



## Vulvovaginal candidiasis: prevalence and antifungal susceptibility of species isolated from women living in recôncavo baiano

*Candidiase vulvovaginal: prevalência e suscetibilidade antifúngica de espécies isoladas de mulheres residentes no recôncavo baiano*

*Candidiasis vulvovaginal: prevalencia y susceptibilidad antifúngica de especies aisladas en mujeres residentes en recôncavo baiano*

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### ABSTRACT

**Background and Objectives:** *Candida albicans* is the species most implicated in vulvovaginal candidiasis, but its adaptive and virulence characteristics have contributed to the increase in *non-albicans* yeast species. Resistance mechanisms make drug treatment difficult. The aim of the study was to evaluate the prevalence of vulvovaginal candidiasis, its etiological agents and antifungal susceptibility profiles. **Methods:** This was an observational, cross-sectional study involving 358 women living in the Recôncavo Baiano. Molecular tests were used to identify the isolated species and antifungal analysis to determine the antifungal susceptibility profile. Fisher's exact test was used to calculate associations. **Results:** The prevalence of vulvovaginal candidiasis was 10,89% (39/358). *C. albicans* was most common (64%-37/58), followed by 12% (7/58) each of *Nakaseomyces glabratus* and *C. parapsilosis*, 7% (4/58) *C. tropicalis*, 3% (2/58) *Meyerozyma guilliermondii* and 2% (1/58) *Pichia kudriavzevii*. Pruritus and bleeding were associated with vulvovaginal candidiasis caused by *C. albicans*. Itraconazole and miconazole showed greater variability in the susceptibility profile. Resistance to itraconazole was reported in *N. glabratus*. **Conclusion:** The results obtained indicate that *C. albicans* continue to be the predominant species in cases of VVC and, together with other species, may present resistance to the antifungals tested.

**Keywords:** Fungi. Vulvovaginitis. Antimycotics. Vulvar Pruritus. Resistance.

### RESUMO

**Justificativa e Objetivos:** *Candida albicans* é a espécie mais comumente implicada na candidiase vulvovaginal, mas suas características adaptativas e de virulência contribuíram para o aumento de espécies de leveduras *não-albicans*. Mecanismos de resistência eficientes e sofisticados têm dificultado a terapia farmacológica e preocupado a comunidade científica, uma vez que o número de antifúngicos é limitado. O objetivo do estudo foi avaliar a prevalência da candidiase vulvovaginal, dos seus agentes etiológicos e perfis de suscetibilidades antifúngicas. **Métodos:** Estudo observacional, de corte transversal, envolvendo 358 mulheres residentes no Recôncavo Baiano. Testes moleculares foram utilizados para identificação das espécies isoladas, bem como antifungograma para determinar o perfil de sensibilidade antifúngica. O Teste exato de Fisher foi utilizado para calcular associações. **Resultados:** A prevalência de candidiase vulvovaginal foi de 10,89% (39/358). *C. albicans* foi mais prevalente (64%-37/58), seguida de 12% (7/58) cada de *Nakaseomyces glabratus* e *C. parapsilosis*, 7% (4/58) *C. tropicalis*, 3% (2/58) de *Meyerozyma guilliermondii* e 2% (1/58) de *Pichia kudriavzevii*. Prurido e sangramento foram associados à candidiase vulvovaginal por *C. albicans*. Itraconazol e miconazol apresentaram maior variabilidade no perfil de sensibilidade. Resistência foi relatada por *N. glabratus* ao itraconazol. **Conclusão:** Os resultados obtidos indicam que *C. albicans* continua sendo a espécie predominante em casos de candidiase vulvovaginal e, juntamente com outras espécies, pode apresentar resistência aos antifúngicos testados.

