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Original Research Article

A STUDY OF NON-ALBICANS CANDIDA SPECIES CAUSING VAGINAL CANDIDIASIS WITH SPECIAL REFERENCE TO ANTIFUNGAL RESISTANCE AND *ERG11* GENE

Bhavana C[1], Shwetha DC[2], Ragini Ananth Kashid[3], Shanthi M[4], Anupma Jyoti Kindo[5], T Premamalini[6], Ms Karthika Kanagasabapathy[7]

[1]*Assistant Professor, Department of Microbiology, BGS Global Institute of Medical Sciences, Bengaluru, India*
[2]*Associate Professor, Department of Microbiology, BGS Global Institute of Medical Sciences, Bengaluru, India*
[3]*Professor and HOD, Department of Microbiology, BGS Global Institute of Medical Sciences, Bengaluru, India*
[4]*Professor and Head, Department of Microbiology, Sri Ramachandra Medical College and Research Institute, Chennai, India*
[5]*Professor, Department of Microbiology, Sri Ramachandra Medical College and Research Institute, Chennai, India*
[6]*Professor, Department of Microbiology, Sri Ramachandra Medical College and Research Institute, Chennai, India*
[7]*PhD scholar, Department of Microbiology, Sri Ramachandra Medical College and Research Institute, Chennai, India*

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Corresponding Author:
Dr. Ragini Ananth Kashid
Professor and HOD, Department of Microbiology, BGS Global Institute of Medical Sciences, Uttarahalli Main Road, Kengeri, Bangalore – 560060
Email: ragini.rajgopal@gmail.com

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ABSTRACT

Background: Vulvovaginal candidiasis represents one of the most prevalent fungal infections among women globally. If not promptly identified and appropriately treated, it can lead to substantial morbidity and recurrent disease. There have been reports highlighting the emergence of drug resistance, especially among non-albicans Candida species. In this context, the study was undertaken to identify the Candida isolates to species level, determine their antifungal susceptibility and to detect the presence of *ERG11* gene, associated with fluconazole resistance.

The results of this prospective study showed that the non-albicans Candida (51.4%) were frequently isolated compared to *Candida albicans* (48.6%). The Candida tropicalis (25.7%) was the predominant species among non-albicans Candida, followed by *Candida glabrata* (13.3%), *Candida krusei* (11.4%) and *Candida parapsilosis* (1%). On evaluation of antifungal susceptibility, by disc diffusion method, it was seen that all the Candida isolates were susceptible to amphotericin B and nystatin. Among *Candida albicans*, 27.5% were resistant to clotrimazole, followed by fluconazole (19.6%) and ketoconazole (17.6%). Among non-albicans Candida species, no resistance was detected to ketoconazole. 66.7% of *Candida tropicalis* were resistant to clotrimazole and 29.6% to fluconazole. 28.6% of *Candida glabrata* were resistant to clotrimazole and 14.3% to fluconazole. 33% of *Candida krusei* were resistant to clotrimazole. In our study, we observed that the resistance to commonly used antifungal drugs was high among non-albicans Candida as compared to *Candida albicans*.

Molecular analysis for the detection of the *ERG11* gene in fluconazole-resistant *Candida tropicalis* isolates demonstrated 100% concordance with the phenotypic resistance observed.

Keywords: Antifungal resistance, *ERG11* gene, Fluconazole resistance, Non-albicans Candida, Vulvovaginal candidiasis.

INTRODUCTION

The genus Candida comprises approximately 163 species, which exist as commensals, colonizing the skin and mucosa of female genital tract and gastrointestinal tract. Certain predisposing factors cause imbalance in the normal human flora, thereby causing transformation of commensals to pathogen, which ultimately leads to the development of superficial and deep candidiasis.[1]

Vulvovaginal candidiasis represents one of the most common superficial infections caused by Candida

species. Around 70% of women will experience vulvovaginal candidiasis at some point in their life. Uncontrolled diabetes, pregnancy, estrogen - containing birth control pills, indiscriminate use of antibiotics, steroid therapy and the intrauterine devices have been identified as risk factors for vulvovaginal candidiasis.[2,3]

