

## Vulvovaginal Candidiasis - Speciation and Antifungal Susceptibility Pattern in a Tertiary Care Hospital, Tirunelveli

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### Abstract:

**Introduction:** Vulvovaginal candidiasis (VVC) refers to a disorder characterized by signs and symptoms of vulvovaginal inflammation due to *Candida* species. Gram's staining is performed to see the presence of yeast and pseudohyphae of *Candida* species. For systemic infection like candidemia, blood culture is done in a biphasic medium like brain-heart infusion agar-broth and incubated at both the temperatures. As drug resistance in *Candida* species is now very common, therefore, antifungal susceptibility testing should be performed. This study aims to determine the prevalence of various *Candida* species among vaginal candidiasis and to determine the antifungal susceptibility pattern of the isolates.

**Methodology:** This is a prospective study with *Candida* isolates from high-vaginal swabs. The samples were screened for *Candida* by microscopy and culture. Speciation of the *Candida* isolates was done on Hi-CHROM *Candida* agar and susceptibility pattern was determined. All the swabs received were subject to microscopy with Gram staining. All the isolates were subjected to antifungal susceptibility testing on Modified Mueller-Hinton agar as per CLSI guidelines. The antifungal agents used were fluconazole, itraconazole, clotrimazole, nystatin and amphotericin-B. The susceptibility pattern was observed by the Kirby-Bauer disk diffusion method.

**Results:** Of all the samples received at the laboratory of the Department of Microbiology, 63 showed *Candida* morphology and were plated on Hi-CHROM agar and their species was noted. Of the 63 *Candida* isolates, 27 were *C. albicans* (42.9%), 14 were *C. glabrata* (22.2%), 15 were *C. dubliniensis* (23.8%), 5 were *C. parapsilosis* (7.9%), *C. krusei* and *C. tropicalis* were 1 each (1.6%). Drug resistance was the most commonly noted in *Candida albicans* (14.8% of all *C. albicans* samples) than in other species.

**Conclusion:** In our study, most drug resistant strains were of *Candida albicans* species, which poses a great threat of recurrences. Moreover, multi-drug resistance patterns have been noted to most commonly use antifungal drugs in *Candida albicans* strains than non-*albicans* species. This makes speciation and antifungal susceptibility a must in treatment of vulvovaginal candidiasis. Doing so will provide proper and complete cure to patients without recurrence due to ineffective antibiotic treatment.

**Keywords:** Vulvovaginal candidiasis, susceptibility, resistance.

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### Introduction

Candidiasis is the commonest fungal disease found in humans affecting skin, nails & internal organs. It is caused by various yeast-like fungi belonging to the genus *Candida*. The infection may be acute or chronic. It is found mainly as a secondary infection in individuals with underlying immunocompromised conditions.

Vulvovaginal candidiasis (VVC) refers to a disorder characterized by signs and symptoms of vulvovaginal inflammation due to *Candida* species, which are commensals that normally colonize skin and mucosal surfaces. It is the second most common cause of vaginitis following bacterial vaginosis. It is estimated that at least 75% of healthy adult women will experience one episode

of vulvovaginal candidiasis during their reproductive phase. The signs and symptoms of VVC include a thick cheese-like discharge associated with intense vaginal and vulvar pruritus, pain, burning, erythema, and/or edema. They are capable of initiating infections in immunocompetent individuals and immunocompromised hosts.[1]

*Candida* sp. infections may present in a asymptomatic manner, but are also responsible for various clinical manifestations ranging from mucocutaneous overgrowth to life threatening disseminated infections like candidemia.[1] There is a significant role of VVC in pregnant women when left untreated, can lead to chorioamnionitis

with subsequent abortion, prematurity, preterm delivery and congenital infection of the neonate. In non-pregnant women it may cause pelvic inflammatory disease resulting in infertility. [2] The lack of specificity of symptoms and signs in vulvovaginal candidiasis explains the need for laboratory confirmation by culture. *Candida albicans* is the most commonly isolated species. More recently, there is an increasing frequency of Non-*Albicans Candida* (NAC) species such as *Candida glabrata*, *Candida parapsilosis*, *Candida tropicalis*, *Candida lusitanae*, *Candida kefyr*, *Candida stellatoidea*, *Candida guilliermondii* and *Candida dubliniensis* which show variable resistance to conventional antifungals and are thus responsible for persistent infections.

