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Speciation Of *Candida*, Their Biofilm Formation and Antifungal Susceptibility Pattern from Cases of Vulvovaginitis in A Tertiary Care Centre.Madhumathy M. S. S<sup>1</sup>, Vijaya Kishore T.<sup>1</sup>, K. Vichitra<sup>1</sup>, Anupma Jyoti Kindo<sup>1</sup><sup>1</sup>Sri Ramachandra Institute of Higher Education and Research. Chennai, India.**Article Information**

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**Keywords***Vulvovaginal candidiasis, polymerase chain reaction, restriction fragment length polymorphism, microbroth dilution, biofilm formation.***ABSTRACT**

**Background:** Vaginal candidiasis (VC) is the most common vaginal infection in women caused by uncontrolled growth of *Candida* species. Chronic odorless curdy white discharge, itching, dysuria, dyspareunia of the lower genital tract are the most common presentations. **Materials and methods:** High vaginal swabs were collected from women attending the obstetrics and gynaecology outpatient department with symptoms of candidiasis. The identification of the yeast was done by phenotypic methods such as Grams stain, culture on Sabouraud dextrose agar (SDA). Germ tube test was performed. For further species identification, isolates were cultured on Hi Crome agar and Tetrazolium Reduction Medium (TRM). Confirmatory analysis by genotypic methods such as Polymerase Chain Reaction (PCR) followed by Restriction Fragment Length Polymorphism (RFLP) to identify species specific *Candida* was performed. Antifungal susceptibility testing (AFST) was performed using microbroth dilution method according to CLSI M60 guidelines. Biofilm detection was done by tube method. **Results:** Out of 448 high vaginal swabs that were collected over a period of one year from June 2023 to May 2024 from patients with symptoms of vulvovaginal candidiasis, 62 samples had moderate polymorphonuclear leucocytes and moderate budding yeast cells in the Grams stain and grew cream-coloured colonies on SDA. Germ tube test was done for all isolates and out of the 62 isolates 29 produced germ tube. The same results were obtained from genotypic analysis with PCR – RFLP to confirm specific species of *Candida*. Out of sixty-two samples that were positive, 29 were confirmed to be *Candida albicans*, 20 as *Candida glabrata*, 10 were *Candida tropicalis* and 3 *Candida krusei*. Following which antifungal susceptibility testing by microbroth dilution method was performed, all the isolates were sensitive to voriconazole and micafungin (100%), followed by caspofungin (94%), amphotericin B (87%), anidulafungin (85%), posaconazole (83%) and itraconazole (69%). 42 isolates (68%) showed resistance to fluconazole. Assessment of biofilms was done by tube method. Twenty-two isolates produced mild biofilms. **Conclusion:** Resistance to antifungal drugs have been increasing in recent times with *Candida* species isolated from high vaginal swabs. Triazole resistance has been increasingly observed in *Candida albicans* species that were once susceptible. Certain *Candida* species like *Candida krusei* show intrinsic resistance to fluconazole. Hence, proper diagnosis of *Candida* up to the species level is recommended. This helps identify fungal strains that are resistant to standard treatments and guides appropriate therapy.

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## INTRODUCTION:

*Candida* species are present as commensals in both humans and animals. Exist as typical elements of human microbiota occupying mucosal surfaces of genital areas, urinary systems, respiratory tract, gastrointestinal system, oral cavity, skin, nails and scalp.<sup>1</sup>

The *Candida* species exists as harmless microorganisms but has the capability to transform from harmless resident state into infectious agents. Pathogenic and opportunistic microorganisms transform their behavior based on host environment changes thus exhibiting either benign or pathogenic characteristics. Standard medical examinations show that superficial *Candida* infections transform into serious systemic infections when patients have extremely weakened immune systems.<sup>1</sup>

*Candida* exists in the vaginal tissues of female bodies throughout their lifetime although many never develop symptoms of infection. The vaginal defense mechanisms together with *Lactobacilli* and immune responses create a balance which allows *Candida* organisms to remain as normal vaginal commensals. The vaginal or vulvar environment shows equilibrium with *Candida* species but any changes in this environment can lead to *Candida* infection called vulvovaginal candidiasis (VVC). This condition is classified as uncomplicated and complicated VVC.<sup>1</sup>

The primary *Candida* species found through high vaginal swab are *Candida albicans*, *Candida glabrata*, *Candida parapsilosis*, *Candida tropicalis* and *Candida krusei*.<sup>2</sup>