Vaginitis is a common inflammatory condition characterized by abnormal vaginal discharge, pruritus, burning sensation, dyspareunia and dysuria, leading to significant gynaecological morbidity among women in reproductive-age group[4] This infection in pregnancy could contribute to adverse maternal outcomes like spontaneous abortion, stillbirth, premature rupture of membranes, inflammation of the placenta or uterus and fetal outcomes including congenital candidiasis, preterm birth, low birth weight and neonatal death[5]

Even though *Candida albicans* is isolated in majority of vulvovaginal candidiasis, worldwide studies indicate a shift towards infections, caused by non-albicans Candida species, especially in complicated and recurrent cases.[6,7]

Non-albicans Candida species including *Candida glabrata*, *Candida krusei*, *Candida tropicalis*, *Candida parapsilosis*, *Candida dubliniensis*, *Candida gulliermondi* and *Candida kefyr* are isolated, as causative agents of vulvovaginal candidiasis.[8,9]

The prevalence of these non-albicans Candida species, has raised an alarm recently, as they show increased resistance and few are intrinsically resistant to most of the antifungal agents, especially fluconazole. Fluconazole is most commonly prescribed as first line of treatment for these infections and available as over the counter drug, which contributes to emergence of drug resistant non-albicans Candida species, leading to therapeutic challenges and treatment failure.[10]

There are various mechanisms of development of resistance against azole antifungals. The key mechanism being alteration in the *ERG11* gene, which encodes for the enzyme lanosterol 14 alpha demethylase. This enzyme plays a crucial role in ergosterol biosynthesis, a very important component of fungal cell membrane and primary target of azole group of drugs. Detection of the *ERG11* gene in fluconazole-resistant Candida isolates indicates that resistance is likely mediated by point mutations or overexpression of *ERG11*, or by alternative mechanisms such as efflux pump activity. These in turn, leads to decreased binding affinity of azoles and finally contributes to antifungal resistance.[10,11]

Therefore, speciation of Candida, antifungal resistance pattern and molecular characterization of resistance determinants such as the *ERG11* gene, is essential to prevent adverse maternal and fetal outcomes. There is dearth of data about the non-albicans Candida species isolated from vulvovaginitis cases and their antifungal susceptibility pattern in our region. Hence, the present study was undertaken, which further helps the clinicians in establishing effective treatment protocols and strengthening antifungal stewardship programme.

MATERIALS AND METHODS

Aims and Objectives

- To isolate and identify Candida species from high vaginal swabs received at the Microbiology laboratory.
- To study the risk factors associated with vaginal candidiasis.
- To determine the antifungal susceptibility pattern of isolated Candida species.
- To detect *ERG11* gene for fluconazole resistance among Candida tropicalis.

Materials and Methods

This prospective cross-sectional study was conducted for a period of 1 year from September 2023 to August 2024, after obtaining the Institutional Ethics Committee clearance.

Patient's demographic details and clinical details including history of diabetes, previous antimicrobial therapy, use of intra-uterine devices and any other risk factors were collected. Among pregnant women, details about weeks of gestation, risk factors, complications during antenatal, natal and post-natal period, pregnancy outcome were documented.

Inclusion Criteria

- All women above 18 years of age, with signs and symptoms of vulvovaginal candidiasis, were included in the study, after obtaining the informed consent.
- All Candida isolates from high vaginal swabs were included in the study.

Exclusion Criteria

- Patients who refused to give consent.
- Patients on any recent antifungal therapy.
- Isolates other than Candida species obtained from high vaginal swabs were excluded from the study.

Methodology

All high-vaginal swabs were subjected to Gram stain. Candida was identified based on the morphology on Gram stain as Gram positive oval budding yeast cells with or without pseudo hyphae. The samples were then inoculated onto blood agar, MacConkey agar, Sabouraud's dextrose agar and incubated aerobically at 37^0C for 24 - 48 hours. On Sabouraud's dextrose agar, smooth, moist, cream to white colored yeast-like colonies were presumptively identified as Candida species. The colonies were subjected to Gram stain and germ tube test. Further speciation was done by using CHROM Candida differential agar, morphology on corn meal agar (Dalmau plate technique), carbohydrate assimilation and fermentation tests.[12,13,14,15]

Germ tube test: The Candida isolate was inoculated into 0.5 ml of human serum, and incubated at 37℃ for 2 to 4 hours. The inoculum was examined microscopically. The germ tubes were identified as

long tube-like projections, arising from the yeast cells, without constriction at their point of origin.