**Descritores:** Fungos. Vulvovaginite. Antimicóticos. Prurido Vulvar. Resistência.

### RESUMEN

**Justificación y Objetivos:** *Candida albicans* es la especie más comúnmente implicada en la candidiasis vulvovaginal, pero sus características adaptativas y de virulencia han contribuido al aumento de especies de levaduras *no albicans*. Mecanismos de resistencia han dificultado el tratamiento farmacológico. El objetivo del estudio fue evaluar la prevalencia de candidiasis vulvovaginal, sus agentes etiológicos y los perfiles de susceptibilidad antifúngica. **Métodos:** estudio observacional transversal onde participaron 358 mujeres residentes en el Recôncavo Baiano. Para identificar las especies aisladas se utilizaron pruebas moleculares y, para determinar el perfil de susceptibilidad antifúngica, pruebas antifúngicas. Se utilizó la prueba exacta de Fisher para calcular las asociaciones. **Resultados:** prevalencia de candidiasis vulvovaginal fue del 10,89 % (39/358). *C. albicans* fue la más prevalente (64%), seguida de un 12% (7/58) de *Nakaseomyces glabratus* y *C. parapsilosis*, un 7% (4/58) de *C. tropicalis*, un 3% (2/58) de *Meyerozyma guilliermondii* y un 2% (1/58) de *Pichia kudriavzevii*. Prurito y sangrado se asociaron a la candidiasis vulvovaginal causada por *C. albicans*. El itraconazol y el miconazol mostraron una mayor variabilidad en el perfil de sensibilidad. Se registró resistencia a itraconazol en *N. glabratus*. **Conclusiones:** Los resultados obtenidos indican que *C. albicans* sigue siendo la especie predominante en los casos de VVC y, junto con otras especies, puede presentar resistencia a los antifúngicos ensayados.

**Palabras Clave:** Hongos. Vulvovaginitis. Antimicóticos. Prurito Vulvar. Resistencia.

## INTRODUCTION

Vulvovaginal candidiasis (VVC) is a common, non-life-threatening fungal infection of the female reproductive tract. This condition is the second leading cause of vulvovaginitis worldwide and has a significant impact on women's physical and mental health and overall functioning.<sup>1</sup> Several risk factors, signs, symptoms, diagnostic methods, microorganism species, virulence factors, and treatment options are relevant to understanding and effectively addressing VVC.<sup>2</sup>

Approximately 75% of women will experience at least one episode of VVC in their lifetime, with *Candida albicans* responsible for approximately 80% to 90% of cases.<sup>3</sup> However, other species, such as *Nakaseomyces glabratus* (*Candida glabrata*), *Candida tropicalis*, *Pichia kudriavzevii* (*Candida krusei*), and *Candida parapsilosis*, may also be involved. Each species has unique characteristics that influence its virulence, response to treatment, and propensity to cause recurrent infections.<sup>4</sup>

As microorganisms of the vaginal microbiota, yeasts of the *albicans* and non-*albicans* species commonly colonize the vaginal lumen asymptotically. However, symptomatic infection results from their excessive growth, followed by epithelial invasion and production of virulence factors that result in mucosal inflammation.<sup>5</sup>

Common symptoms may vary in intensity and frequency, including hyperemia, pruritus, and burning in the genital region. In addition, pain may occur during sexual intercourse and urination. These symptoms are often accompanied by thick, white vaginal discharge, consisting of desquamated epithelium, immune cells, yeast, and vaginal fluid. The recurrent presence of these signs and symptoms, with episodes more than four times throughout the year, characterizes recurrent candidiasis.<sup>6</sup>

Several factors increase women's susceptibility to fungal vulvovaginitis. One of the main risk factors is the indiscriminate use of antibiotics, which can alter the balance of the vaginal microbiota, favoring the overgrowth of *albicans* and non-*albicans* species. Other factors include pregnancy, uncontrolled diabetes mellitus, compromised immune system, prolonged use of corticosteroids, oral contraceptives, smoking, and the use of synthetic underwear that promotes moisture.<sup>7</sup>

The diagnosis of VVC is usually clinical, based on symptoms and physical examinations. Laboratory diagnosis can be made presumptively by identifying the presence of yeast through direct Gram and Papanicolaou staining tests and confirmatory mycological tests, such as culture or molecular tests.<sup>2,8</sup>

Although the Papanicolaou test plays an important role in women's health by identifying inflammatory processes and yeast-like structures, it is not able to

distinguish the species of fungus involved in vulvovaginitis or assess the sensitivity profile to antifungal agents. Thus, mycological diagnosis is essential because it covers these particularities.<sup>9</sup>

Treatment of VVC usually involves drug therapy with antifungal agents, which can be administered orally, topically, or intravaginally. The most commonly prescribed drugs are azoles, which include compounds such as ketoconazole, miconazole, clotrimazole (imidazoles), fluconazole, and itraconazole (triazoles) and polyene agents such as nystatin. However, sensitivity to antifungals varies between species, and resistance mechanisms have compromised drug therapy, highlighting the need for specific identification of the yeast and sensitivity testing to establish appropriate therapy.<sup>10,11</sup>

Considering the scarcity of scientific studies related to VVC in the state of Bahia, the objective of this study was to evaluate the prevalence of vulvovaginal candidiasis, its etiological agents, and antifungal susceptibility profiles. We hope that the results will have a positive impact on the health of women in this region by providing clinical and laboratory information that can help in safe diagnosis and a more effective therapeutic approach against this infection.

