

# Correlation of *Candida* species and symptoms among patients with vulvovaginal candidiasis in Maringá, Paraná, Brazil

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**Summary** Vulvovaginal candidiasis (VVC) is an infection caused by abnormal yeast growth in the mucosa of the female genital tract which is commonly diagnosed in gynecology. The aim of this study was to correlate the frequency of yeasts and their respective species in asymptomatic women with different clinical manifestation of VVC; evaluate possible relationships between number of fungus colonies and symptoms in this pathology. All patients who visited the laboratory within a period of five months, for routine examinations of vaginal secretion, independent of the presence or absence of symptoms of VVC were included in this study. Of these, women with immunodeficiency or with an infection of the genital tract by another agent were excluded. *Candida albicans* was the most frequently yeast isolated (60%). Among non-*C. albicans* yeasts, 61.5% were isolated of the asymptomatic women, 38.7% from patients with VVC and 11.1% of those from patients with RVVC. *C. albicans* was associated with symptoms of VVC and while, the presence of non-*C. albicans* yeasts with asymptomatic women. However, there was no association between the number of fungal colonies and symptoms.

**Key words** Vulvovaginal candidiasis, Non-*C. albicans* yeasts, Asymptomatic, Recurrence

## Correlación de especies de *Candida* y síntomas entre pacientes con vulvovaginitis candidiásica en Maringá, Paraná, Brazil

**Resumen** La vulvovaginitis candidiásica (VVC) es una afección originada por el crecimiento anormal de levaduras en la mucosa del aparato genital femenino y es un diagnóstico frecuente en la ginecología. La presente pesquisa tiene como objetivo determinar la frecuencia, cantidad y especies de levaduras aisladas en las muestras vaginales de mujeres asintomáticas o con diferentes manifestaciones de VVC. Fueron incluidas todas las mujeres que durante cinco meses acudieron al laboratorio para un examen rutinario de secreción vaginal, independiente de la presencia o ausencia de síntomas de VVC. De éstas, fueron excluidas las mujeres con inmunodeficiencia o infección del aparato genital provocada por otro agente. *Candida albicans* fue la levadura más aislada (60%) y las levaduras no-*C. albicans* fueron aisladas en 61.5% de las mujeres asintomáticas, en el 38,7% de pacientes con VVC y en el 11,1% de aquellas con VVC recurrente. *C. albicans* fue asociada a síntomas de VVC; por otro lado se puso en evidencia la presencia de levaduras no-*C. albicans* en mujeres asintomáticas. Sin embargo, no hubo relación entre cantidad de colonias de hongos y síntomas.

**Palabras clave** Vulvovaginitis candidiásica, *Candida no albicans*, Asintomática, Recurrente

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Vulvovaginal candidiasis (VVC) is a disease caused by abnormal growth of yeasts in the mucosa of the female genital tract and it is frequently diagnosed in the daily practice of gynecology, with a significant increase during the last years [16]. Approximately 75% of adult women will experience at least one episode of VVC during their lifetime, among which approximately 40 to 50% will experience further episodes and 5% will develop the recurrent type (RVVC). On the other hand, studies show that 20 to 25% of healthy and totally asymptomatic women exhibit positive vaginal secretion cultures for *Candida albicans* [12].

According to Sobel [12], 80 to 90% of VVC cases are caused by *Candida albicans*, while 10 to 20% are due to other non-*C. albicans* yeasts (*Candida tropicalis*, *Candida glabrata*, *Candida krusei*, *Candida parapsilosis*). However, it seems that there is an increase in non-*C. albicans* yeast frequency in certain populations [10].

In this study we intend to correlate frequency of yeasts and their respective species in asymptomatic females and those with different clinical manifestation of VVC. The possible relationships between number of fungus colonies and the symptomatology of this pathology were investigated.

## Materials and methods

This study was carried out in the Laboratory of Teaching and Research in Clinical Analysis of the State University of Maringá - Maringá, Paraná - Brazil, during the period of April to August 2002. It was carried out on those females who visited the laboratory for examinations of vaginal secretion, independent of the presence or absence of symptoms of VVC. Of these, women with a previous history of immunodeficiency, including AIDS, or with an infection of the genital tract by another agent were excluded.