HiMedia CHROM agar: Candida species were differentiated based on color and colony morphology on Candida differential agar (Figure 1). The culture media contains chromogenic substrate, which react with species-specific enzymes released by Candida, leading to distinct color production (Table 1).

HiMedia Corn meal agar: The Candida isolate was inoculated onto Cornmeal agar by Dalmau technique. One streak of the isolate was made on the culture media by ploughing, followed by 4 additional streaks across the first one. Anaerobic condition was created by placing coverslip (22 x 22 mm) over the inoculum, such that the streak projected beyond the coverslip. The cornmeal agar was then incubated at room temperature for 48 to 72 hours. Following which, the plate was placed on the microscope stage and the edge of coverslip was examined under low-power and high-power objective. The Candida species were identified based on their distinct morphological features as shown in Figure 2.

Carbohydrate assimilation test (Auxanographic plate method – Haley and Standard modification) using HiMedia Carbohydrate discs: A yeast suspension was prepared by inoculating 24-to-72-hour Candida culture into sterile distilled water. The suspension was mixed with the molten yeast nitrogen base, and poured into a 15 x 150 mm petri plate. The media was kept at room temperature to allow it to solidify. Carbohydrate impregnated discs containing lactose, sucrose, maltose, xylose, galactose were placed on the surface of the medium and incubated for 18 to 24 hours at 30℃. A positive carbohydrate assimilation test was indicated by visible growth surrounding the respective carbohydrate disc (Figure 3).

Carbohydrate fermentation test: This test was performed by inoculating Candida isolate in a media containing peptone (1%), NaCl (0.5%), Andrade's indicator (0.005%) and carbohydrates (2%) including glucose, maltose, sucrose, lactose, galactose and trehalose. Gas production was detected by placing inverted Durham's tube. The inoculated tubes were incubated at 37℃ for 24 to 48 hours and examined for change in color to pink, indicating acid production along with gas in Durham's tube (Figure 3).

Antifungal susceptibility testing

Disc diffusion method: Antifungal susceptibility testing was performed using Kirby Bauer disc diffusion method on Mueller-Hinton agar (MHA) supplemented with 2% glucose and 0.5 µg/ml methylene blue. The following antifungal discs procured from HiMedia were used - fluconazole (10 µg), ketoconazole (10 µg), clotrimazole (10 µg), amphotericin B (100 U) and nystatin (100 U).

Five well-isolated colonies of Candida were selected and emulsified in 5 ml of sterile saline and the turbidity of the suspension was matched to 0.5 McFarland. A sterile cotton swab was dipped into the Candida suspension and inoculated on the prepared media by lawn culture technique, streaking the swab uniformly over the agar surface as per standard guidelines. Antifungal discs were placed on the inoculated culture media which were then incubated at 35℃ for 24 to 48 hours. The zone diameter was measured and the results were interpreted as per CLSI (Clinical and Laboratory Standards Institute) M44 guidelines for Fluconazole.[16] For other antifungal agents including clotrimazole, ketoconazole, amphotericin B and nystatin, the zone diameter was interpreted based on previously published studies. [17-20]

The growth on MHA after incubation was as shown in Figure 4.

E-test (Epsilometer test): Fluconazole Minimum Inhibitory Concentration (MIC) was obtained by performing E-test. The inoculum preparation and lawn culture was performed in the same manner as described in disc diffusion method. Fluconazole E-test (HiMedia) strip was placed onto the inoculated agar surface and plates were incubated at 35℃ for 24-48 hours. The MIC value was read at the point where elliptical zone of inhibition intersects the gradient markings on the strip. Any pinpoint and isolated colonies, hazy appearance within the zone of inhibition were ignored. In such cases, MIC was determined at the point on the scale where a clear and marked reduction in growth was observed. Interpretation of the MIC values was done in accordance to CLSI guidelines. The results of fluconazole sensitivity by E-test method were interpreted and appeared as in Figure 4.