Oral azole medications like fluconazole and itraconazole yield similar cure outcomes to topical treatments while patients find oral formulations more convenient. Oral azoles present the danger of toxic effects to patients. The antifungal effectiveness of both oral and topical azoles is reduced when treating VVC with *Candida glabrata* or *Candida krusei* instead of *Candida albicans*. Non-*Candida albicans* species infections and VVC cases associated with risk factors become highly difficult to treat and necessitate robust treatment strategies.<sup>1</sup>

Millions of female patients experience Vulvovaginal candidiasis each year which makes it a substantial public health problem. VVC's morbidity rate leads to substantial mental agony because it produces pain and discomfort alongside self-image deterioration and anxiety which then degrades work performance and relationships of all kinds. VVC also results in substantial direct and indirect economic costs and increases susceptibility to human immunodeficiency virus (HIV) infection.<sup>3</sup>

The failure to treat vulvovaginal candidiasis (VVC) leads to multiple complications which may result in pelvic inflammatory disease after infertility, an ectopic pregnancy and pelvic abscess formation while increasing the risk of spontaneous abortion with accompanying menstrual disorders.

Fast detection and proper treatment of vulvovaginal candidiasis (VVC) in at-risk patient populations is essential for averting additional health complications. Research advancements have not eliminated VVC as a common medical problem since the cause factors and their biological origins remain unknown to science.<sup>1</sup>

The accurate determination of *Candida* species in clinical environments becomes essential because it enables medical staff to provide patients with exact treatment methods. Hence this study aims to identify *Candida* up to species level and to study the drug sensitivity pattern along with its biofilm formation.

## MATERIALS AND METHODS:

This study was conducted in a tertiary care hospital in Chennai, India from June 2023 to May 2024. The present study enrolled 448 female patients, who came to the obstetrics and gynaecology outpatient's department with symptoms suggestive of vulvovaginal candidiasis.

## STRAIN COLLECTION:

High vaginal swabs were carefully collected aseptically from the posterior vaginal fornix using speculum and posterior vaginal wall retractor. The swabs were then transferred to the microbiology laboratory and processed immediately.

## PHENOTYPIC METHODS:

Initially a Grams stain from the high vaginal swab was made to look for budding yeast cells with or without pseudohyphae following which a culture was made on Sabouraud Dextrose agar (SDA) (HiMedia, Mumbai, India) and incubated at 37 °C for 24-48 h. The colonies from SDA plate were subjected to Gram stain to confirm the growth of *Candida* and were further sub-typed by streaking on HiCrome *Candida* differential agar (HiMedia,

Mumbai, India) and tetrazolium reduction medium and incubated at 37 °C for 24–48 hours<sup>4</sup>. The pigments produced by different species of *Candida* are listed in Table 1. The colonies from SDA plate were also subjected to germ tube test to check for the production of germ tubes. Germ tube test is done by mixing 3–4 colonies in 0.5 ml human serum and incubated at 37 °C for 24 – 48 hours, examined under microscope for the formation of germ tube<sup>5</sup>. Colonies suggestive of *C. albicans* were confirmed by positive germ tube test.

**Table 1**

<i>Candida</i> species	Colour produced on Hicrome agar	Colour produced on tetrazolium reduction medium
<i>Candida albicans</i>	Light green	Light pink
<i>Candida glabrata</i>	Cream to white	Pale pink
<i>Candida tropicalis</i>	Steel blue to purple	Dark maroon
<i>Candida krusei</i>	Purple, fuzzy	Dry and pink

**GENOTYPIC METHODS:**

Further confirmation was done by genotypic methods. Polymerase Chain Reaction – Restriction Fragment Length Polymorphism (PCR-RFLP) was done.

**POLYMERASE CHAIN REACTION – RESTRICTION FRAGMENT LENGTH POLYMORPHISM.**

The culture positive isolates were subjected to PCR – RFLP method. A loop full of 24 hours fresh colony

from Sabouraud Dextrose Agar (SDA) was used for this method.

**COLONY PCR**

Primers ITS 1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS 4 (5'-TCCTCCGCTTATTGATATGC-3') were used as forward and reverse primers Colony PCR is designed to screen the target DNA directly from the colony. PCR with specific primers allows amplification of the target region. In this method, the colonies are transferred directly to the PCR master mix. DNA is released from the cell by extending the initial denaturation temperature.

The reaction mixture is prepared by adding the reagents mentioned in Table 2. An isolated yeast colony is picked up with a sterile toothpick or a sterile tip and suspended in the reaction mixture. This is followed by amplification as per the reaction conditions mentioned in Table 3<sup>6</sup>.

