AT A TERTIARY CARE HOSPITAL IN INDIA, ANTIFUNGAL SUSCEPTIBILITY PATTERN OF *CANDIDA ALBICANS* AND NON *CANDIDA ALBICANS* SPECIES ISOLATES

ABSTRACT

Vaginitis, also known as vulvovaginal candidiasis (VVC), is a common fungal illness that affects women of all ages. After bacterial vaginosis, vulvovaginal candidiasis is the second most frequent cause of vaginitis, and it affects 40% of women who have vaginal discharge. Candida is a fungal disease and one of the most frequent human opportunistic fungus. Standard Candida isolation procedures were used to process the samples. Germ tube tests and Candida agar medium were used to identify Candida species. The disc diffusion technique was used to test antifungal sensitivity on Mueller Hinton Agar (MHA) supplemented with 2 percent glucose and 0.5 g/ ml methylene blue dye. *Candida albicans* accounted for 42 (36.3 percent) of the 350 Candida isolates, followed by *Candida glabrata* (24.1 percent), *Candida tropicalis* (22.5 percent), Candida krusei (12.3%), and Candida parapsilosis (9.7%). Amphotericin Bis is the most active antifungal drug against Candida isolates, with a sensitivity pattern of 106/116 (91.3%). Ketoconazole, on the other hand, had the highest resistance.24 (20.6 percent). In light of the growing tide of antimicrobial resistance to fungal agents, the current study suggests that species-level identification of Candida isolates should be encouraged.

Introduction

Vulvovaginal candidiasis (VVC), often known as vaginitis, is a common fungal illness that affects women of all ages. After bacterial vaginosis, vulvovaginal candidiasis is the second most frequent cause of vaginitis, and it affects 40% of women who have vaginal discharge. Candida is a fungal infection and one of the most frequent human opportunistic fungi [1]. Although the genus Candida has approximately 350 species, only a handful have been identified as causing opportunistic human disease [2]. *Candida albicans, Candida glabrata, Candida tropicalis, Candida krusei, Candida parapsilosis, Candida dubliniensis, Candida guillermondii,* and *Candida kyfe* are among the *Candida* species that cause illness in humans [3-6]. Candida species are found on the mucosal surfaces of the human gastrointestinal system, genitourinary tract, and mouth as part of the natural flora. It causes a wide range of illnesses, from minor infections to life-threatening invasive and haematogenic infections [7]. Vaginal candidiasis is the most frequent fungus that infects the female genital system across

the world [8, 9]. They, along with bacteria, are the most common cause of vaginitis, which is characterised by vaginal pruritis, thick white vaginal discharge, itching, vulva inflammation, and dyspareunia [10]. Vaginal candidiasis can be characterised as straightforward or difficult depending on the clinical presentation and antifungal treatment. The most common cause of uncomplicated vaginal Candidiasis is Candida albicans, which produces mild to severe symptoms. Non-albicans Candida species are the most prevalent cause of complex vaginal candidiasis, which is common among immunocompromised people and pregnant women. *Candida albicans* is the most common cause of VVC, however additional species classified as Candida non-albicans (*C. glabrata, C. tropicalis, C. krusei, C. parapsilosis, C. gullermondii*) have also been found. The treatment of C. glabrata, the second most prevalent yeast, is considered a severe therapeutic challenge [11]. Candida albicans and Candida non-albicans species are closely related but differ in epidemiology, virulence characteristics, and fungal susceptibility, hence Candida species identification is critical for effective management [12]. The most prominent risk factors for azoles resistance among Candida isolates from vulvovaginitis candidiasis patients include prolonged treatment and increasing antifungal usage for recurrent candidiasis [13]. Women with vaginal candidiasis have a higher risk of contracting HIV [14]. Several investigations have found a substantial link between candida and diabetes [15-17], and premature birth [18]. Pregnancy, uncontrolled diabetes, antibiotic usage, oral contraceptive use, immunological suppression status, excessive perfume use, and contraceptive use are all risk factors for VC [19]. The treatment for VC is quite gentle and only lasts a few weeks. It is a strong risk factor for other sexually transmitted diseases if left untreated [20]. A brief course of azole-based antifungal treatment for verified cases of VC is effective, safe, and cheap [21].

MATERIALAND METHODS

This cross-sectional investigation took place in the Department of Microbiology of a tertiary care hospital in central India from January 2018 to February 2019. The research comprised 350 individuals who complained of vaginal discharge and were seen at the Obstetrics and Gynecology department.

Specimen collection:

Specimens were taken from the vaginal or cervix with a sterile cotton swab to avoid contamination by other organisms. For each patient, two swabs were taken. One was utilised for direct smear inspection, while the other was inoculated on Sabouraud's dextrose agar and

incubated aerobically at 37°C. Gram staining and a 10% KOH preparation were used to examine direct smears.

