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Antifungal Susceptibility Pattern of *Candida albicans* and Non *Candida albicans* Species Isolates at a Tertiary Care Hospital in India

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Vulvovaginal candidiasis (VVC) is a widespread fungus that affects women of all ages. After bacterial vaginosis, vulvovaginal candidiasis is the second most prevalent cause of vaginitis, affecting 40% of women with vaginal discharge. Candida is a fungus that is one of the most common opportunistic fungi in humans. The samples were processed using standard Candida isolation techniques. Candida species were identified using germ tube tests and Candida agar media. On Mueller Hinton Agar (MHA) supplemented with 2 percent glucose and 0.5 g/ ml methylene blue dye, the disc diffusion method was employed to investigate antifungal sensitivity. *Candida albicans* was responsible for 42 (36.3%) of the 350 Candida isolates, followed by *Candida glabrata* (24.1%), *Candida tropicalis* (22.5%), *Candida krusei* (12.3%), and *Candida parapsilosis* (12.3%). (9.7 percent). With a sensitivity pattern of 106/116, amphotericin Bis is the most effective antifungal medication against Candida isolates (91.3 percent). The resistance to ketoconazole, on the other hand, was the highest (20.6 percent). In light of the rising tide of antimicrobial resistance to fungal medicines, the current study suggests that species-level identification of Candida isolates should be encouraged.

Keywords: Vulvovaginal candidiasis; Candida albicans; vaginitis.

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1. INTRODUCTION

The fungal illness vulvovaginal candidiasis (VVC), often known as vaginitis, affects women of all ages. After bacterial vaginosis, vulvovaginal candidiasis is the second most prevalent cause of vaginitis, affecting 40% of women with vaginal discharge. Candida is an opportunistic fungus that is found in humans [1]. Only a handful of the 350 species in the genus Candida have been recognised as causing opportunistic human illness [2]. *Candida albicans*, Candida glabrata, Candida tropicalis, Candida krusei, Candida parapsilosis, Candida dubliniensis, Candida guillermondii, and Candida kyfe are some of the Candida species that cause illness in humans [3-6]. Candida species can be found on the mucosal surfaces of the human gastrointestinal system, genitourinary tract, and mouth as part of the normal flora. It can induce minor infections all the way up to life-threatening invasive and haematogenic infections [7]. Vaginal candidiasis is the most frequent fungus that affects the female genital system over the world [8,9]. The most common symptoms of vaginitis are vaginal pruritis, thick white vaginal discharge, itching, vulva inflammation, and dyspareunia [10]. The most common symptoms of vaginitis include vaginal pruritis, thick white vaginal discharge, discomfort, vulva inflammation, and dyspareunia. Depending on the clinical presentation and antifungal therapy, vaginal candidiasis can be classified as easy or tough. *Candida albicans* is the most prevalent cause of simple vaginal Candidiasis, which causes mild to severe symptoms. Candida species other than *Candida albicans* are the most common cause of complicated vaginal candidiasis, which affects immune compromised persons and pregnant women. *Candida albicans* is the most prevalent cause of VVC, however other Candida non-albicans species (*C. glabrata, C. tropicalis, C. krusei, C. parapsilosis, C. gullermondii*) have been discovered as well. The treatment of C. glabrata, the second most common yeast, is considered revolutionary. Although *Candida albicans* and Candida non-albicans are closely related, their epidemiology, pathogenicity features, and susceptibility to fungal infection are distinct, making Candida species identification critical for effective management [11,12]. Prolonged therapy and increased antifungal use for recurrent candidiasis are the most important risk factors for azoles resistance among Candida isolates from vulvovaginitis candidiasis patients [13]. Women with vaginal candidiasis have a higher risk of contracting HIV [14]. A substantial link between candida and diabetes [15-17], as well as early delivery [18], has been established in several investigations. VC is caused by pregnancy, uncontrolled diabetes, antibiotic use, oral contraceptive use, immunological suppression, excessive perfume use, and contraceptive use [19]. The VC therapy is painless and only lasts a few weeks. If left untreated, it is a major risk factor for other sexually transmitted infections [20]. For confirmed instances of VC, a brief course of azole-based antifungal therapy is effective, safe, and inexpensive [21].