## METHODS

An observational, cross-sectional study conducted between August 2021 and November 2023, comprising women aged between 18 and 65 years old, residing in the Recôncavo Baiano region: Governador Mangabeira, Cruz das Almas, and Sapeaçu. The municipalities were chosen based on their proximity to the Maria Milza University Center (UNIMAM) laboratory, where the initial microbiological tests were carried out, at distances of approximately 5, 8, and 20 km, respectively. Three Primary Care Center, one in each municipality, were selected because they had better physical structures in the collection rooms, a higher flow of patients, and the availability of nurses to perform the collections.

Women who spontaneously sought public health services to undergo a Pap smear participated in the study, agreeing to sign the Free and Informed Consent Form. Pregnant women, postpartum women, women with no history of sexual activity, and women who had undergone treatment for any urogenital tract pathology in the last 12 months were excluded. Cervical-vaginal samples were collected at the time of the cytopathological examination of the cervix, using a sterile swab containing Amies transport medium.

### Cervical samples

The cervicovaginal samples collected were cultured in Sabouraud Dextrose Agar (SDA) (ACUMEDIA, USA)

supplemented with chloramphenicol and, after 48 hours of incubation at 35±1°C, positive cultures were subjected to Gram staining to confirm yeast. Direct examination of the sample by Gram staining was not used to assess the presence of yeast, but rather a Papanicolaou cytological examination. Hyphae or pseudo-hyphae morphology was not considered a criterion for pathogenicity, since non-albicans yeast species, even if they do not produce them, can be responsible for infectious processes because they present several other virulence factors.<sup>12</sup>

Species identification was performed using the molecular technique PCR-RFLP (Polymerase Chain Reaction - Restriction Fragment Length Polymorphism) technique, where the intergenic spacer region (ITS) of rDNA was amplified with the primers ITS1 (5'TCCGTAGGTGAACCTGCGG3') and ITS4 (5'TCCTCCGCTTATTGATAGC3') with GoTaq® Green Master Mix (PROMEGA). The reaction was performed in a MyCycler thermocycler (Bio Rad) under the following conditions: 2 min at 94°C, 35 cycles of 1 min at 95°C, 1 min at 51°C, 2 min at 72°C, and 10 min at 72°C. The negative control was performed with Milli-Q water and the positive control with the standard strain *C. albicans* ATCC 90028. The PCR product was used in restriction with *Hpa* II and *Fsp*B I enzymes (Thermo Fisher Scientific), separately.<sup>13</sup>

Antifungal susceptibility tests were performed based on the disk diffusion technique as described in protocol M44, using disks of fluconazole (25 µg), itraconazole (10 µg), miconazole (50 µg), nystatin (100 IU), clotrimazole (50 µg), and ketoconazole (50 µg) - CECON.<sup>14</sup>

Signs and symptoms of vaginal bleeding after sexual intercourse, itching and burning in the vaginal region, presence of thick white vaginal discharge (leucorrhea), and changes in the cervix/vagina, including edema, hyperemia, excoriations, fissures, and/or lesions, were evaluated based on information recorded by the nurse responsible for collecting the cytopathological examination request.

The diagnosis of VVC was defined when study participants simultaneously presented a positive yeast culture and any of the signs/symptoms described above.

Data were analyzed using GraphPad InStat version 3.05. Fisher's exact test, with a significance level of 5%, was performed to determine the association of VVC with possible risk factors, clinical signs, and symptoms. Descriptive statistics were presented in the form of tables and graphs.

This study is part of a project and was approved by the Research Ethics Committee (REC) of Maria Nilza University (UNIMAM), opinion No. 7,281,350, CAAE No. 36887120.6.0000.5025. During the study, strict patient confidentiality was maintained.

RESULTS

The study consisted of 358 women, with a mean age of 41 ± 11.8 years, years old, with 10.9% (39/358) between 18 and 24 years old, 53.3% (191/358) between 25 and 45 years old, and 35.8% (128/358) between 46 and 65 years old. Clinical signs and symptoms were described in 56.7% (203/358) of the study participants, while 43.3% (155/358) of the women were asymptomatic.