Identification: Gram staining revealed gram positive budding fungal yeast cells, which validated Candida development on Sabouraud's dextrose agar. On the basis of colony morphology and gram stain analysis, Candida growth on SDA was established. Candida species were identified after growth.

Species identification- Standard mycological procedures were used to identify Candida isolates, including the germ tube test, sugar fermentation and assimilation, colony colour on Hi Chrome Candida agar, and chlamydospore development on Corn meal agar.

Antifungal susceptibility testing: Antifungal susceptibility testing was performed by disk diffusion method using Mueller-Hinton Agar, 2% Glucose with Methylene Blue Dye Medium as per CLSI guidelines (C.L.S.I. document M44-A2, 2009.). The inoculum was made by separating five separate colonies with a diameter of about 1 mm from a Candida species culture that had been cultured for at least 24 hours. Colonies were suspended in 5 mL of sterile saline, and the turbidity was adjusted visually by comparing the transmittance of the inoculums to that of a 0.5 McFarland standard.

The disc diffusion technique was used to assess antifungal susceptibility. Using disk dispenser (Oxoid™), fluconazole disk (10 µg), itraconazole (10 µg), voriconazole (10 µg), clotrimazole (10 µg) and nystatin (100 IU) antifungal discs (Thermo Scientific™ Oxoid™) were applied on MHA (Thermo Scientific™ Oxoid™) as recommended by the Clinical Laboratory Standard Institute (CLSI) M44A document.

The plates were incubated at 37°C for 24 hours before being read. 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 [SDD], and resistant R) (Table 1). Quality control was carried out using American Type Culture Collection (ATCC) 90028 quality control strains.

Table 1: Interpretative breakpoints of antifungal agents

	Sensitive	intermediate/SDD	Resistant

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

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.

RESULTS & DISCUSSION

350 high vaginal swabs yielded a total of 116 Candida species. Non-albicans Candida (NAC) accounted for 63.7 percent of the 116 Candida isolates, whereas *C. albicans* accounted for 42 percent. As indicated in figure 1, *C. glabrata* was found 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%). Table 1 shows the results of Candida species speciation using Candida HiChrom agarcolor of the colony and Germ tube test. *Candida albicans* had green colour colonies and a germ tube, *Candida glabrata* had purple colour colonies and a germ tube, *Candida krusei* had pink colour colonies and a germ tube, *Candida tropicalis* had blue colour colonies and a germ tube, and *Candida parapsilosis* had cream colour colonies and a germ tube.

Table 2 shows the sensitivity pattern of different antifungal agents for the 116 Candida isolates tested (73 isolates, 62.9 percent) were sensitive to fluconazole, (104 isolates, 89.6%) were sensitive to Voriconazole, (86 isolate, 74.1 percent) were sensitive to Ketoconazole, (94 isolates 81 percent) were sensitive to Nystatin, and (106 isolates 99.2%) were sensitive to Amphotericin B.

In the case of *Candida albicans* (n=42), 35 isolates (83.3%) were susceptible to Fluconazole, 34 isolates (80.9%) were susceptible to Voriconazole, 32 isolates (76.1%) were susceptible to Ketoconazole, 38 isolates (90.4%) were susceptible to Nystatin, and 40 isolates (95.2%) were susceptible to Amphotericin B.

For 76 isolates of Non *albicans candida* the 26 isolates of *Candida glabrata*, (21 isolates, 80.7%) were susceptible to Fluconazole, (24 isolates, 92.3%) were susceptible to

Voriconazole, (19 isolates, 73.0%) susceptible to Ketoconazole, (22 isolate 84.6%) was susceptible to Nystatin and (24isolates 92.3%) was susceptible to Amphotericin B.

Of the 24 isolates of *Candida Tropicalis*, (18 isolates, 75%) were susceptible to Fluconazole, (23 isolates, 95.8%) were susceptible to Voriconazole, (17 isolates, 70.8%) susceptible to Ketoconazole, (18 isolate 75%) was susceptible to Nystatin and (23 isolates 95.8%) was susceptible to Amphotericin B.

Of the 16 isolates of *Candida Parapsilosis*, (13 isolates, 81.2%) were susceptible to Fluconazole, (16 isolates, 100%) were susceptible to Voriconazole, (13 isolates, 81.2%) susceptible to Ketoconazole, (11 isolate 68.7%) was susceptible to Nystatin and (14 isolates 87.5%) was susceptible to Amphotericin B.