2. MATERIALS AND METHODS

From January 2018 to February 2019, a cross-sectional study was conducted at the Department of Microbiology of a tertiary care hospital in central India. The participants in the study were 350 women who visited the Obstetrics and Gynecology department with vaginal discharge.

2.1 Collection of Specimens

To avoid contamination by other organisms, samples were obtained from the vaginal or cervix with a sterile cotton swab. Two swabs were obtained from each subject. One was used for direct smear examination, while the other was inoculated and cultured aerobically on Sabouraud's dextrose agar. Direct smears were examined using Gram staining.

Gram staining revealed gram positive budding fungal yeast cells, confirming Candida development on Sabouraud's dextrose agar. Colony morphology and gram stain analyses were used to detect Candida growth on SDA. Candida species were identified after they grew.

To identify Candida isolates, standard mycological procedures such as the germ tube test, sugar fermentation and assimilation, colony colour on Hi Chrome Candida agar, and chlamydospore development on Corn meal agar were used.

Antifungal defencelessness difficult was carried out with the disc diffusion technique with Mueller-Hinton Agar, 2 percent Glucose, and Methylene Blue Dye Medium, according to CLSI standards (C.L.S.I. document M44-A2, 2009.). Five distinct colonies with a diameter of around 1 mm were separated from a Candida species culture that had been cultivated for at least 24 hours to make the inoculum. The turbidity was accustomed visually by comparing the

transmittance of the inoculums to that of a 0.5 McFarland benchmark suspended in 5 mL of sterile saline.

Antifungal susceptibility was determined using the disc diffusion approach. Antifungal discs (Thermo ScientificTM OxoidTM) containing fluconazole (10 g), itraconazole (10 g), voriconazole (10 g), clotrimazole (10 g), and nystatin (100 IU) were applied to MHA (Thermo Scientific TM Oxoid TM) using a disc dispenser (Oxoid TM) as suggested by the scientific Laboratory criterion Institute M44.

Before being read, the plates were incubate for 24 hours at 37°C. For each antifungal disc, the sizes of zones of inhibition were measured in millimetres with a ruler. The CLSI criteria were used to interpret all antifungal susceptibility tests (susceptible S, susceptible dose dependent relative [SDD], and resistant R) (Table 1). Superiority manage was carried out with American Type Culture Collection (ATCC) 90028 quality control strains.

As quality control, *C. albicans* ATCC 90028 and *C. parapsilosis* ATCC 22019 were utilised. Himedia Laboratories in India provided all of the culture media, antifungal disc, and control strains.

3. RESULTS AND DISCUSSION

A total of 116 Candida varieties are found in 350 elevated vaginal swabs. NAC accounted for 63.7 percent of the 116 Candida isolates, while *C. albicans* was responsible for 42 percent. Fig. 1 shows that *C. glabrata* was detected in 26/116 (22.4%) of NAC, followed by *C. tropicalis* in 24/116 (20.6%), *C. parapsilosis* in 16/116 (13.7%), and *C. krusei* in 10/112 (8 percent). The findings of Candida species speciation utilising Candida HiChrom agarcolor outpost and germ tube test are shown in Table 2. *Candida albicans* colonies were green with a germ tube, *Candida glabrata* colonies were purple with a germ tube, *Candida krusei* colonies were pink with a germ tube, and *Candida tropicalis* colonies were blue with a germ tube. *Candida parapsilosis* had cream colour colonies and a germ tube.

Table 2 demonstrates the compassion pattern of various antifungal drugs for the 116 Candida isolates tested: fluconazole (73 isolates, 62.9 percent), Voriconazole (104 isolates, 89.6%), Ketoconazole (86 isolates, 74.1 percent), Nystatin (94 isolates 81 percent), and Amphotericin B (106 isolates 99.2%).