All patients answered a standard questionnaire on their symptoms of vulvovaginal candidiasis (vaginal discharge, vulvovaginal itching, vulvovaginal burning sensation, dysuria and dyspareunia) [4,8]. The characteristic vaginal discharge was classified by the health agent when sample was taken, according to Odds et al. [8]. All women involved in the research signed a term of consent in which they declared to know that the collected material would be used for research, as mandatory by the Ethical Commission in Research Involving Humans of State University of Maringá (Reg. 007/2002; Report 013/2002; CI 032/2002).

Cervical and vaginal specimens for the culture were collected with the aid of a disposable vaginal speculum (Vagispec, Brazil) and immediately spread in Petri dishes containing the culture medium Sabouraud Dextrose Agar «SDA» (Difco, USA) supplemented with 50 mg/ml of chloramphenicol (Sigma, USA). The dishes were incubated at 37 °C, for 48-72 h, then a pool of the grown colonies were subcultured in CHROMagar Candida (Probac, France) to investigate the purity of the culture and the color of the colonies. From this selective and differential medium the yeasts were identified according to classical methods [5,6]. The numbers of yeasts colonies isolates were assigned according to Odds [8].

Cervical-vaginal samples were also collected for smears in microscope slides, which were dyed by Gram and Papanicolaou stains for the evaluation of the presence of yeasts and other infectious agents. Specific microorganisms were identified by microbiological and serological methods.

Patients with positive yeast culture were distributed into three groups: asymptomatic women who presented none or only one symptom out the five mentioned above, VVC women who presented two or more symptoms in a single occurrence in the past of one year, RVVC women who presented two or more symptoms in three or four occurrences during the year.

Statistical analysis was Mantel-Haenszel's  $\chi^2$  test [14]; Prism 3.00 (Graphpad Software, Inc., USA) was used throughout the analysis. A p-value less than 0.05 was regarded as significant.

## Results

One hundred and sixty-one patients with 14 to 66-year-old were evaluated. Positive fungus culture had a frequency of 21.7%, or 35 patients. *C. albicans* was isolated in 60% (21/35). The percentage of non-*C. albicans* yeasts may be subdivided into 25.7% of *C. glabrata* (9/35), 5.7% *C. parapsilosis* (2/35), 5.7% *Saccharomyces cerevisiae* (2/35), and 2.9% *Trichosporon* sp (1/35). Table 1 shows the relative and absolute frequencies of these species in the three groups of patients analyzed. In addition, according to figure 1, the isolation of *C. albicans* was proportional to the presence of symptoms, and the inverse occurred with non-*C. albicans*.

According to table 1, Mantel-Haenszel's  $\chi^2$  test showed that there is a correlation between the isolated yeast and the presence of symptoms. *C. albicans* was more frequent among the symptomatic women, while non-*C. albicans* were more frequent in asymptomatic ones ( $p = 0.03$ ). These analyses corroborate the trend revealed in table 1, or rather, isolation of *C. albicans* increases in proportion to severity of vulvovaginitis, whereas the opposite occurs in the case of non-*C. albicans* yeasts. Table 2 shows the colony count and their relation with the symptoms of VVC analyzed. Only 33 samples were used in this table and the statistical analysis did not show association between the number of colonies and the five symptoms evaluated in this study.

## Discussion

Yeasts were isolated from the vaginal secretions of 21.7% of women, this finding is in agreement with Bauters et al. [2], who isolated yeasts in 20.1%. Abu-Elteen [1] detected yeasts in 48.4% of fertile women and in 51.5% of

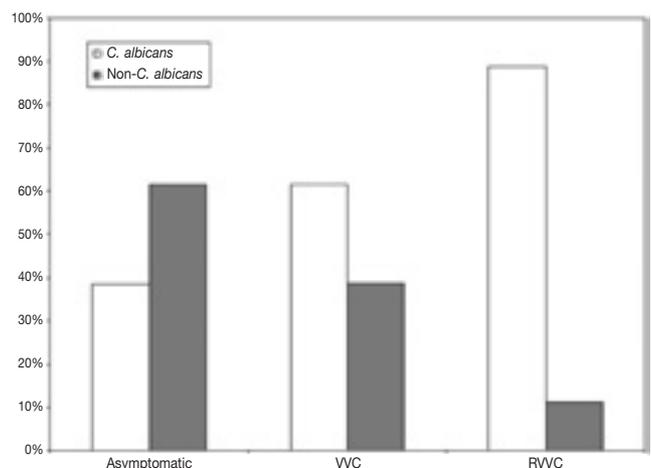


Figure 1. Distribution of *C. albicans* and non-*C. albicans* yeasts.