Candida albicans ATCC 14053 and *Candida parapsilosis* ATCC 22019 were used as controls. Results were interpreted as per CLSI guidelines.[16]

Detection of *ERG11* gene: Fluconazole resistant *Candida tropicalis* isolates were further subjected to PCR for the molecular detection of ERG11 gene.

DNA extraction and PCR amplification of *ERG11* gene of *Candida tropicalis*

DNA was extracted from all the *Candida tropicalis* isolates by Phenol-Chloroform method. PCR amplification of ERG11 gene was performed for all *Candida tropicalis* isolates using CT *ERG11* 1F 5'-TCTGACATGGTGTGTGTGTG-3' and CT ERG11 3R 5' CAAGGAATCAATCAAATCTCTC-3' primers. Following amplification, the PCR products underwent 1.5% agarose gel electrophoresis with ethidium bromide staining and visualized under ultraviolet illumination. After gel electrophoresis, positive control (*Candida tropicalis* ATCC 750) and negative control (Nuclease free water) were satisfactory. *ERG11* gene of *Candida tropicalis* isolates yielded a band at approximately 1500bp.

Statistical Analysis

Data was analyzed by percentage analysis, using SPSS version 22 software.

RESULTS

504 vaginal swabs were collected in female patients with symptoms of vulvovaginitis. 105 (20.8%) Candida species were isolated from these patients.

Most cases of vulvovaginal candidiasis were seen among the age group of 21 - 30 years (54.7%), followed by 31- 40 years (24.2%), 41-50 years (8.4%), 11 - 20 years (8.4%) and least in 51 - 60 years (4.2%) of age group. (Figure 5)

52.4% of Candida species were isolated from antenatal care (ANC) patients and the remaining 47.6% were from non-ANC group of patients. The distribution of cases among different trimesters of pregnancy is as depicted in Table 2.

In our study, non - albicans Candida (51.4%) were isolated more compared to *Candida albicans* (48.6%). Among non-albicans Candida, *Candida tropicalis* (25.7%) was predominant, followed by *Candida glabrata* (13.3%), *Candida krusei* (11.4%) and *Candida parapsilosis* (1%) as depicted in Table 3.

40 (38.1%) Candida isolates were resistant to clotrimazole, followed by 32 (30.5%) resistant to fluconazole and 21 (20%) isolates were resistant to ketoconazole. None of the Candida isolates were resistant to amphotericin B and nystatin.

Species-wise comparison of antifungal resistance pattern, among the Candida species is as follows: 27.5% of *Candida albicans* were resistant to clotrimazole, followed by fluconazole (19.6%) and ketoconazole (17.6%). *Candida tropicalis* showed 66.7% resistance to clotrimazole and 29.6% resistance to fluconazole. 28.6% of *Candida glabrata* were resistant to clotrimazole and 14.3% were resistant to fluconazole. No resistance was observed for ketoconazole among *Candida tropicalis* and *Candida glabrata* isolates. Excluding azoles, to which *Candida krusei* is intrinsically resistant, 33% resistance to clotrimazole was observed. The *Candida parapsilosis* isolate showed no resistance to any of the antifungal agents tested (Table 4).

We observed increased resistance to antifungal agents among non-albicans Candida for clotrimazole, fluconazole and ketoconazole when compared to *Candida albicans* (Figure 6).

All the 8 *Candida tropicalis* strains resistant to fluconazole were subjected to PCR testing to detect the *ERG11* gene. All 8 isolates were positive for *ERG11* gene (Figure 7).

The various risk factors observed in our study were as in Table 5. Among ANC cases, gestational diabetes was observed in 7.3% of cases, hypothyroidism in 5.5% and Koch's disease, associated with Systemic Lupus Erythematosus (SLE) in 1.8% of cases. Among non -ANC cases, uncontrolled diabetes mellitus, hypothyroidism and post-Intra Uterine Device (IUD) insertion status were observed in 4% each, followed by Chronic Kidney Disease (CKD) in 2% of cases.

We observed various maternal and fetal outcomes in our study, which is as shown in Table 6.