**Table 2**

Colony per reaction mixture				
S.no	Reagents	Available concentration	Required concentration	Volume taken (µl)
1	Master mix	2X	1X	25
2	Forward primer (ITS 1)	50pM	50pM	1
3	Restriction primer (ITS4)	50pM	50pM	1
4	Nuclease free water	-	-	23
TOTAL				50

**Table 3**

Colony per - reaction condition				
S.no	Steps	Temperature	Time	No. Of cycles
1	Initial denaturation	95°C	10 minutes	
2	Denaturation	95 °C	30 seconds	
3	Annealing	56 °C	30 seconds	35 cycles
4	Extension	72 °C	30 seconds	
5	Final extension	72 °C	10 minutes	

**AGAROSE GEL ELECTROPHORESIS:**

Amplified products were analysed by 1.5% agarose gel electrophoresis. In 1X TAE buffer for approximately 45 minutes at 50 V and viewed under UV light and photographed. The isolates which showed positive bands in the gel were subjected to RFLP.

**RESTRICTION FRAGMENT LENGTH POLYMORPHISM:**

Restriction fragment length polymorphism (RFLP) technique involves fragmenting the homologous DNA sample using restriction enzyme, which can cleave DNA at specific sites producing fragments of varying length. The fragments are electrophoresed

on agarose gel and analyzed. Digestion with restriction enzyme produces different banding patterns of each species. This can be used in the identification of medically important *Candida* species as mentioned in Table 4.

The interpretation is based on the number and size of the bands in the agarose gel electrophoresis. Each medically important *Candida* species gives different sizes of PCR - RFLP products which help in determining the species (Table 4)<sup>6</sup>.

**Table 4**

**Medically Important Candida Species**

<i>Candida</i> species	Amplicon size	Restriction fragment size
------------------------	---------------	---------------------------

<i>C.albicans</i>	535	297, 238
<i>C.glabrata</i>	608	371, 155, 82
<i>C.tropicalis</i>	524	340, 184
<i>C.krusei</i>	510	261, 249

In a sterile 200 µl PCR tube the following ingredients were added and were incubated at 37 °C for 2 hours [6]. (Table 5)

**Table 5**  
**Rflp Reaction Mixture**

Reagents	Available concentration	Final concentration	Volume
Nuclease free water	-	N/A	7.5
Enzyme buffer	10X	1X	2
Restriction enzyme (Msp 1)	10U/µl	5U	0.5
PCR product	-	N/A	10
TOTAL			20

**ANTIFUNGAL SUSCEPTIBILITY TESTING:**

Antifungal susceptibility testing was done according to CLSI M60 guidelines document entitled ‘Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard – Second Edition’, intended for testing yeasts that cause invasive infections. A total of eight antifungal drugs (stock prepared from pure drug available in powder form) were used along with their range of concentration tested were: amphotericin B, fluconazole, itraconazole, voriconazole, posaconazole, anidulafungin, caspofungin diacetate, micafungin sodium. The reagents used were: RPMI (Roswell Park Memorial Institute) 1640, DMSO (dimethyl sulfoxide), Double autoclaved distilled water, 4 N NaOH (pH indicator solution -7)<sup>7</sup>.

RPMI 1640 medium was prepared with L-glutamine, phenol red, 0.2% glucose and 0.165 moles per liter MOPS buffer without sodium bicarbonate was prepared by dissolving 44.9-gram RPMI 1640 powder in 900 ml sterile distilled water. The pH was adjusted to 7.0 using 4M sodium hydroxide and volume made up to 1 liter with sterile distilled water. This was then a filter sterilized using 0.22µ membrane filter and pre-incubated for 24 hours for sterility checking. It was stored at 4°C until use.

A 24-hour old culture grown on SDA was used. Using a sterile inoculation loop suspension is prepared in sterile distilled water and adjusted at 0.5 McFarland standard. 10 µl of this suspension is added to 5 ml of RPMI 1640 (1:500 dilution).

For a highest concentration of 16 µg/ml, 4µl of stock solution with concentration 1600 µg/ml is added to 196 µl of RPMI 1640 in the first well of the microtiter plate and there after serially diluted in the

remaining wells containing 100 µl of RPMI 1640. To 100 µl of drug solution in each well, 100 µl of inoculum is added and mixed well. [7]

The readings were taken after sufficient growth appeared in the growth control well and absence of growth in the sterility well. The plates were checked after a period of 24 hours and 48 hours.

**QUALITY CONTROL**

Sterility check was performed for all the media before use. ATCC *Candida albicans* 90028 and *Candida parapsilosis* ATCC 2209 were used as reference strains. The test values are considered valid if the readings of the QC strains were within the expected range.

