



Candida Speciation and Antifungal Susceptibility in Vulvovaginitis: Insights from Clinical Isolates

S R Patil (Professor and Head)¹, Priyanka Mane (Assistant Professor)¹, S K Pawar (Associate Professor)¹ and Sanjay S Patil (Professor)^{2,*}

¹Department of Microbiology, Krishna Institute of Medical Sciences, Krishna Vishwa Vidyapeeth (Deemed to be university), Karad, Maharashtra, India

²Department of Ob & Gyn, Krishna Institute of Medical Sciences, Krishna Vishwa Vidyapeeth (Deemed to be university), Karad, Maharashtra, India.

Corresponding author: Dr. S R Patil (Professor and Head)

Department of Microbiology, Krishna Institute of Medical Sciences, Krishna Vishwa Vidyapeeth (Deemed to be university), Karad, Maharashtra, India

(Received: 28 October 2023

Revised: 08 November

Accepted: 02 December)

KEYWORDS

Candida, Candida albicans, CHROM agar, Antifungal susceptibility testing, Vaginitis.

Abstract: Background: Vulvovaginal candidiasis presents a worldwide concern owing to its connections with economic burdens, sexually transmitted infections, and the potential for infection to ascend the genital tract, resulting in various complications for both pregnant and non-pregnant women. It ranks second only to bacterial vaginosis and impacts roughly 40-62% of women in their reproductive years. Moreover, there has been a notable rise in resistance to antifungal treatments over the past decade. As such, it is crucial to accurately identify Candida strains down to the species level and perform susceptibility testing for antifungal agents as part of the management of Candidal infections. **Methods:** During a 6-month period, 60 Candida species were obtained from 100 clinically diagnosed vaginitis cases. The growth of these isolates on Sabouraud dextrose agar followed established protocols, and subsequent Candida speciation was carried out using CHROM agar. Antifungal susceptibility testing was conducted utilizing the Etest method. **Results:** Out of the 100 patients with vaginitis, 60 tested positive for Candida species. The isolated Candida species were categorized into four types: *C. albicans* (25 cases, 42.8%), *C. krusei* (20 cases, 35.7%), *C. tropicalis* (10 cases, 12.5%), and *C. glabrata* (5 cases, 8.9%). Antifungal susceptibility testing results revealed that all Candida isolates exhibited 100% susceptibility to amphotericin B, nystatin, and voriconazole. However, *C. krusei* isolates demonstrated 100% resistance to fluconazole, while *C. glabrata* isolates exhibited 100% resistance to ketoconazole. **Conclusion:** In our current research, *C. albicans* emerged as the most prevalent species, closely followed by

C. krusei. The process of initially presuming the identity of Candida species, followed by confirming it, proves essential in facilitating the prompt commencement of suitable antifungal therapies. Notably, the relatively elevated resistance observed in Non-albicans Candida species to commonly prescribed antifungal agents such as fluconazole and ketoconazole underscores the importance of routinely conducting antifungal susceptibility testing for all Candida isolates.

1. INTRODUCTION

Vulvovaginal candidiasis (VVC) ranks as the second most prevalent mucosal infection, trailing only behind bacterial vaginosis, and it affects a significant number of otherwise healthy women in the childbearing age group [1], [2]. Research estimates suggest that approximately 70% of women in their childbearing years encounter at least one episode of VVC, with

around half of them experiencing at least one recurrence [3], [4]. Beyond the discomfort and the financial expenses linked to medication and healthcare, various studies have proposed that vaginal candidiasis could potentially heighten a woman's susceptibility to contracting othersexually transmitted diseases, including Human Immunodeficiency Virus (HIV) [2]. Untreated vaginal candidiasis in pregnant women can have severe



consequences, potentially leading to conditions such as chorioamnionitis, which

may result in abortion, prematurity, preterm delivery, and even congenital infections in the newborn. In non-pregnant women, untreated vaginal candidiasis can contribute to pelvic inflammatory disease, which can, in turn, lead to infertility [1], [2], [5]. The primary symptoms associated with VVC typically include itching in the vulvar and/or vaginal area and the presence of a thick, curd-like vaginal discharge. However, it's

tropicalis, with increasing frequency. These NAC species are known for their varying resistance levels to conventional antifungal treatments, often contributing to persistent infections. To prevent the inadvertent selection of less susceptible NAC species through empirical antifungal treatments or prophylaxis, it becomes crucial to routinely perform Candida isolates' speciation during specimen processing [7], [8]. The current study was conducted with the aim of assessing the prevalence of different Candida species in cases of vaginal candidiasis and evaluating the susceptibility of these isolates to antifungal treatments. This data is essential as it can offer valuable insights into the development of effective strategies for prevention and potential treatment approaches for vaginal candidiasis.