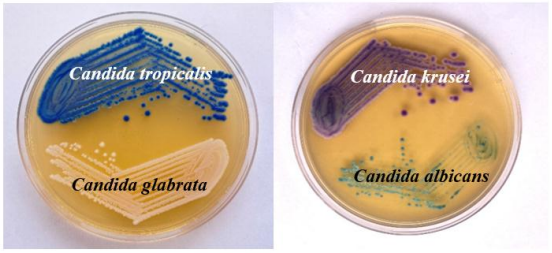

Figure 1: Candida species on Chrom agar

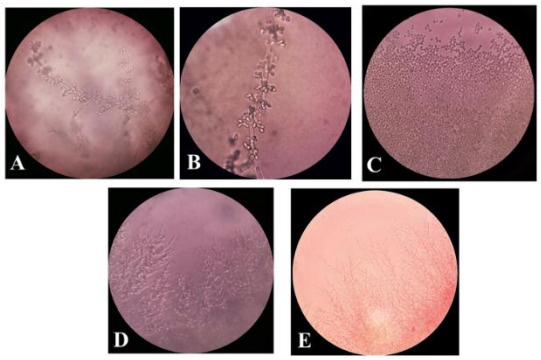

Figure 2: Morphology of Candida species on Corn meal agar

(A) *C. albicans* (B) *C. tropicalis* (C) *C. glabrata* (D) *C. krusei* (E) *C. parapsilosis*

Figure 3: Carbohydrate assimilation and Carbohydrate fermentation test

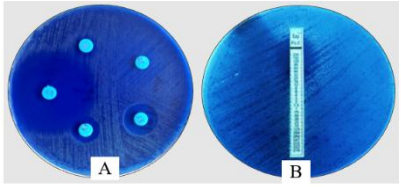

Figure 4: Antifungal susceptibility testing by (A) Disc diffusion method (B) Etest method

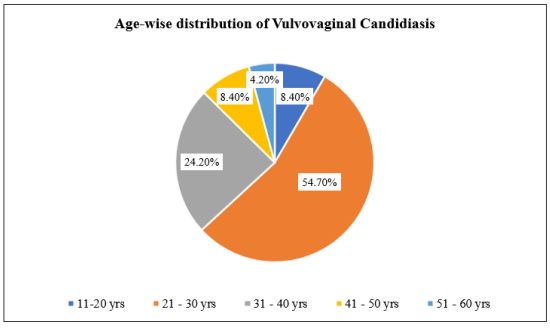

Figure 5: Age-wise distribution of vulvovaginal candidiasis

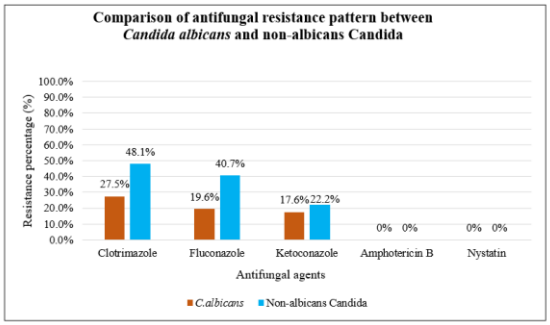

Figure 6: Comparison of Antifungal resistance pattern between *Candida albicans* and non-albicans Candida species.

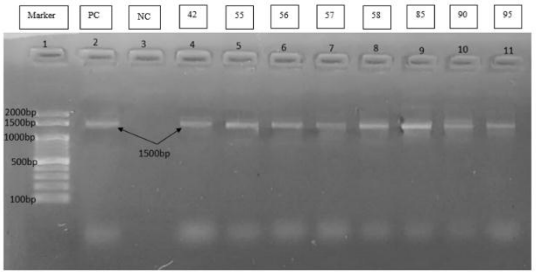

Figure 7: PCR for *ERG 11* gene
Lane 1 - Marker (from 100bp to 2000bp),
Lane 2 - Positive control (ATCC *C. tropicalis* 750),
Lane 3 - Negative control (nuclease free water),
Lane 4 to 11 - *C. tropicalis* strains (42,55,56,57,58,85,90,95) for *ERG 11* gene.

