies cannot be readily explained. However, it should be noted that azoles are the predominant medication used in the treatment of fungal infections. And Choukri et al. (2014) [11] cited *non-albicans Candida* species seem to be less responsive to treatment with imidazoles.

Moreover, non-albicans *Candida* species are usually associated with cases of relapse and multidrug resistance, associated with more severe cases of infection, especially when predisposing factors are associated, such as immunosuppression [12]. Therefore, it is important to know the prevalence of different species so that treatment can be better directed. And since the species of *Candida* other than albicans are usually associated with cases of relapse and multidrug resistance, associated with more severe cases of infection, especially when predisposing factors are associated, such as immunosuppression[12]. Therefore, it is important to know the prevalence of the different species, so that the treatment can be better directed.

Corroborating with Choukri et al. (2014) [11], the results of this study confirmed the susceptibility of *C. albicans* to the most frequently used topical agents and may support the use of alternative agents to imidazoles, in the treatment of vulvovaginal candidiasis caused by non-albicans *Candida* species. The widespread use of antifungal agents has caused an increase in pathogens related to non-albicans *Candida* species [13]. Thus, to determine the therapeutic approach and prognosis, and to guide epidemiological investigations, it is important to identify the *Candida* spp. present in infected patients.

5. FINAL CONSIDERATIONS

This study was the first time to determine the prevalence in the capital of the state of Amazonas, Brazil, and it was possible to observe the widespread use of antifungal agentes

caused an increase in pathogens related to *non-albicans Candida* species. Thus, to determine the therapeutic approach and prognosis, and to guide epidemiological investigations, it is important to identify *Candida* spp. present in infected patients.

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