Original Research Article

Speciation and antifungal susceptibility pattern of *Candida* isolates from vulvovaginitis patients attending a tertiary care hospital in South India

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Abstract

Background: Vulvovaginal candidiasis is a global issue of concern due to its association with economic costs, sexually transmitted infections, and ascending genital tract infection leading to several complications in both pregnant as well as non-pregnant women. It is second to bacterial vaginosis affecting approximately 50–72% of women of reproductive age group. Resistance to antifungal agents has increased during the last decade. Thus, identification of *Candida* up to species level and its antifungal susceptibility testing is essential in the management of Candidal infections.
Aim: To determine the prevalence of various *Candida* species among vaginal candidiasis and to determine the antifungal susceptibility pattern of the isolates.
Materials and methods: A total of 56 *Candida* species were isolated from 200 clinical diagnosed cases of vaginitis over 6 months period. Growth on Sabouraud dextrose agar were evaluated according to standard protocol and further processed for Candida speciation on CHROM agar. Antifungal susceptibility testing was performed using the Etest method as recommended by Clinical and Laboratory Standards Institute (CLSI) M27-A3 document.
Results: Out of 200 vaginitis patients, 56 were positive for *Candida* species. All the isolates were speciated comprising four species – *C. albicans* 24 (42.8%), *C. krusei* 20 (35.7%), *C. tropicalis* 7 (12.5%), and *C. glabrata* 5 (8.9%). Antifungal susceptibility testing result of all Candida isolates were

100% susceptible to amphotericin B, nystatin and voiconazole. *C. krusei and C.glabrata* isolates were showed 100% resistance to fluconazole and ketoconazole respectively.

Conclusion: In the present study, *C. albicans* was most common species followed by *C. krusei.* Presumptive identification followed by confirmation of Candida species helps to initiate early appropriate antifungal treatment. The relatively higher resistance shown by Non-abicans Candida species to commonly prescribed antifungals (fluconazole and ketoconazole) emphasizes the need for routine antifungal susceptibility testing of all *Candida* isolates.

Key words

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

Introduction

Vulvovaginal candidiasis (VVC) is second to bacterial vaginosis as most common mucosal infections that affect large number of otherwise healthy women of childbearing age group [1, 2]. It is estimated that around 75% of all women experience at least one episode of VVC during their childbearing years, of which about half have at least one recurrence [3, 4].

In addition to discomfort and the cost associated with medication and health care, several studies have suggested that vaginal candidiasis may increase a woman's risk of contracting other sexually transmitted diseases such as Human immunodeficiency virus (HIV) [2].

Vaginal candidiasis in pregnant women if untreated can lead to chorioamnionitis with subsequent abortion, prematurity, preterm delivery and congenital infection of the neonate and pelvic inflammatory disease resulting in infertility in non-pregnant women [1, 2, 5].

The principal symptoms of VVC are vulvar and/or vaginal pruritus and a thick curd/cheese like vaginal discharge. However, painful urination and/or dyspareunia are also common [1]. The lack of specificity of symptoms and signs in vulvovaginal candidiasis explains the need for laboratory confirmation by culture [6].

Candida albicans is the most commonly isolated species. More recently, non-albicans Candida (NAC) species such as Candida glabrata, Candida parapsilosis, and Candida tropicalis have been recovered with increasing frequency, which are known for their variable resistance to conventional antifungals and are thus responsible for persistent infections. To avoid selection of less susceptible NAC species by empirical antifungal treatment or prophylaxis, speciation of Candida isolates is essential in routine specimen processing [7, 8].

The present study was thus undertaken to determine the prevalence of various Candida species among vaginal candidiasis and to determine the antifungal susceptibility pattern of the isolates. Such data will provide important information in developing effective strategies for prevention and possible treatment option for vaginal candidiasis.

Materials and methods

This cross-sectional study of duration of six months, from June 2018 to November 2018 was performed in the Department of Medical Microbiology, Apollo Institute of Medical Sciences and Research (AIMSR), Chittoor, AP, South India. A total of 200 women attending the Outpatient department of Obstetrics and Gynecology with complaints of vaginal discharge, itching, dyspareunia, low backache, and pain in the lower abdomen were enrolled for the study by simple random sampling.

Inclusion criteria

Women of age group of 18–45 years clinically diagnosed as vaginitis were included in the study.