II. MATERIAL AND METHODS

This cross-sectional study spanned a duration of six months. A total of 100 women who presented complaints including vaginal discharge, itching, dyspareunia, low backache, and lower abdominal pain were selected for participation in the study using a straightforward random sampling method.

Inclusion Criteria: The study included women in the age range of 16 to 40 years who were clinically diagnosed with vaginitis.

Exclusion Criteria: The study excluded women who had previously undergone antifungal treatment and those who presented with evident vaginal bleeding.

Prior to sample collection, informed verbal and written consent were obtained from each patient, and the study received approval from the Institutional Ethics Committee.

Aseptically, a pair of high vaginal swabs were collected from the posterior vaginal fornix of the subjects using a vaginal speculum and a posterior vaginal wall retractor. These swabs were subsequently inoculated onto

important to note that painful urination and/or discomfort during sexual intercourse (dyspareunia) are also frequently reported symptoms [1]. Because these symptoms are not specific and can overlap with other conditions, confirming the diagnosis through laboratory culture becomes essential [6]. *Candida albicans* stands out as the most frequently encountered species. However, there has been a notable rise in the recovery of non-*albicans* *Candida* (NAC) species, such as *Candida glabrata*, *Candida parapsilosis*, and *Candida*

Sabouraud's dextrose agar (SDA) tubes supplemented with chloramphenicol and CHROM agar plates. Additionally, direct smears were prepared from the samples, and Gram's staining was conducted following standard protocols. The culture tubes and plates that were inoculated were placed in an incubator set at 37°C for a period of 48 hours. Colonies showing characteristics indicative of *Candida* species were subjected to further identification and speciation using various methods, including Gram's staining, Germ tube test, Chlamydospore formation on corn meal agar, and biochemical tests. Additionally, *Candida* isolates were processed for speciation using CHROMagar. Following an incubation period of 24-48 hours at 37°C, *Candida* species were differentiated based on the type of growth and the color of the isolates on CHROMagar *Candida* plates [7], [9]:

- *C. albicans*: Green
- *C. tropicalis*: Blue to purple
- *Candida krusei*: Pinkish-purple and fuzzy
- *C. glabrata*: Cream to white

Antifungal susceptibility testing was conducted for all *Candida* isolates using the Etest method to ascertain the Minimum Inhibitory Concentration (MIC).

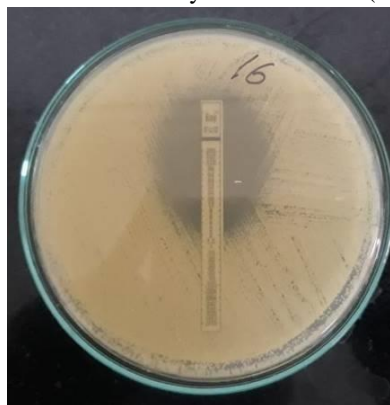


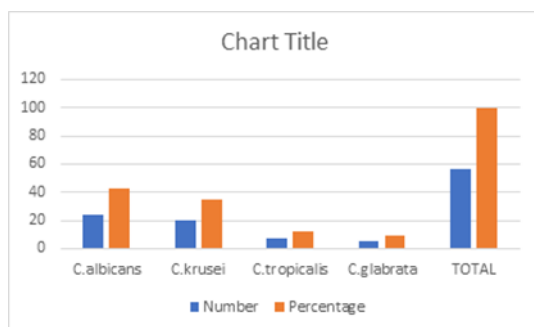
FIGURE 1: The Etest result of a *Candida albicans* isolate

tested against fluconazole.

| Age group | Number | Percentage |
|---------------|--------|------------|
| <20 years | 8 | 7.5 |
| 20 - 24 years | 14 | 14 |
| 25 - 40 years | 72 | 72 |
| >40 years | 7 | 6.5 |
| TOTAL | 100 | 100 |

TABLE 1: Age distribution of cases.

Candida isolates obtained from vulvovaginal candidiasis patients were placed on Sabouraud dextrose agar (SDA) and then incubated at 36°C under moist conditions for a period ranging from 2 to 4 days. Yeast colonies with a diameter greater than 2 mm on the SDA were suspended in a 0.84% sterile saline solution to achieve a turbidity level equivalent to a 0.5 McFarland standard.



ity to amphotericin B, nystatin, and voriconazole. Specifically, all *C. albicans* (100%) and *C. tropicalis* (100%) isolates, as well as 60% of *C. glabrata* isolates, exhibited susceptibility to fluconazole. However, *C. krusei* isolates were uniformly resistant to fluconazole (100%). Regarding ketoconazole, *C. albicans* displayed 100% susceptibility, while other species displayed varying levels of resistance. All *C. glabrata* isolates (100%) were resistant to ketoconazole, along with 60% of *C. krusei* isolates, and one *C. tropicalis* isolate (33.3%) also exhibited resistance to ketoconazole.