Table 1: Cultural characteristics of Candida species on CHROM agar

Candida species	Color on CHROM agar
Candida albicans	light green
Candida dubliniensis	pale green
Candida tropicalis	blue to purple
Candida glabrata	cream to mauve
Candida krusei	purple, fuzzy
Candida parapsilosis	white to cream
Candida kefyr	cream to white with slight purple centre or brown

Table 2: Distribution of vulvovaginitis cases among different trimester of pregnancy

Sl. No.	Trimester of pregnancy	No. of ANC cases (n = 55)	Percentage (%)
1	1st Trimester	19	34.5
2	2nd Trimester	04	7.3
3	3rd Trimester	32	58.2

Table 3: Distribution of Candida species.

	No. of isolates (n = 105)	Percentage (%)
Candida albicans	51	48.6%
Candida tropicalis	27	25.7%
Candida glabrata	14	13.3%
Candida krusei	12	11.4%
Candida parapsilosis	01	1%

Table 4: Antifungal susceptibility pattern of Candida (species wise)

	Clotrimazole		Fluconazole		Ketoconazole		Amphotericin B		Nystatin	
	S	R	S	R	S	R	S	R	S	R
C. albicans (n=51)	37 (72.5%)	14 (27.5%)	41 (80.4%)	10 (19.6%)	42 (82.4%)	09 (17.6%)	51 (100%)	-	51 (100%)	-
C. tropicalis (n=27)	9 (33.3%)	18 (66.7%)	19 (70.4%)	8 (29.6%)	27 (100%)	-	27 (100%)	-	27 (100%)	-
C. glabrata (n=14)	10 (71.4%)	04 (28.6%)	12 (85.7%)	02 (14.3%)	14 (100%)	-	14 (100%)	-	14 (100%)	-
C. krusei (n=12)	08 (67%)	04 (33%)	NA	NA	NA	NA	12 (100%)	-	12 (100%)	-
C. parapsilosis (n=1)	1 (100%)	-	1 (100%)	-	1 (100%)	-	1 (100%)	-	1 (100%)	-

Table 5: Risk factors observed among vulvovaginitis cases

Sl. No.	Risk factors observed among ANC cases	Total no. of ANC cases (n = 55)	Percentage (%)
1	Gestational diabetes	04	7.3
2	Hypothyroidism	03	5.5
3	Koch's disease, associated with SLE	01	1.8
Sl. No.	**Risk factors observed among non - ANC cases**	**Total no. of non - ANC cases (n = 50)**	**Percentage (%)**
1	Uncontrolled Diabetes mellitus	02	4
2	Hypothyroidism	02	4
3	Post IUD insertion	02	4
4	Chronic Kidney Disease (Stage IV)	01	2

Table 6: Maternal and fetal outcome among vulvovaginitis cases

Sl. No.	Maternal outcome	No. of ANC cases (n = 55)	Percentage (%)
1	Emergency LSCS	09	16.4
2	Preterm labour	04	7.3
3	Spontaneous rupture of membranes	01	1.8
4	Preterm Premature Rupture of Membranes	01	1.8
Sl. No.	**Fetal outcome**	**No. of ANC cases (n = 55)**	**Percentage (%)**
1	Respiratory distress	02	3.6
2	Intra Uterine Growth Retardation	01	1.8
3	Low birth-weight baby	01	1.8
4	Early onset sepsis	01	1.8

DISCUSSION

Vulvovaginal candidiasis is one of the most frequently encountered genito-urinary tract infections. It commonly affects young and middle-aged women during their reproductive years. Patients usually present with symptoms including curd-like vaginal discharge, burning sensation, pruritis and dyspareunia. Nearly 70% of female experience this infection once in their lifetime.[1]

Various predisposing factors for the development of vulvovaginal candidiasis includes pregnancy, diabetes mellitus, prolonged usage of antibiotics, glucocorticoids, oral contraceptives, intrauterine devices and immunosuppressive conditions.[2,21,22] In our study, risk factors like diabetes mellitus, hypothyroidism, obesity, tuberculosis, Systemic Lupus Erythematosus (SLE), post Intra Uterine Device insertion and chronic kidney disease were

observed among women with vulvovaginal candidiasis.