In the instance of *Candida albicans* (n=42), 35 isolates (83.3%) were sensitive to Fluconazole, 34 isolate (80.9%) to Voriconazole, 32 isolates (76.1%) to Ketoconazole, 38 isolates (90.4%) to Nystatin, and 40 isolates (95.2%) to Amphotericin B. The 26 isolates of Candida glabrata were responsive to Fluconazole (21 isolates, 80.7 percent), Voriconazole (24 isolates, 92.3 percent), Ketoconazole (19 isolates, 73.0 percent), Nystatin (22 isolates, 84.6 percent), and Amphotericin B (24 isolates, 92.3 percent).

Fluconazole sensitivity was found in 18 isolates (75%), Voriconazole sensitivity was found in 23 isolates (95.8%), Ketoconazole sensitivity was found in 17 isolates (70.8%), Nystatin sensitivity was found in 18 isolates (75%), and Amphotericin B sensitivity was found in 23 isolates (95.8%). (13 isolates, 81.2 percent) were sensitive to Fluconazole, (16 isolates, 100%) were susceptible to Voriconazole, (13 isolates, 81.2 percent) were susceptible to Ketoconazole, (11 isolates, 68.7%) were susceptible to Nystatin, and (14 isolates, 87.5 percent) were susceptible to Amphotericin B. 4 isolates (50%) were sensitive to Fluconazole, 7 isolates (87.5%) were susceptible to Voriconazole, 5 isolates (62.5%) were susceptible to Ketoconazole, 6 isolates (75%), were susceptible to Nystatin, and 6 isolates (75%), were susceptible to Amphotericin B.

The rate of isolation of NAC was 63.7 percent in our investigation, compared to 36.2 percent for *C. albicans*. Kikani B et al. [22] (55.6 percent vs 44.4 percent), Deepa Babin et al [23] (64.5 percent vs 35.5 percent), and Namrata et al. [24] have all found higher NAC isolation than *C. albicans* (53 percent vs 47 percent). However, Tehran [25] (65.1 percent versus 34.9 percent), Sudan [26] (92 percent vs 8%), Egypt [27] (60.3 percent vs 39.7 percent), Turkey [28] (59.9% vs 40.1 percent), and India [29] have reported greater isolation of the most prevalent species, *C. albicans*, than NAC (66 percent vs 34 percent). After *C. albicans, C. glabrata* was the instant mainly prevalent isolate (24.1%) in the current investigation. In instances of VVC, it has been found to be the second most frequent isolate in Saudi Arabia [30] (31%), Turkey [31] (34.5%), Australia [32] (20%), Egypt [33] (12.7%), and India [34] (11 percent). *C. tropicalis* was the third most common isolate in the current investigation, following *C. albicans*

Table 1. Interpretative break points of antifungal agents

	Susceptible	Intermediate/SDD	Unwilling
Amphotericin B (20 µg)	≥15	10-14	<10
Fluconazole (10 µg)	≥19	15-18	≤14
Clotrimazole (10 µg)	≥20	12-19	≤11
Voriconazole (10 µg)	≥17	14-16	≤13
Nystatin (100 U)	≥15	10-14	<10

Table 2. Categorization of vaginal Candida isolates

Candida species	Colony on chrome agar	Germ tube test
Candida albicans	Light green	+
Candida glabrata	Purple	-
Candida tropicalis	Dark blue	Later produced
Candida krusei	Pink	-
Candida parapsilosis	Cream	-

Table 3. Occurrence allocation of c species in positive culture

Candida species	No of patients (n=116)	Percentage (%)
C. albicans	42	(36.2%)
C. glabrata	26	(22.4%)
C. tropcalis	24	(20.6%)
C. parapsilosis	16	(13.7%)
C. Krusei	10	(8.6%)