Smoking, multiparity (two or more pregnancies), and hormone therapy use were reported by 5.6% (20/358), 68.4% (255/358), and 0.84% (3/358) of the study participants, respectively. Among the contraceptive methods evaluated, oral contraceptives, regardless of the route of administration, were the most widely used (24.9% - 89/358), while intrauterine devices (IUDs) were the choice of only one participant.

Yeasts were isolated in 15.6% (56/358) of the participants. However, 58 species were identified, as two participants had positive cultures for two different yeast species. The distribution of species showed a frequency of 63.8% (37/58) of *C. albicans* and 36.2% (21/58) of non-albicans yeasts (Table 1).

Table 1. Frequency of yeast isolated from women in the Recôncavo Baiano region, from 2021 to 2023.

Yeast species	N (%)
<i>Candida albicans</i>	37 (63.8)
<i>Nakaseomyces glabrata</i>	7 (12.1)
<i>Candida parapsilosis</i>	7 (12.1)
<i>Candida tropicalis</i>	4 (6.9)
<i>Meyerozyma guilliermondii</i>	2 (3.4)
<i>Pichia kudriavzevii</i>	1 (1.7)

The prevalence of VVC in this study was 10.89%, with the diagnosis established in 39 of the 358 participants who simultaneously presented a positive mycological culture for yeast and any of the clinical signs/symptoms evaluated. Cases of colonization were described in 4.75% (17/358) of asymptomatic participants. Although signs and symptoms were associated with VVC ( $p=0.0227$ ), the association remained only when the isolated yeast was of the albicans species ( $p=0.0098$ ) as opposed to non-albicans species ( $p=0.5554$ ). The signs/symptoms and their associations with VVC are described below (Table 2).

**Table 2.** Clinical signs and symptoms associated with vulvovaginal candidiasis in women from the Recôncavo Baiano region, 2021 to 2023.

Variable	Total		<i>Candida albicans</i>		Non-albicans yeasts	
	N (%)	<i>p</i> value	N (%)	<i>p</i> value	N (%)	<i>p</i> value
<b>Leukorrhea</b>						
Yes	23 (59.0)	0.8423	18 (64.3)	0.5314	05 (45.5)	0.1773
No	16 (41.0)		10 (35.7)		06 (54.5)	
<b>Altered cervix/vagina</b>						
Yes	11 (28.2)	0.4720	07 (25.0)	0.5201	04 (36.4)	0.3291
No	28 (71.8)		21 (75.0)		07 (63.6)	
<b>Bleeding after sexual intercourse</b>						
Yes	05 (12.8)	0.0143	04 (14.3)	0.0226	01 (9.0)	0.4006
No	34 (87.2)		24 (85.7)		10 (91.0)	
<b>Itching</b>						
Yes	24 (61.5)	0.0026	20 (71.4)	0.0004	04 (36.4)	0.5216
No	15 (38.5)		08 (28.6)		07 (63.6)	
<b>Burning sensation</b>						
Yes	11 (28.2)	0.5325	07 (25.0)	0.3960	04 (36.4)	0.4023
No	28 (71.8)		21 (75.0)		07 (63.6)	
<b>Odor</b>						
Yes	04 (10.3)	0.4116	04 (14.3)	0.4982	0 (0.0)	-
No	35 (89.7)		24 (85.7)		11 (100)	

Considering possible risk factors for VVC, age was not associated with this pathology in this population ( $p=0.3001$ ), but women between 18 and 24 years of age had a lower prevalence (13%) of VVC, followed by the group aged 46 to 65 years (26%). The 25-45 age group had the highest number of VVC cases (61%). Smoking was also not associated with VVC; however, the results suggest that women who smoke may be 2.1 times more likely to develop fungal vulvovaginitis ( $p=0.1308$ , RR 2.123, 95% CI 0.8211; 5.490), especially when the isolated pathogen is of the non-albicans species ( $p=0.0838$ , R.R. 3.185, 95% CI 0.9877; 10.269).

Analyzing the antifungal sensitivity profile, *C. albicans* isolates did not show resistance to the antifungals tested, but showed intermediate sensitivity to fluconazole in 3% (1/37), itraconazole in 19% (7/37), miconazole in 16% (6/37), and clotrimazole in 3% (1/37); all remaining isolates were sensitive to the antifungals tested.