During pregnancy, factors like decreased immunity, elevated level of estrogen, glycogen deposition, low vaginal pH can be attributed as potential risk factors for the development of infection.[23]

In our study, the following maternal outcomes were observed: Emergency LSCS (16.4%), preterm labour (7.3%), spontaneous rupture of membranes and preterm premature rupture of membranes (1.8% each). The fetal outcomes include respiratory distress (3.6%), Intra-uterine growth retardation (IUGR), low birth-weight baby and early onset sepsis (1.8% each). Various studies have observed similar outcomes like preterm labor, premature rupture of membranes, chorioamnionitis, IUGR, congenital infections and respiratory distress in the neonate.[5,18,24] This highlights the need for early identification and management of the infection in pregnancy to prevent adverse outcomes. In our study, we observed better maternal and fetal outcomes with no mortality.

We observed in our study that Candida species were responsible for 20.8% of vulvovaginitis cases, highlighting their role as an important etiological agent. Similar findings are seen in studies done by Rawat S et al,[22] (24.5%) and Kalaiarasan K et al,[25] (23.7%).

Majority of cases (55%) were seen among the age group of 21 to 30 years. Thus, indicating the higher burden of infection among women of reproductive age group. These findings are similar to many other studies. Bashir G et al,[26] and Chopra et al,[27] documented 53.8% and 45.1% of vulvovaginitis cases, between the age group of 21 to 30 years, respectively.

In our study, the isolation of non-albicans Candida species (51.4%) was more compared to that of *Candida albicans* (48.6%). Many other studies like Nagalakshmi R et al,[9] and Lavanya V et al,[28] have reported similar findings. The reasons could be indiscriminate use of over-the-counter antifungal agents, treatment of candidiasis for long duration without speciation, prescribing effective antifungals against *Candida albicans* only. These factors contribute to selection of non-albicans Candida over *Candida albicans* in causing these infections.[21,28]

In most laboratories, identification and reporting of Candida isolates other than *Candida albicans* is restricted to the level of non-albicans Candida species. However, certain non-albicans Candida species show resistance to commonly used antifungal agents such as fluconazole. This may lead to inappropriate therapy and treatment failure. Hence, species-level identification of Candida, followed by antifungal susceptibility testing, should be made available as a part of routine laboratory testing.

Candida albicans contributed to 48.6% of all the species isolated in our study. Similar findings were reported by Yadav et al,[29] and Mokhtar GA et al,[30] who documented a 44% isolation rate of *Candida albicans* in their studies on vulvovaginitis.

In the present study, *Candida tropicalis* was the predominant non-albicans Candida species isolated from vulvovaginitis cases, which contributed to 25.7% of overall cases. The finding is in concordance with studies by Venugopal D et al,[31] and Gulnar and Multani et al,[32] which reported similar rate of occurrence of *Candida tropicalis*, around 27% and 24% respectively, contributing to most common non-albicans Candida species isolated.

We found in our study, that *Candida glabrata* was isolated in 13.3% of isolates, which is consistent with studies by Purohit GK et al, (18.8%)[33] and Chopra et al, (16.9%)[27].

Candida krusei accounted to 11.4% of cases in our study, in consistent with studies by Kumar S et al, (10.3%)[34] and Kalaiarasan K et al, (9.8%)[25].

Candida parapsilosis accounted for 1% of Candida species. Similar results are seen in studies by Singhal et al, (0.9%) [35] and Kalaiarasan K et al, (3.9%) [25].

In the current study, no resistance was detected against amphotericin B and nystatin. Few studies including Gulnar and Multani et al,[32] and Shrestha P et al,[36] showed similar findings. The reasons for low resistance to these drugs could be due to their restricted use, because of high cost, adverse effects like renal toxicity, and they are used only in complicated and recurrent cases of vulvovaginitis.[1,37]

In our study, 66.7% of *Candida tropicalis* were resistant to clotrimazole, as seen in the study by Khan et al, (73.30%) [38]. This was followed by 30% of isolates resistant to fluconazole, similar to study done by Gupta and Gadekar et al, (24%) [39]. No resistance was observed to ketoconazole among *Candida tropicalis*, similar finding was seen in Thanooja et al.[40]