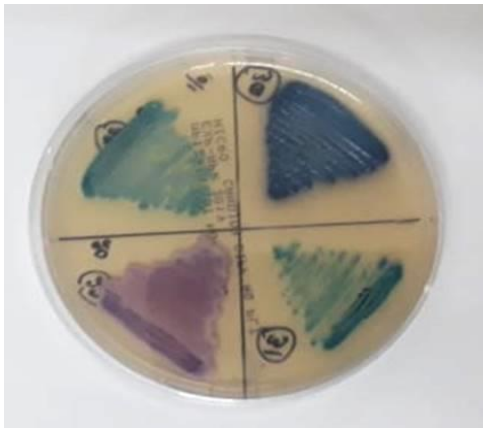
Exclusion criteria

Women on prior antifungal treatment and those with frank vaginal bleeding were excluded from the study.

Prior verbal and written consent was obtained from every patient before sample collection, and the study was approved by the Institutional ethics committee.

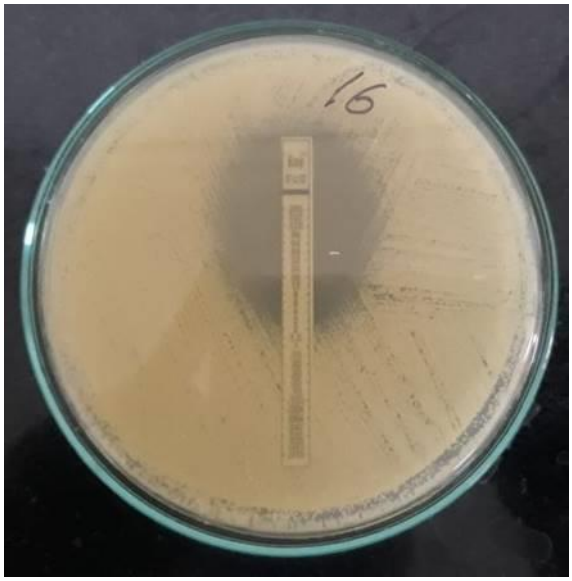
A pair of high vaginal swabs was obtained from the posterior vaginal fornix of the subjects aseptically with the help of a vaginal speculum and posterior vaginal wall retractor. These were then inoculated on Sabourauds dextrose agar (SDA) tubes with chloramphenicol and CHROM agar plates. Direct smears were prepared from the sample and Gram's staining was done according to the standard protocol. The inoculated culture tubes and plates were incubated at 37°C for 48 hours. Colonies suggestive of Candida species were further identified and speciated by Gram's staining, Germ tube test, Chlamydospore formation on corn meal agar and biochemical tests. The isolates were further processed for Candida speciation on CHROMagar (HiMedia, Mumbai, India). After incubation at 37 °C for 24–48 h, candida species were differentiated based on type of the growth and colour of 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 (**Figure – 1**).

Figure - 1: CHROM agar plate showing the different *Candida* species.



Antifungal susceptibility testing was performed and interpreted for all the isolates of Candida using the Etest method to determine the Minimum Inhibitory Concentration (MIC) as recommended by Clinical and Laboratory Standards Institute (CLSI) M27-A3 document guidelines [10]. As a basis for the treatment of vulvovaginal candidiasis practised in our hospital, the susceptibility testing was done for the following antifungal drugs: Amphotericin B (AP), Fluconazole (FLC), Ketoconazole (KET), Voriconazole (VRC) and Nystatin (NYT).

Figure - 2: The Etest result of a *Candida albicans* isolate tested against fluconazole.



Candida isolates from vulvovaginal candidiasis patients were inoculated on SDA and incubated at 35ºC in moist condition for 3 to 4 days. Yeast colonies (> 1 mm diameter) on SDA were suspended in 0.85% sterile saline solution to adjust to a turbidity of a 0.5 McFarland standard. A sterile cotton swab was used to spread 500 µl fungal suspension evenly on a plate of Mueller Hinton Agar, Modified for antifungal testing (HiMedia, Mumbai, India). Etest strips of FLC (0.016~256 µg/ml), KET (0.002~32 µg/ml), VRC (0.002~32 µg/ml), NYT (0.002~32 µg/ml and AP (0.002~32 µg/ml) were placed on plates that had been dried for 15 minutes at room temperature. The MIC of each drug was determined after incubation for 24 hours at 35º C in moist condition (**Figure – 2**). C. albicans

ATCC 90028 was used as reference strain. The MIC values of each of the isolates against the five antifungals were recorded and the susceptibility pattern interpreted according to CLSI M27-A3 guidelines [10, 11].