**Table 1.** Yeasts species isolated from women, according to clinical categories.

Species	Asymptomatic		VVC		RVVC		Total	
	No	%	No	%	No	%	No	%
<i>Candida albicans</i>	5	38.5	8	61.5	8	88.9	21	60
Non- <i>C. albicans</i>	8	61.5	5	38.5	1	11.1	14	40
<i>Candida parapsilosis</i>	1	7.7	0	0	1	11.1	2	5.7
<i>Candida glabrata</i>	7	53.8	2	15.5	0	0	9	25.7
<i>Saccharomyces cerevisiae</i>	0	0	2	15.5	0	0	2	5.7
<i>Trichosporon</i> sp	0	0	1	7.7	0	0	1	2.9
Total	13	100	13	100	9	100	35	100

**Table 2.** Colony count and their relationship to the symptoms under analysis.

Symptoms		1 - 99 colonies (n=11)	≥ 100 colonies (n=22)	p value
Vaginal discharge	Yes	7	12	0.71934
	No	4	10	
Vulvovaginal itching	Yes	2	10	0.24922
	No	9	12	
Burning sensation	Yes	3	12	0.26593
	No	8	10	
Dysuria	Yes	4	4	0.39148
	No	7	18	
Dyspareunia Yes	Yes	3	9	0.70260
	No	8	13	

those with infertility problems. In relation to the species, this research showed a predominance of *C. albicans*, accounting for 60% of the total of the yeasts isolated. Verghese et al. [15] found *C. albicans* in 40.5%, and Bauters et al. [2] in 68.3%.

*Candida glabrata* was the second most frequently isolated yeast (25.7%), coinciding with the results of other authors [7,11]. This species is represented in 16.3% and 38.1% of the cases in the studies of Bauters et al. [2] and Verghese et al. [15], respectively. The isolation rate of *C. glabrata* in the asymptomatic patients was 53.8% (Table 1). These data are similar to those of Oriel et al. [9], who found that more than half of women with *C. glabrata* were asymptomatic. It is not surprising that in asymptomatic women, the ratio of isolation of non-*C. albicans* yeasts was higher than for patients with vaginal symptoms. This is due to the fact that the typical clinical manifestations of infections by these yeasts are characterized by less intensive symptoms than those caused by *C. albicans* [13].

The genus and species of yeasts isolated from vaginal secretions has been poorly associated to the symptoms of VVC. Dan et al. [3], found similar results to those findings in this study, despite their study was carried out on selected patients with symptoms of VVC. There is a statistical tendency to a high frequency of non-*C. albicans* yeasts in asymptomatic women. On the other hand, *C. albicans* is associated with VVC and RVVC. This seems to be a general tendency, but further studies should be carried out in patients selected and non-selected for clinical aspects of VVC to confirm this tendency.

Table 1 shows that *Saccharomyces cerevisiae* was isolated in two VVC women (2/35). Dan et al. [3] reported the same yeast in a single case (1.7%).

Our study did not find association between colony count and the five symptoms valued in this study (Table 2). This fact is in contrast with results by Odds et al. [8] who detected a relationship between number of colonies and vaginal discharge and itching.

This study showed that although the most isolated yeast from vaginal secretions is still *C. albicans*, this is only true for patients with symptoms. The isolation of non-*C. albicans* yeasts was not low (40%), but was more frequent in the asymptomatic women, representing 61.5% of the yeasts found in this group. These findings suggest that in the vagina, colonization by yeasts is associated with non-*C. albicans* yeasts and that the evolution to the symptoms of VVC or RVVC would depend, among other factors, on replacement by *C. albicans*. If this idea is correct, the routine practice of carrying out culture identifications of yeasts could contribute to clarifying the challenge of differentiating colonization's of infection by yeasts in vaginal secretions.

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