When the analysis was performed on non-albicans yeasts, resistance was detected in only 4.76% (1/21) of the isolates, which were of the species *N. glabrata*, against itraconazole. Still with regard to itraconazole, 4.76% (1/21) of *C. tropicalis* and 9.52% (2/21) of *M. guilliermondii* showed intermediate sensitivity, while 9.52% (2/21) of *C. tropicalis* and 4.76% (1/21) of *P. kudriavzevii* showed intermediate sensitivity to miconazole. The remaining isolates were sensitive to the antifungals tested.

DISCUSSION

Information on the prevalence of VVC in the Recôncavo Baiano region is lacking in academic literature. Therefore, understanding the epidemiological profile of this pathology and its causative agents in this

population can help public managers and the scientific community to direct actions for prevention, early diagnosis, and appropriate treatment.

Although the Recôncavo Baiano is located in a tropical region, where the combination of high temperatures and relative humidity usually favors yeast growth, the findings of this study revealed a prevalence of VVC lower than the national average estimated at 18%.<sup>15</sup> This discrepancy may be related to methodological variations employed in studies, especially when based on self-reports or only on clinical criteria.<sup>2,9,16</sup> Robust methodologies, with VVC diagnosis defined by the presence of signs/symptoms and mycological tests, including molecular tests, as used in this study, may have allowed a more precise distinction between asymptomatic colonization and active infection, avoiding an overestimation of VVC frequency.

Additionally, environmental, behavioral, and cultural factors specific to the studied population may have contributed to the lower prevalence observed. The Recôncavo Baiano region has semi-urban and rural characteristics, which may favor protective habits, such as the predominant use of light and cotton clothing and less exposure to urban factors associated with VVC, such as stress, obesity, and industrialized diets.<sup>7,11,16</sup>

Furthermore, data on the fungal species responsible for VVC cases in the study are similar to scientific studies that indicate a predominance of the species *C. albicans*, with an increasing number of non-albicans clinical yeasts, the most frequently isolated remaining *C. tropicalis*, *N. glabratus*, *C. parapsilosis*, and *P. kudriavzevii*.<sup>3,7,17,18</sup>

In this context, the predominance of *C. albicans* as the etiological agent of VVC, as well as its association with the presence of clinical signs and symptoms, can be



explained by the intrinsic virulence characteristics of this yeast. *C. albicans* has a high capacity for adhesion to the vaginal epithelium, formation of hyphae and pseudohyphae, production of hydrolytic enzymes (proteases and phospholipases), and the ability to invade the immune system and cause an intense inflammatory response. These factors favor not only colonization but also the transition to the pathogenic form, resulting in more evident clinical manifestations.<sup>5,11,12</sup>

In contrast, *non-albicans* clinical yeasts tend to be less aggressive and cause less immune inflammatory stimulation, which may explain their lower frequency as causative agents of VVC and the absence of association with the clinical signs and symptoms investigated. However, the greater involvement of these species in fungal vulvovaginitis represents a significant epidemiological change and may be a warning sign for the possible impact of self-medication, prolonged use of azoles, or changes in the immunological profiles of patients.<sup>4,12,19,20</sup>

The findings of the present study, which identified vulvar pruritus as a symptom associated with fungal vulvovaginitis, contrast with the current clinical guidelines in Brazil. The Brazilian Protocol for Sexually Transmitted Infections recommends empirical treatment of VVC based on signs such as white, lumpy vaginal discharge and/or vulvar erythema, not considering pruritus as a criterion for therapeutic conduct.<sup>8</sup> However, growing evidence reiterating pruritus as the only independent predictor for VVC strengthens the implementation of a newly proposed algorithm, in which this clinical symptom is included in the differential diagnosis of vulvovaginitis in women with abnormal vaginal discharge, including VVC.<sup>1,2,21,22</sup>

A unique finding in this study was the association between vaginal bleeding after sexual intercourse and the presence of VVC caused by *C. albicans*. Although this symptom does not commonly occur in the typical clinical presentation of VVC, it can be pathophysiologically justified when considering the invasive and inflammatory power of this species, which, combined with local hypervascularization induced by inflammation, makes the vaginal epithelium more susceptible to microtrauma, especially after sexual intercourse. This manifestation may also be exacerbated in contexts of hypoestrogenism, use of vaginal douches, or vigorous sexual intercourse, factors not evaluated in the present study, but which may act as additional modulators of the tissue response.<sup>18</sup>

Although age and smoking do not represent risk factors for VVC in this population, the observation that women between 18 and 45 years of age are more affected by fungal vulvovaginitis is consistent with the literature. It is believed that increased hormone levels, common in this age group, as well as contraceptive use and pregnancy, may promote a reduction in the

inhibitory activity of epithelial cells against yeasts, which explains increase in candidiasis in women of reproductive age.<sup>23</sup>