Results

A total of 200 women were included in the study. The age distribution of total cases were: in less than 19 years of age, 15 (7.5%), in 19–24 years of age group, 28 (14%) cases, in 25–40 years of age, 144 (72%) cases, and in more than 40 years of age 13 (6.5%) cases (**Table – 1**).

Out of 200 vaginitis patients, 56 were positive for Candida species. Age distribution of confirmed cases of vaginal discharge in less than 19 years age group was 2(3.5%), in 19–24 years age group, 07 (12.5%) cases, in 25–40 years age group 46 (82.1%) cases, and in more than 40 years age group, 1 (1.7%) case.

All the isolates were speciated comprising four species; C. albicans 24 (42.8%), C. krusei 20 (35.7%), C. tropicalis 7 (12.5%), C. glabarata 5 (8.9%) (**Table – 2**).

Table - 1: Age distribution of cases.

Age group	Number	Percentage
<19 years	15	7.5
19 – 24 years	28	14
25 – 40 years	144	72
>40 years	13	6.5
TOTAL	200	100

Table - 2: Distribution of Candida species.

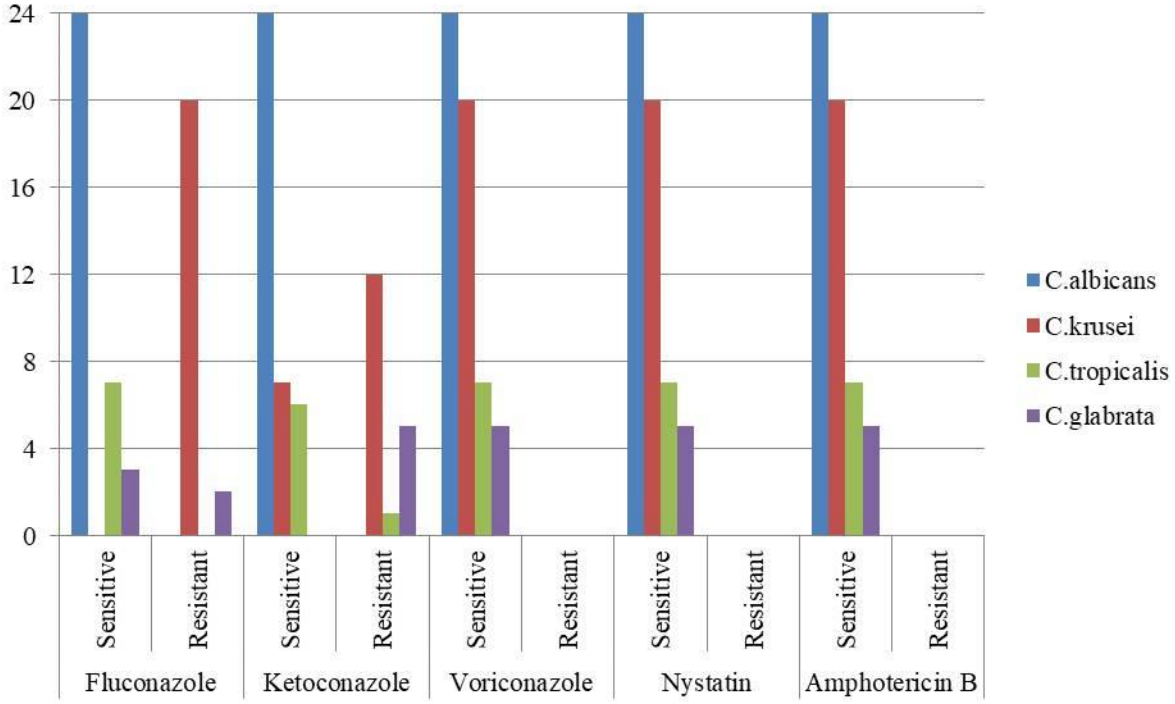
Candida species	Number	Percentage
C.albicans	24	42.8
C.krusei	20	35.1
C.tropicalis	07	12.5
C.glabrata	05	8.9
TOTAL	56	100

Table - 3: Susceptibility patterns of Candida isolates based on MIC by E-test after 24 hours incubation.