Gram's staining is performed to see the presence of yeast and pseudohyphae of *Candida* species. The yeast cells are approximately 4-8  $\mu\text{m}$  with budding and pseudohyphae. the Gram's smear may be visible as Gram-negative also due to over-decolorization otherwise all the fungi are Gram-positive in nature, The presence of pseudohyphae shows colonization and tissue invasion hence their demonstration in the direct smear of tissue is highly significant. Some do species not form pseudohyphae like *Candida glabrata*, *C. rugosa* and *C. lusitanae*, this fact must be kept in mind while doing microscopy.[8]

Over-the-counter medications favor infection by drug-resistant species. Hence, speciation of *Candida* isolates and its antifungal susceptibility is essential in routine specimen processing to avoid selection of drug resistant NAC species by empirical antifungal treatment or prophylaxis.[2][3]

The clinical specimens can be cultured on Sabouraud dextrose agar with antibacterial antibiotics and incubated at 25°C and 37°C. The colonies appear in 2-3 days as cream colored, smooth and pasty. Sometimes growth may be observed after an overnight incubation as seen in bacteria. The bacterial culture media like blood agar may also be used for growing the *Candida* species. The LPCB mount is prepared from the colonies to examine for the presence of yeast cells and pseudohyphae. Very rarely true hyphae may be seen. Gram staining may also be performed from the culture isolates. For systemic infection like candidemia, blood culture is done in a biphasic medium like brain-heart infusion agar-broth and incubated at both the temperatures.[4]

The clinical specimens can be cultured on CHROMagar *Candida*, which is a differential medium for yeasts, which exhibits chromogenic property according to the species grown, can be used to differentiate the various species.[5]

The recent clinical practice guidelines for the management of candidiasis were released by IDSA

in 2016. As far as the treatment of oral and mucocutaneous lesions of candidiasis is concerned, 1% gentian violet is locally applied. Nystatin can be used for the resistant mucosal lesions as suspension containing 2,00,000 units/ml. The azole creams like clotrimazole, miconazole, ketoconazole and econazole are also topically applied. For the systemic lesions intravenous infusion of amphotericin B is indicated. The azoles like ketoconazole, fluconazole and itraconazole are given orally. Itraconazole solution is also available for topical application. Amphotericin B can be combined with flucytosine in systemic candidiasis. Flucytosine should not be given alone because of apprehension of emergence of drug resistance in the yeasts of this genus.[4]

As drug resistance in *Candida* species is now very common, therefore, antifungal susceptibility testing should be performed by determining MICs as per the CLSI guidelines of approved document M27-A or EUCAST guidelines. This can play a major role in the management of resistant candidal infections, particularly, caused by non-*albicans Candida* species. In addition, Etest may also provide the simplest, most reproducible method of antifungal susceptibility testing and allow hospitals to develop suitable antifungal biograms.

This study aims 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 options for vaginal candidiasis.

#### Materials and Methods

This is a prospective study of census samples with *Candida* isolates from high-vaginal swabs received at the laboratory of the Department of Microbiology, Tirunelveli Medical College Hospital, Tirunelveli. The samples were screened for *Candida* by microscopy and culture. Speciation of the *Candida* isolates was done on Hi-CHROM *Candida* agar and susceptibility pattern was determined. All cultures of high vaginal swabs showing *Candida* colony morphology were included in the study, whereas improper and inadequate sample collected were excluded.