IV. DISCUSSION

Vulvovaginal candidiasis (VVC) ranks as the second most prevalent infection affecting symptomatic women in the vulvovaginal region, making up approximately 18% to 40% of cases [12]–[14]. In the current study, vaginal candidiasis was identified in 56 (28%) symptomatic women. This finding aligns with the research of Ahmad A, et al. [13] and Olowe, et al. [14]. However, it is worth noting that our prevalence rate is

III. RESULTS

The study comprised a sample of 100 women. The age breakdown of the participants was as follows: 8 (7.5%) were under 20 years old, 14 (14%) fell within the 20-24 age group,

72 (72%) were aged between 25 and 40, and 6 (6.5%) were over 40 years old.

Every *Candida* isolate demonstrated complete susceptibil-

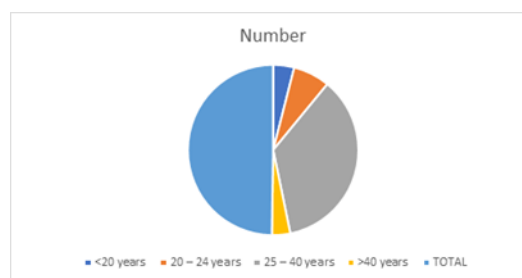


FIGURE 2: Distribution of *Candida* species.

lower than the rate reported by ER Ylander, et al.

[15] Variations in the socio-demographic profiles, immune system health of patients, administration of broad-spectrum antibiotics and immune-suppressing medications, as well as hormonal factors have been recognized as contributing factors to the disparities observed in the frequency of initial and/or recurring episodes of vulvovaginal candidiasis across various research studies [12]. The utilization of CHROM agar for *Candida* species speciation, which relies on color differentiation, presents a swift, user-friendly, and dependable approach for distinguishing clinically significant *Candida* species, as opposed to the labor-intensive traditional methods. Particularly in developing nations, CHROM agar emerges as a straightforward phenotypic testing alternative to molecular assays. It boasts both remarkable sensitivity and specificity in the accurate identification of *Candida* species [7], [9]. Consistent with prior research findings, the current study also revealed that *C. albicans* was the predominant species isolated, constituting 44.8% of cases. Among the non-*albicans* *Candida* species, *C. krusei* took the lead at 36.7%, followed by *C. tropicalis* at 18.5%, and *C. glabrata* at 9.9%. Notably, *C. albicans* exhibited a significantly greater propensity for adhering to vaginal epithelial cells when compared to other *Candida* species. This heightened adhesive capability may elucidate the relatively higher prevalence of

C. albicans in cases of vaginal candidiasis [2]. In the azole group of antifungals, *C. krusei* isolates displayed complete resistance to fluconazole, while 40% of *C. glabrata* isolates exhibited resistance. These results align with the findings reported by Sasikala G [2] and Bitew A, et al. [12]. Further- more, all five *C. glabrata* isolates demonstrated resistance to ketoconazole, as did 60% of *C. krusei* isolates and one *C. tropicalis* isolate, consistent with the observations of MondalS, et al. [19]. The notably elevated resistance levels observed in these non-*albicans* *Candida* (NAC) species toward commonly prescribed antifungals underscore the importance of conducting routine antifungal susceptibility testing for all *Candida* isolates.

V. CONCLUSION

In the current study, vulvovaginal candidiasis had an inci- dence of 28%, with *C. albicans* being the most frequently isolated species. The initial presumptive identification fol- lowed by confirmation of *Candida* species is crucial for en- abling prompt initiation of appropriate antifungal treatment, thereby reducing

REFERENCES

- [1] Nnadi, D. C., & Singh, S. (2017). The prevalence of genital *Candida* species among pregnant women attending antenatal clinic in a tertiary health center in North-west Nigeria. *Sahel Medical Journal*, 20(1), 33-37.
- [2] Jindal, N., Gill, P., & Aggaarwal, A. (2007). An epidemiological study of vulvovaginal candidiasis in women of childbearing age. *Indian Journal of Medical Microbiology*, 25(3), 175-176.
- [3] Sobel, J. D., Faro, S., Force, R. W., Foxman, B., & Ledger, W. J. (1998). Vulvovaginal candidiasis: Epidemiologic, diagnostic, and therapeutic considerations. *American Journal of Obstetrics and Gynecology*, 178(2), 203-211.
- [4] Vijaya, D., Dhanalakshmi, T. A., & Kulkarni, S. (2014). Changing trends of vulvovaginal candidiasis. *Journal of Laboratory Physicians*, 6(1), 28-30.
- [5] Neerja, J., Aruna, A., & Paramjeet, G. (2006). Significance of *Candida* culture in women with vulvovaginal symptoms. *Journal of Obstetrics and Gynecology of India*, 56(3), 139-141.
- [6] Vijaya, D., Harsha, T. R., & Nagarathamma, T. (2011). *Candida* specia- tion using chrom agar. *Journal of Clinical and Diagnostic Research*, 5(4), 755-757.