**BIOFILM DETECTION – TUBE METHOD**

Trypticase soy broth with 1% glucose was prepared and distributed in sterile glass test tubes. All the tubes were autoclaved at 121° C for 15 minutes. A loop full of colony from overnight culture plate was inoculated into the broth and incubated for 24 to 48 hours at 37° C. The tubes were decanted and washed with phosphate buffered saline (PBS) with a pH of 7.3 and dried. The dried tubes were stained with 0.1% crystal violet (CV). All the tubes were washed with distilled water to remove excess stain and then dried in an inverted position to observe biofilm formation [8]. A visible film lining the wall and the bottom of the tube was considered positive and was divided into mild, moderate and severe biofilm formation depending on the intensity of the crystal violet staining. Only the ring formation at the liquid interface was considered negative.

**RESULTS:**

A total of 448 isolates were collected from high vaginal swabs were collected over a period of one year, from June 2023 to May 2024, out of which 62 isolates were identified as *Candida* species using conventional phenotypic methods, then confirmed by PCR – RFLP.

**DEMOGRAPHIC AND CLINICAL DETAILS**

**AGE DISTRIBUTION:**

Out of the 62 positive isolates 31 isolates belonged to women aged between 20 to 30 years of age (50%), 25 isolates belonged to women aged between 30 to 40 years of age (40.32%), 2 samples from women aged 18 to 20 years of age (3.23%) and two samples from women 60 to 70 years of age (3.23%) (Figure 1).

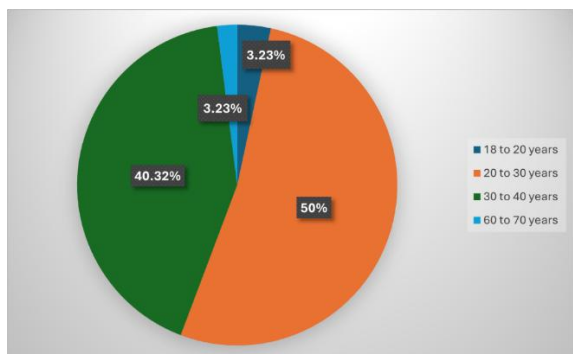


Figure 1 – Age distribution of *Candida* species

### CHARACTERIZATION OF *CANDIDA* SPECIES

All the 62 isolates underwent phenotypic characterization. Colony morphology of all the isolates observed on Sabouraud Dextrose Agar (SDA) after incubation and was seen as cream colonies (Figure 2). Once growth was confirmed on SDA, the colonies were cultured on to HiCrome agar (Figure 3A) and TRM agar (Figure 3B) to observe pigment production. The colour of the pigments produced is listed in Table 1.



2(A) 2(B)  
 Figure 2 – (A)(B) *Candida* colonies on SDA



3(A) 3(B)  
 Figure 3 – (A) Pigment production on HiCrome agar  
 (B) Pigment production on tetrazolium reduction medium

Microscopic observation of the isolates grown on Sabouraud dextrose agar revealed budding yeast cells with or without pseudohyphae (Figure 4).

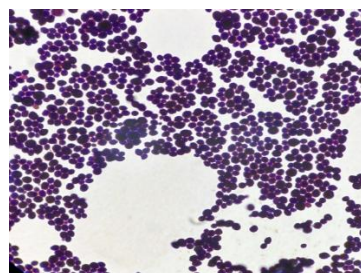


Figure 4 – Gram stain showing budding yeast cells.

Germ tube test was performed for all the isolates. 29 isolates showed the presence of Germ tube suggesting to be *Candida albicans* species (Figure 5).



Figure 5 – Germ tube formation

Out of the 62 isolates, 29 were found to be *C. albicans*, 20 were found to be *C. glabrata*, 10 were found to be *C. tropicalis*, 3 were found to be *C. krusei* phenotypically. This was followed by genotypic identification by PCR-RFLP.

Colony PCR was carried out using universal ITS1 as forward and ITS4 as reverse primers. All the 62 isolates produced a band confirming it as genus *Candida*.

From the PCR performed, 29 samples had product size of ~510 bp, 20 samples had product size of ~870bp, 10 samples had a product size of ~520bp, 3 samples had a product size of ~580bp (Figure 6).