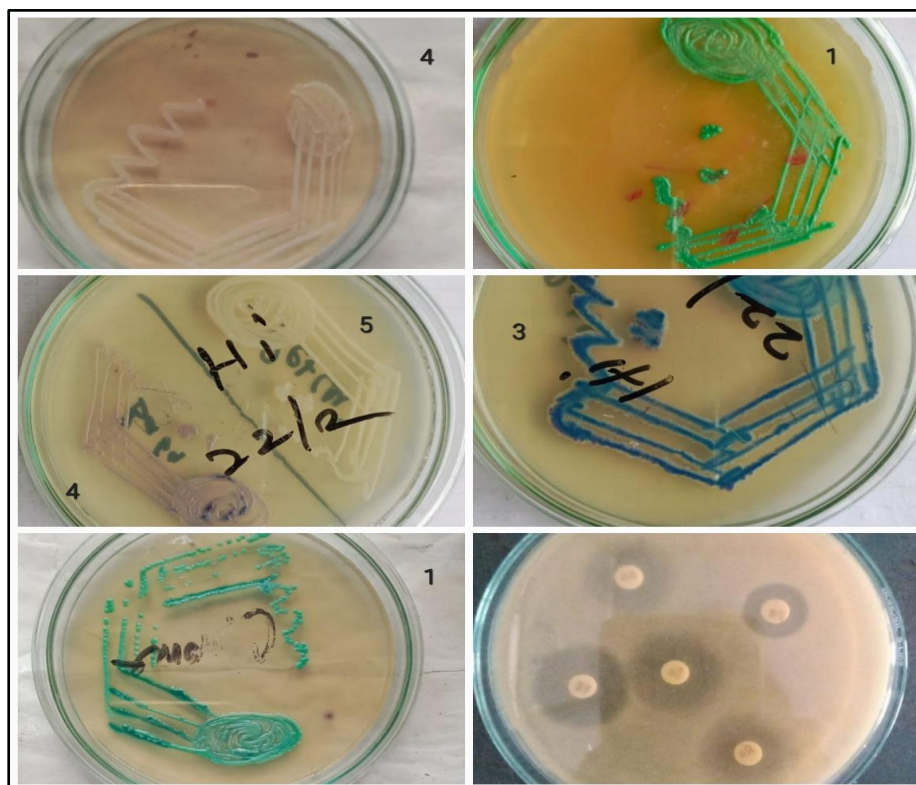
All the swabs received were subject to microscopy with Gram staining. 63 of the smears showed Gram positive budding yeast cells with/without pseudohyphae which were inoculated in Sabouraud Dextrose Agar and incubated at 37°C for 48 hours. The *Candida* isolates were inoculated in Hi-CHROM agar, speciation was done based on the pigmentation observed. All the isolates were subjected to antifungal susceptibility testing on Modified Mueller-Hinton agar as per CLSI guidelines. The antifungal agents used were

fluconazole, itraconazole, clotrimazole, nystatin and amphotericin-B. The susceptibility pattern was observed by the Kirby-Bauer disk diffusion method. The received Candida isolates were further segregated based on age groups to identify the age distribution of candida infections.

**Observations and Results:** Of all the samples received at the laboratory of the Department of Microbiology, 63 showed Candida morphology and

were plated on Hi-CHROM agar and their species was noted. The speciation was done based on the pigmentation observed as follows

1. Light green to bluish green - *Candida albicans*
2. Purple - *Candida glabrata*
3. Dark green - *Candida dubliniensis*
4. Cream colour - *Candida parapsilosis*
5. Pink - *Candida krusei*
6. Blue - *Candida tropicalis*[8]



**Figure 1: Speciation in Hi-CHROM agar**

Of the 63 Candida isolates, 27 were *C. albicans* (42.9%), 14 were *C. glabrata* (22.2%), 15 were *C. dubliniensis* (23.8%), 5 were *C. parapsilosis* (7.9%), *C. krusei* and *C. tropicalis* were 1 each (1.6%). Among the non-albicans species, *Candida*

*dubliniensis* was the majority followed by *Candida glabrata*.

*Candida albicans* was the most commonly isolated species (42.9%) and the rest were non-albicans candida.