With regard to smoking, it is important to note that the research data suggest that participants who smoke are more likely to develop fungal vulvovaginitis. This observation is justified by the fact that nicotine, present in tobacco, is responsible for altering the local immunomodulatory response, which can lead to changes in the genital tract microbiota, favoring the development of candidiasis.<sup>24</sup>

The evaluation of *C. albicans* isolates revealed an in vitro susceptibility profile without resistance determination, although cases of intermediate sensitivity were observed. The presence of intermediate phenotypes may indicate a possible adaptive process related to repeated, prolonged, or inappropriate use of topical or systemic antifungals. In addition, mutations in the *ERG11* gene and biofilm formation capacity have been documented as relevant mechanisms in reducing susceptibility to azoles.<sup>4,20</sup> This finding highlights the importance of considering epidemiological surveillance strategies, even in community settings, especially in cases of recurrent infection.

Analysis of the in vitro susceptibility profile of non-*albicans* clinical yeasts showed greater phenotypic heterogeneity compared to *C. albicans* isolates, although resistance was detected in only one case, corresponding to the species *N. glabratus* against itraconazole. This finding is consistent with the literature, which describes *N. glabratus* as a yeast that is naturally less susceptible to azoles due to characteristics such as low membrane permeability and constitutive activation of efflux pumps.<sup>19,20,25</sup>

Because it is not a notifiable infection, few health professionals request diagnostic tests for VVC to distinguish between the causes of vaginitis, which can lead to excessive prescription of antifungals and misuse of antibacterials. This inappropriate use favors the resistance of opportunistic pathogens, which makes antifungal susceptibility testing essential, both for accurate treatment and for the detection of possible antifungal resistance.<sup>4,20</sup>

The changes in the in vitro susceptibility profile for the antifungals tested in this study require attention in light of current therapeutic recommendations for the management of VVC. In cases of a single episode of VVC, local treatment (vaginal ovules), usually based on imidazole, or oral treatment with fluconazole is indicated. In the therapeutic approach to recurrent VVC, systemic use of fluconazole or itraconazole and local application of imidazoles are recommended. In primary care, the antifungals miconazole, in the form of a vaginal cream, and fluconazole, itraconazole, and nystatin, for oral administration, are available for the treatment of VVC.<sup>3,10</sup>

The finding of strains with resistance or intermediate sensitivity to antifungals, even in an outpatient population such as that in this study, has significant implications for epidemiological surveillance and clinical practice. This reality is especially concerning in tropical regions with low regulation of access to medicines, as is often the case in inland areas of Brazil, including the Recôncavo Baiano.

Although the data do not indicate established clinical resistance, they may reflect an early stage of fungal adaptation to the drugs, which would compromise the response to treatment, especially in patients with recurrent VVC. Therefore, the integration of epidemiological surveillance, accurate mycological diagnosis, and rationalization of antifungal use would be essential to contain emerging cases of resistance and ensure the effectiveness of recommended therapies.

The findings of this study conclude that, although the prevalence of VVC in the outpatient population of the Recôncavo Baiano was lower than the national average, *Candida albicans* remains the main etiological agent associated with signs and symptoms, while non-*albicans* species showed no clinical association. Detection of isolates with reduced sensitivity, even outside the hospital environment, reinforces the importance of mycological monitoring and rationalization of antifungal use in primary care, contributing to the prevention of resistance and the adequacy of therapeutic approaches.

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## AUTHORS' CONTRIBUTIONS

**Cássia Vargas Lordêlo** contribuiu para a concepção, delineamento do estudo, coleta, análise e interpretação dos dados; redação, revisão crítica relevante do conteúdo intelectual do manuscrito. **Jakeline Souza Torres** contribuiu para a realização das coletas, análises e interpretação dos dados. **Alessandra dos Santos Campos** contribuiu para a realização das análises e interpretação dos dados. **Manuela Fraga Fernandes e Silva** contribuiu para a realização das análises e interpretação dos dados. **Ricardo David Couto** contribuiu para a realização das análises e interpretação dos dados; revisão crítica relevante do conteúdo intelectual do manuscrito. **Tânia Fraga Barros** contribuiu para a concepção e delineamento do estudo, análise e interpretação dos dados; revisão crítica relevante do conteúdo intelectual do manuscrito.

All authors approved the final version to be published and are responsible for all aspects of the work, including ensuring its accuracy and integrity.

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