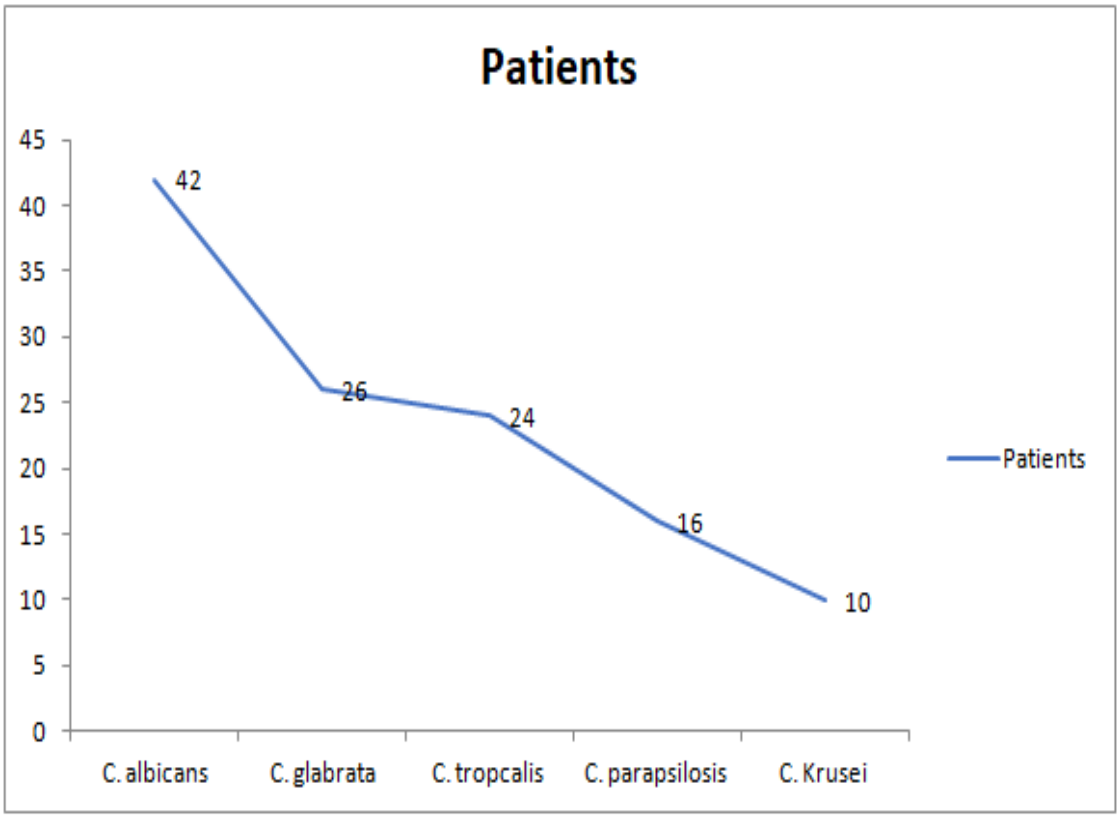

Fig. 1. Frequency distribution of c species in positive culture

Table 4. Antifungal vulnerability outline of *candida albicans* and candida *non albicans* species