Of the 8 isolates of *Candida krusei*, (4 isolates, 50%) were susceptible to Fluconazole, (7 isolates, 87.5%) were susceptible to Voriconazole, (5 isolates, 62.5%) susceptible to Ketoconazole, (6 isolate 75%) was susceptible to Nystatin and (6 isolates 75%) was susceptible to Amphotericin B.

Table 1: Characterization 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 2: Frequency distribution 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%)

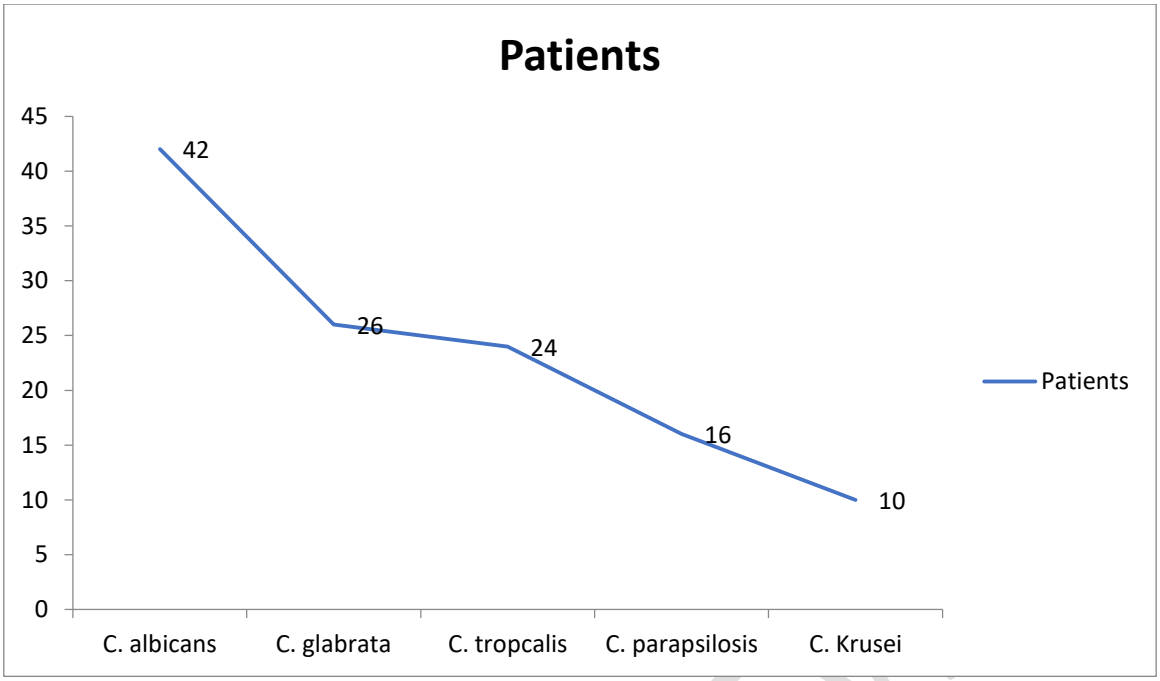

Fig1: Frequency distribution of c species in positive culture

Table 3: Antifungal susceptibility pattern 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) C.glabrata (n=28)	*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.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

The rate of isolation of NAC was 63.7 percent in our investigation, compared to 36.2 percent for *C. albicans*. Kikani B et a1 [22] (55.6 percent vs 44.4 percent), Deepa Babin et a1 [23] (64.5 percent vs 35.5 percent), and Namrata et a1 [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 second most 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* and *C.* glabrata. *C. tropicalis* isolation rates in cases VVC ranged 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 a1 [40] (27%) reported greater rates of resistance, whereas Zomorodian et a1 [41] (3.4%), Colombo et a1 [42] (6%), Kikani et al [43] (8.2%), and Pfaller et a1 [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 a1 [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 a1 [50] (6.45%) and Dalia Saad El Feky et a1 (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. Resistance to Ketoconazole was greater (20.6 percent) than resistance to voriconazole (9.1%) in this investigation, presumably because Ketoconazole is more often used than Voriconazole. Resistance to Ketoconazole is a reason for worry, not only because it is a cost-effective therapy for candidiasis, but also because it is the most often used azole. As a result, care should be used while prescribing or using Ketoconazole. Voriconazole, on the other hand, appears to be a superior alternative, not only because of the lesser resistance seen with this antifungal, but also because of its more efficient binding to the Candida species [51] cytochrome P-450 isoenzyme. Resistance to Amphotericin B was found in 8.6% of *Candida* species in this investigation, compared to 1.37 percent in Kashid et al [52] and zero percent in Negri et [53]. In our investigation, amphotericin B resistance was found to be 4.7 percent in *C.*

albicans, which is similar to the figures reported by Capoor et al.1[54] and Badiee et al.1[55] (4.3 percent and 7 percent respectively).

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. 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.

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