**Table 1: Age-wise distribution of different Candida species**

Age group	C.albicans	C.glabrata	C.dubliniensis	C. parapsilosis	C.krusei	C.tropicalis
15-24	6	3	2	2	-	-
25-34	7	3	3	1	-	-
35-44	8	5	5	1	1	1
45-54	6	2	4	1	-	-
55-64	-	1	1	-	-	-

Most commonly affected age group was 35-44 years followed by 25-34 years and 45-54 years which shows the middle age has more common incidence. The resistance patterns were obtained by Kirby-Bauer disk diffusion method using Fluconazole, Itraconazole, Clotrimazole, Nystatin and Amphotericin-B as the antifungal agents.

**Table 2: Drug resistance pattern of different Candida species: S - Sensitive, SDD - Susceptible dose dependent, R- Resistance**

Species	Drug	S%	SDD%	R%
Candida albicans	Fluconazole	24 (88.9%)	2 (7.4%)	1 (3.7%)
	Itraconazole	24 (88.9%)	2 (7.4%)	1 (3.7%)
	Clotrimazole	23 (85.2%)	2 (7.4%)	2 (7.4%)
	Nystatin	25 (92.6%)	1 (3.7%)	1 (3.7%)
	Amphotericin-B	23 (85.2%)	3 (11.1%)	1 (3.7%)
Candida glabrata	Fluconazole	14 (100%)	-	-
	Itraconazole	14 (100%)	-	-
	Clotrimazole	13 (92.8%)	-	1(7.1%)
	Nystatin	14 (100%)	-	-
	Amphotericin-B	14 (100%)	-	-
Candida dubliniensis	Fluconazole	15 (100%)	-	-
	Itraconazole	15 (100%)	-	-
	Clotrimazole	15 (100%)	-	-
	Nystatin	15 (100%)	-	-
	Amphotericin-B	15 (100%)	-	-
Candida parapsilosis	Fluconazole	5 (100%)	-	-
	Itraconazole	5 (100%)	-	-
	Clotrimazole	5 (100%)	-	-
	Nystatin	5 (100%)	-	-
	Amphotericin-B	5 (100%)	-	-
Candida krusei	Fluconazole	-	-	1 (100%)
	Itraconazole	1 (100%)	-	-
	Clotrimazole	1 (100%)	-	-
	Nystatin	1 (100%)	-	-
	Amphotericin-B	1 (100%)	-	-
Candida tropicalis	Fluconazole	1 (100%)	-	-
	Itraconazole	1 (100%)	-	-
	Clotrimazole	1 (100%)	-	-
	Nystatin	1 (100%)	-	-
	Amphotericin-B	1 (100%)	-	-

Drug resistance was the most commonly noted in *Candida albicans* (14.8% of all *C. albicans* samples) than in other species. Nystatin had highest sensitivity followed by other antifungals. Other Species did not develop any resistance except in one sample of *Candida glabrata* to cotrimazole and one sample of *Candida krusei* to Fluconazole

#### Discussion:

There is a drastic rise in non-*albicans* species as compared to the report by Seeniammal et.al.[6] which was done in 2015 over a period of 1.5 years in the same tertiary care hospital showed 18% whereas our study shows 57.1%, which corresponds to the report by Kandati Jithendra et. al. [7] (56.19%). The incidence of *Candida albicans* (42.9%) in our study coincides with Lavanya V et.al [8]. (42.8%) and Kandati Jithendra et. al. (43.81%). However, there was a stark difference in incidence reported by Seeniammal S et. al. (82%). An increased isolation of *Candida dubliniensis* from culture samples is observed as compared to Seeniammal S et. al. And other similar studies. The incidence of *Candida glabrata* (22.2%) from our study corresponds to the report by Cita Rosita Sigit

Prakoewa et. al. [9] who reported 20%. The incidence in our study is 22.2% whereas it is 8% in the report by Seeniammal et. al. And 8.2% by Lavanya V et. al. There has been isolation of *Candida parapsilosis* in our study which was not seen by Seeniammal et.al. The incidence of *Candida tropicalis* and *Candida krusei* in our study is 1.6% each, while it was reported to be 6% and 4% respectively by Seeniammal et. al. And 12.5% and 35.7% respectively by Lavanya V et. al.