Candida species	Antifungal														
	Amphotericin B (20μg)			Fluconazole (10μg)			Voriconazole (10μg)			Ketoconazole(30μg)			Nystatin (100 U)		
	S ≥15 (%)	**DDS 10-14(%)**	**R ≤9 n(%)**	**S ≥19 n (%)**	**DDS 15-18 n (%)**	**R ≤14 n (%)**	**S ≥17 n (%)**	**DDS 14-16 n (%)**	**R ≤13 (%)**	**S ≥15**	**DDS**	**R ≤9**	**S ≥15**	**DDS**	**R≤10**
C.albicans (n=42)	40 (95.2)	0 (0.0)	2(4.7)	35(83.3)	4(9.5)	3(7.1)	34(80.9)	3(8.8)	5(19.5)	32(80.9)	3(8.8)	7(16.6)	38(90.4)	0(0.0)	4(9.5)
C. non albicans (n=74)	*26*(92.8)	0(0.0)	2(7.1)	23(82.1)	2(7.6)	3(11.5)	26(92.8)	0 (0.0)	2(7.1)	21(75)	2(7.6)	5(17.85)	24(85.7)	1(3.5)	3(11.5)
C.glabrata (n=28)															
C.tropicalis(n=25)	*24*(96)	0 (0.0)	1(4.0)	19(76)	0 (0.0)	6(25)	24(96)	0 (0.0)	1(4)	18(72)	2(8)	5(20)	19(75)	1(4.1)	5(20.8)
C.parapsilosis (n=12)	*10*(83.3)	0 (0.0)	2(16.6)	10(83.3)	0 (0.0)	2(12.5)	12(100)	0 (0.0)	0 (0.0)	9(83.3)	2(8)	5(20)	9(75)	0 0.0)	3(25)
C. krusei (n=9*)*	6*(66.6%)*	0 (0.0)	3(33.3)	5(55.5)	0 (0.0)	4(44.4)	8(88.8)	0 (0.0)	1(11.1)	6 (66.6)	1(11.1)	2(22.2)	7(77.7)	0 0.0)	3(33.3)
Total	106(91.37)	0(0.0)	10(8.6)	92(79.3)	6(5.1)	18(15.5)	104(89.6)	3(2.5)	9(7.7)	86(74.1)	10(8.6)	24(20.6)	97(83.6)	2(1.7)	18(15.5)

S - Sensitive. DDS - Dose dependent Susceptible, R – Resistant

and *C.* glabrata. *C. tropicalis* segregation rates in cases VVC range from 4% to 26.4 percent [34-36]. The disc diffusion technique revealed that 15.5 percent of Candida isolates were resistant to fluconazole in our investigation. This finding is similar to resistance reported by Lee et al. [37] (17.1%) and Kustimur et al. [38] (16 percent). However, Ooga et al. [39] (25%) and Negri et al. [40] (27%) reported greater rates of resistance, whereas Zomorodian et al [41] (3.4%), Colombo et al. [42] (6%), Kikani et al. [43] (8.2%), and Pfaller et al. [44] reported lower rates of resistance (9.9 percent). In comparison to our study, there was a reduced rate. In our study, 7.1 percent of *C. albicans* had fluconazole resistance. Our findings are similar to those of Capoor et al. [45] (21.8 percent). Doddaiah V et al. [46], on the other hand, found it in 8.6% of their *C. albicans* isolates. Several workers have reported fluconazole resistance in *C. tropicalis* (10-11%) and *C. glabrata* (31-33%), but none of our isolates were resistant [47-49]. Voriconazole resistance was found in 7.7 % of our isolates. Das P et al. [50] (6.45%) and Dalia Saad El Feky et al (7.9%) have come to similar conclusions.

Voriconazole resistance was found in 21.1 percent of *C. albicans* isolates and 50 percent of *C. parapsilosis* isolates in our investigation. In this study, resistance to ketoconazole was higher (20.6%) than resistance to voriconazole (9.1%), possibly because ketoconazole is more commonly used than Voriconazole. Ketoconazole resistance is concerning, not only because it is a cost-effective candidiasis treatment, but also because it is the most often used azole. As a result, while prescribing or using Ketoconazole, caution should be exercised. Voriconazole, on the other hand, appears to be a better option, not only because of its lower resistance, but also because of its more effective binding to the Candida species' cytochrome P-450 isoenzyme [51]. In this study, Amphotericin B resistance was observed in 8.6% of Candida species, compared to 1.37 percent in Kashid et al. [52] and zero percent in Negri et al. [53]. Amphotericin B resistance in *C. albicans* was found to be 4.7 percent in our study, which is similar to the results reported by Capoor et al. [54] and Badiee et al. [55]. (4.3 percent and 7 percent respectively).

4. CONCLUSION

With a sensitivity pattern of 106/116, amphotericin Bis is the most effective antifungal medication against Candida isolates (91.3 percent). The resistance to ketoconazole, on the other hand, was the highest (20.6 percent). In light of the rising tide of antimicrobial resistance to fungal medicines, the current study suggests that species-level identification of Candida isolates should be encouraged.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT AND ETHICAL APPROVAL

As per international standard or university standard guideline participant consent and ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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