C. species (Number)	Antifungal	S (%)	SDD (%)	R (%)
C. albicans (24)	Fluconazole	24(100)	-	-
	Ketoconazole	24(100)	-	-
	Voriconazole	24(100)	-	-
	Nystatin	24(100)	-	-
	Amphotericin B	24(100)	-	-
C. krusei (20)	Fluconazole	-	-	20(100)
	Ketoconazole	7 (35)	1 (5)	12 (60)
	Voriconazole	20(100)	-	-
	Nystatin	20(100)	-	-
	Amphotericin B	20(100)	-	-
C. tropicalis (7)	Fluconazole	7(100)	-	-
	Ketoconazole	6(66.7)	-	1(33.3)
	Voriconazole	7(100)	-	-
	Nystatin	7(100)	-	-
	Amphotericin B	7(100)	-	-
C. glabrata (5)	Fluconazole	3(60)	-	2(40)
	Ketoconazole	-	-	5(100)
	Voriconazole	5(100)	-	-
	Nystatin	5(100)	-	-
	Amphotericin B	5(100)	-	-

Values are presented as numbers (:). S: Susceptible, SDD: Susceptible-dose dependent, R: Resistant

Figure - 3: Comparative antifungal susceptibility patterns of Candida species isolates.



The antifungal susceptibility pattern of all Candida isolates were recorded as Susceptible (S), Resistant (R) and Susceptible-dose dependent (**Table - 3**).

All the Candida isolates were 100% susceptible to amphotericin B, nystatin and voriconazole. All the isolates of C. albicans (100%) and tropicalis (100%) and 60% isolates of C. glabrata were susceptible to fluconazole whereas C. krusei isolates showed 100% resistance to fluconazole. While C. albicans showed 100% susceptibility to ketoconazole, other species showed varying resistance with all the isolates of C. glabrata (100%), 12 isolates of C. krusei (60%) and one isolate of C. tropicalis (33.3%) showing resistance to ketoconazole (**Figure – 3**).

Discussion

VVC is the second most common infection of the vulvovaginal area of symptomatic women accounting for about 17% to 42% [12, 13, 14].

In the present study, vaginal candidiasis was found in 56 (28%) symptomatic women, which is similar to Ahmad A, et al. [13] and Olowe, et al. [14], but lower than the prevalence rate reported, by ER ylander, et al. [15].

Differences in socio-demographic characteristics, immune– status of patients, treatment with broad spectrum antibiotics and immune suppressive drugs, and hormonal influence have been identified as some of the factors for differences in the prevalence of the occurrence and/or recurrent vulvovaginal candidiasis among studies [12].

In the present study, the highest incidence of VVC was seen in the 25-40 years age group, which correlates with previous studies [2, 12].

Vaginal discharge culture is the most sensitive method compared to Gram stain and other tests [16].

Speciation of Candida species by CHROM agar on the basis of colour diferentiation offered a rapid, convenient and reliable method for identification of clinically important Candida species when compared with cumbersome traditional techniques. In developing countries, CHROM agar can be taken as a simple phenotypic test alternative to molecular based

assay. CHROM agar has high sensitivity as well as specificity for the identification of Candida species [7, 9].

As has been previously reported in several studies, in the present study too, C. albicans was the most common species isolated at 42.8%. Among the non-albicans Candida species, C. krusei (35.7%) was most common followed by C. tropicalis (12.5%) and C. glabrata (8.9%). C. albicans adhere to vaginal, epithelial cells in significantly higher number than other Candida species. This could explain relative higher frequency of C. albicans in vaginal candidiasis [2].

However, many studies have shown that NAC species have more isolation rate than C. albicans which suggest the emergence of non-albicans Candida species as important pathogens [17, 18]. As per the current trend, the prevalence of non-albicans Candida was significantly higher in present study. The possible reason for this may be the indiscriminate use of antifungals which eliminates more sensitive C. albicans and selects azole resistant non-albicans candida [19].

Among the azole group of antifungals, resistance to fluconazole was shown by C. krusei isolates with 100% resistance and 40% of C. glabrata isolates. These findings are similar to that reported by Sasikala G [2] and Bitew A, et al. [12]. All the five C. glabrata isolates were found to be resistant to ketoconazole, along with 60% C.krusei isolates and one C. tropicalis, which is similar to Mondal S, et al. [20]. The relatively higher resistance exhibited by these NAC species to commonly prescribed antifungals emphasizes the need for routine antifungal susceptibility testing of all Candida isolates.

Conclusion

The incidence of vulvovaginal candidiasis in the present study was 28% with C. albicans as the most common species isolated. Presumptive identification followed by confirmation of Candida species helps to initiate early appropriate antifungal treatment there by reducing the morbidity and mortality. Among commonly used antifungal drugs fluconazole and ketoconazole was the least effective for NAC spp, with higher resistance against them shown by C. krusei and C. glabrata. Changing trends in the antifungal susceptibility pattern recommends routine antifungal susceptibility testing of Candida isolates in clinical microbiology laboratories.

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