Age-wise distribution of the different species was done and it was found that the most number of cases was seen in the 35-44 years age group (33.3%) and the least number of cases in the 55-64 age group (3.2%). This increased number of cases in the 35-44 year age group is probably due to poor hygiene and sedentary lifestyle of Indian homemakers. This combined with the humidity, provides excellent habitat for *Candida* to flourish. The very low incidence of infections in the 55-64 years age group could be due to comparatively lower oestrogen levels than the reproductive age group, which does not favour binding of *Candida albicans* [4] or due to negligence of caretakers.

Of the 63 *Candida* samples, 6 showed complete resistance to certain drugs (9.5%) and 6 showed dose-dependent susceptibility (9.5%). The remaining were sensitive. Of the 6 drug resistant samples, the majority (4) were *Candida albicans* samples. Moreover, 2 samples showed multi-drug resistance to the azole group (Fluconazole + Itraconazole and Itraconazole+ Clotrimazole). *Candida krusei* is intrinsically resistant to fluconazole. [10] Similarly, 3 of the 6 dose dependant susceptible samples showed multi drug patterns (Nystatin+ Clotrimazole + Amphotericin-B, Amphotericin-B+ Itraconazole and Itraconazole + Clotrimazole). The sample resistant to amphotericin-B showed dose-susceptible sensitivity to both Itraconazole and Clotrimazole while other 2 dose-dependent susceptible samples were sensitive to all other drugs.

### Conclusion

In *Candida* infections, a rise in non-*albicans* species of *Candida* recently has been noted as suggested by previous studies. Most drug resistant strains were of *Candida albicans* species, which poses a great threat of recurrences. Moreover, multi-drug resistance patterns have been noted to most commonly use antifungal drugs in *Candida albicans* strains than non-*albicans* species. This makes speciation and antifungal susceptibility a must in treatment of vulvovaginal candidiasis. Doing so will provide proper and complete cure to patients without recurrence due to ineffective antibiotic treatment.

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### References

1. Prakoeswa, C., Puspitorini, D., Widya, Y., Anggraeni, S., Astari, L., Ervianti, E. and Suyoso, S. Profile of *Candida* Species in Vulvovaginal Candidiasis using Conventional Methods.
2. Lavanya V, Pavani P, Kailasanatha Reddy B. Speciation and antifungal susceptibility pattern of *Candida* isolates from vulvovaginitis patients attending a tertiary care hospital in South India. IAIM, 2019; 6(2): 62-68.
3. Seeniammal S, Selvakumar M, Nirmaladevi P. Clinicomycological study of vulvovaginal candidiasis. Indian J Sex Transm Dis 2021; 42:57-61.
4. J. Chander, Textbook of Medical Mycology, Jaypee Brothers Medical Publishers Pvt. Limited, 2017.
5. Odds FC, Bernaerts R. CHROMagar *Candida*, a new differential isolation medium for presumptive identification of clinically important *Candida* species. J Clin Microbiol. 1994 Aug; 32(8):1923-9.
6. Seeniammal S, Selvakumar M, Nirmaladevi P. Clinicomycological study of vulvovaginal candidiasis. Indian J Sex Transm Dis 2021; 42:57-61
7. Jithendra K, Madhavulu B, Rama P, Munilakshmi P, Avinash G. *Candida* Speciation from Vaginal Candidiasis and Its Antifungal Susceptibility. Int. J. Curr. Med. App. Sci. 2015 Feb; 3(5):144-8.
8. Lavanya V, Pavani P, Kailasanatha Reddy B. Speciation and antifungal susceptibility pattern of *Candida* isolates from vulvovaginitis patients attending a tertiary care hospital in South India. IAIM, 2019; 6(2): 62-68.
9. Prakoeswa, C., Puspitorini, D., Widya, Y., Anggraeni, S., Astari, L., Ervianti, E. and Suyoso, S. Profile of *Candida* Species in Vulvovaginal Candidiasis using Conventional Methods.
10. Berkow EL, Lockhart SR. Fluconazole resistance in *Candida* species: a current perspective. Infect Drug Resist. 2017 Jul 31; 10:237-245.