morbidity and mortality. Notably, among commonly employed antifungal medications, fluconazole and ketoconazole demonstrated the lowest efficacy against non-*albicans* *Candida* (NAC) species, with *C. krusei* and

C. glabrata displaying heightened resistance to these drugs. Given the evolving trends in antifungal susceptibility pat- terns, it is advisable to conduct routine antifungal suscep- tibility testing for *Candida* isolates in clinical microbiology laboratories.

FUNDING

This research did not receive any specific grant from funding agencies in the public, commercial, or nonprofit sectors.

CONFLICTS OF INTEREST

The authors declared no conflict of interest.

AUTHORS' CONTRIBUTIONS

All authors equally contributed to preparing this article.

- [7] Agarwal, S., Manchanda, V., Verma, N., & Bhalla, P. (2011). Yeast identification in routine clinical microbiology laboratory and its clinical relevance. *Indian Journal of Medical Microbiology*, 29(2), 172-177.
- [8] Khadka, S., et al. (2017). Isolation, speciation and antifungal susceptibility testing of *Candida* isolates from various clinical specimens at a tertiary care hospital, Nepal. *BMC Research Notes*, 10, 218.
- [9] Song, Y. B., et al. (2015). Antifungal susceptibility testing with Etest for *Candida* species isolated from patients with oral candidiasis. *Annals of Dermatology*, 27(6), 715-720.
- [10] Negri, M., Henriques, M., Svidzinski, T. I., Paula, C. R., & Oliveira, R. (2009). Correlation between Etest, disk diffusion, and microdilution methods for antifungal susceptibility testing of *Candida* species from infection and colonization. *Journal of Clinical Laboratory Analysis*, 23(5), 324-330.
- [11] Bitew, A., & Abebaw, D. (2018). Vulvovaginal candidiasis: species distribution of *Candida* and their antifungal susceptibility pattern. *BMC Women's Health*, 18(1), 94.
- [12] Ahmad, A., & Khan, A. U. (2009). Prevalence of *Candida* species and potential risk factors for vulvovaginal candidiasis in Aligarh, India. *European Journal of Obstetrics, Gynecology, and Reproductive Biology*, 144(1), 68-71.
- [13] Olowe, O. A., Makanjuola, O. B., Olowe, R., & Adekanle, D. A. (2014). Prevalence of vulvovaginal candidiasis, trichomoniasis and bacterial vaginosis among pregnant women receiving antenatal care in Southwestern Nigeria. *European Journal of Microbiology and Immunology*, 4(4), 193-197.
- [14] Erylander, E., Berglund, A.-L., Krassny, C., & Petrini, B. (2004). Vulvovaginal candida in a young sexually active population: prevalence and association with orogenital sex and frequent pain at intercourse. *Sex Transm Infect*, 80, 54-57.
- [15] Omar, A. A. (2001). Gram stain versus culture in the diagnosis of vulvovaginal candidiasis. *EMHJ-Eastern Mediterranean Health Journal*, 7(6), 925-934.
- [16] Chakraborti, A., Ghosh, A., Batra, R., Kaushal, A., Roy, P., & Singh, H. (1996). Antifungal susceptibility patterns of the non-*C. albicans* species and the distribution of the species which were isolated from *Candidaemia* cases over a 5 year period. *Indian Journal of Medical Research*, 104, 171-6.
- [17] Mokaddas, E. M., Al-Sweih, N. A., & Khan, Z. U. (2007). Species distribution and antifungal susceptibility of *Candida* bloodstream isolates in Kuwait: a 10-year study. *Journal of Medical Microbiology*, 56(2), 255-259.
- [18] Nyirjesy, P., & Sobel, J. D. (2003). Vulvovaginal candidiasis. *Obstetrics and Gynecology Clinics*, 30(4), 671-684.
- [19] Mondal, S., Mondal, A., Pal, N., Banerjee, P., Kumar, S., & Bhargava, D. (2013). Species distribution and in vitro antifungal susceptibility patterns of *Candida*. *Journal of Institute of Medicine Nepal*, 35(1), 45-49.