We observed that 28.6% and 14.3% of *Candida glabrata* isolates were resistant to clotrimazole and fluconazole respectively. Similarly, Bashir G et al,[26] showed 21% resistance to clotrimazole and Gupta and Gadekar et al,[39] showed 19% resistance to fluconazole. No isolates were resistant to ketoconazole, which was similar to studies by Sivagamasundari et al.[41]

Candida krusei exhibits intrinsic resistance to azoles, therefore azoles are not recommended for treating the infections caused by *Candida krusei*. In our study, *Candida krusei* showed 23% resistance to clotrimazole, which is comparable to the findings of Syed S et al,[42] who reported a resistance rate of 29%.

In the present study, single isolated *Candida parapsilosis* did not show any resistance to the antifungal agents tested.

Among *Candida albicans*, 27% were resistant to clotrimazole, comparable to study by Phung et al,[43] and Shrestha P et al,[36] who observed 31.65% and 24.9% of resistance respectively. 20% of the isolates were resistant to fluconazole in this study, similar to studies by Ali et al, (19.4%) [21] and Gupta and Gadekar et al, (16.7%) [39]. 18% were resistant to ketoconazole, similar to study conducted by Hussen et al (18.9%).[44]

When compared to *Candida albicans*, non-albicans Candida showed increased resistance to all the antifungal agents tested. Many recent studies across India and even globally including Richter SS et al,[6] and Bashir G et al,[26] shows similar results.

Overall, Candida species showed increased resistance to routinely used antifungal agents in our hospital, including fluconazole and clotrimazole. In such situations, alternative antifungal agents may need to be considered for effective treatment.

The emergence of antifungal resistance complicates the treatment, increases the likelihood of recurrent infections, and poses a significant challenge in effective management of vulvovaginal candidiasis.

Hospital antifungal susceptibility pattern should therefore be periodically reviewed, to guide rational treatment of vulvovaginal candidiasis. This will help in developing and updating institutional antifungal stewardship programme and policies. These further emphasize the importance of performing species level identification and antifungal susceptibility testing of Candida at the laboratory level.

In the present study, all the 8 isolates of fluconazole resistant *Candida tropicalis* were positive for *ERG11* gene. This was in accordance to the study by Nigam et al,[45] where *ERG11* gene was detected in all the fluconazole resistant strains of Candida species. Another study by Abdu AB et al.[46] showed that 88.89% of azole resistant Candida strains were positive for *ERG11* gene.

A better understanding of various molecular mechanisms involved in drug resistance among antifungal agents, is essential for improving the management of vulvovaginal candidiasis. Studies on detection of *ERG11* gene, among Candida species are limited. Therefore, research focusing on molecular characterization of antifungal resistance among clinical isolates of Candida are necessary to initiate prompt and effective treatment strategies and improve patient outcomes in vulvovaginitis cases.

Limitations of the study

- Due to limited resources, the minimum inhibitory concentration (MIC) for other antifungal agents could not be determined for all the isolates in the present study. Future studies should incorporate detailed antifungal susceptibility testing, including MIC determination for all the isolates, to provide more accurate results.
- Further research focusing on the *ERG11* gene, including detailed analysis of gene mutations, could not be undertaken, which is required for the better understanding of the mechanisms of antifungal resistance.

CONCLUSION

The present study shows the increasing antifungal resistance among cases of vulvovaginitis. *Candida tropicalis* emerged as the most common non-albicans Candida species isolated and also exhibited increased resistance to commonly used antifungal agents, namely clotrimazole and fluconazole. These findings underscore the importance of species-level identification of Candida isolates and routine antifungal susceptibility testing as part of standard diagnostic protocols. Furthermore, future studies focusing on the genetic mechanisms of antifungal resistance are warranted for better understanding of resistance patterns.

In conclusion, the diagnosis of vulvovaginal candidiasis should not rely solely on clinical findings. Laboratory confirmation is important to accurately identify the Candida species and determine the antifungal susceptibility. This helps in selecting appropriate antifungal agent, improve the patient management, and to prevent development of antifungal resistance.

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Informed consent: Informed consent was obtained from the patients before considering the samples for the study.

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