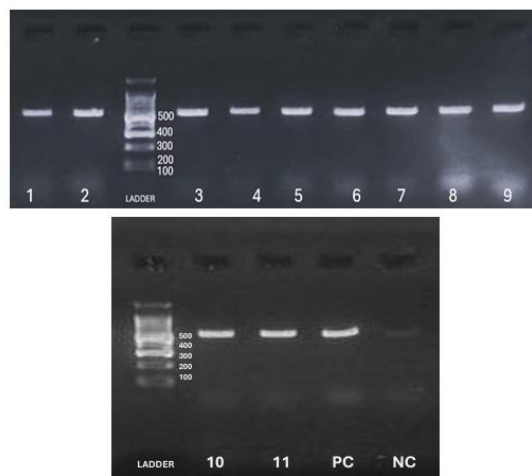


Figure 6. *Candida* Genus Specific Pcr

DNA ladder (1-100bp)

1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 – Representative clinical isolates

PC – Positive control

NC – Negative control

The PCR product obtained was further used to do RFLP for species identification. Out of the 62 isolates, 29 were found to be *C. albicans*, 20 were found to be *C. glabrata*, 10 were found to be *C. tropicalis*, 3 were found to be *C. krusei* thus correlating with the phenotypic findings. ATCC *Candida albicans* 90028 was used as positive control (PC) (Figure 7).

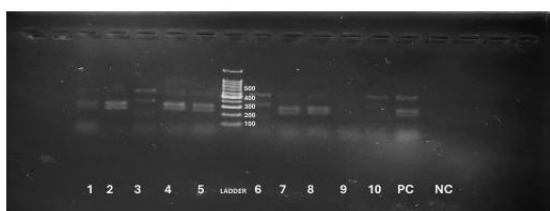


figure 7 candida species specific rflp

DNA ladder (1-100bp)

1, 2, 3, 4, 5, 6, 7, 8, 9, 10 - Representative clinical isolates

PC – Positive control

NC – Negative control

Table 7

Isolate	Drugs	Sensitive	Resistant	Gmic
<i>Candida albicans</i>	Amphotericin B	23	6	0.7
	Fluconazole	16	13	4.50
	Itraconazole	20	9	3.15
	Voriconazole	29	0	0.17
	Posaconazole	25	4	0.94
	Caspofungin	25	4	0.44
	Micafungin	29	0	0.19
<i>Candida glabrata</i>	Amphotericin B	19	1	0.47
	Fluconazole	0	20	5.20
	Itraconazole	11	9	2.94
	Voriconazole	20	0	0.15
	Posaconazole	16	4	0.79
	Caspofungin	20	0	0.43
	Micafungin	20	0	0.18
<i>Candida tropicalis</i>	Amphotericin B	9	1	0.54
	Fluconazole	4	6	5.02
	Itraconazole	9	1	2.91
	Voriconazole	10	0	0.15
	Posaconazole	8	2	0.84
	Caspofungin	10	0	0.42
	Micafungin	10	0	0.18
<i>Candida krusei</i>	Amphotericin B	3	0	0.51

The results obtained from the phenotypic and genotypic methods were similar (Table 6).

Table 6  
 Results Obtained by Phenotypic and Genotypic Methods

Candida species	Phenotypic and genotypic results
<i>C. albicans</i>	29
<i>C. glabrata</i>	20
<i>C. tropicalis</i>	10
<i>C. krusei</i>	3

DETERMINATION OF ANTIFUNGAL SUSCEPTIBILITY PATTERN OF CANDIDA SPECIES.

The MICs of all the drugs tested were within the accepted range as per CLSI M60 guidelines for the reference strain.

Amongst the antifungal agents tested, all the isolates were sensitive to voriconazole and micafungin (100%), followed by caspofungin (94%), amphotericin B (87%), anidulafungin (85%), posaconazole (83%) and itraconazole (69%).

42 isolates (68%) showed resistance to fluconazole. The sensitivity pattern with the mean is tabulated in Table 7.

	Fluconazole	0	3	5.80
	Itraconazole	3	0	3.55
	Voriconazole	3	0	0.14
	Posaconazole	3	0	0.75
	Caspofungin	3	0	0.50
	Micafungin	3	0	0.17
	Anidulafungin	3	0	2.42

**BIOFILM FORMATION:**

Out of the 62 isolates tested for biofilm production, 22 produced mild biofilms and 40 did not produce biofilms. The most common biofilm producer was *Candida glabrata* 10 out of 22 followed by 9 *Candida albicans* isolates (table 8). Among the biofilm producer's resistance was seen to fluconazole, followed by itraconazole and then posaconazole. Two isolates showed resistance to anidulafungin (Figure 8).

**Table 8**  
Total number of biofilm production in *Candida* isolates

Isolate	Mild biofilm production
<i>C.albicans</i>	9
<i>C.tropicalis</i>	3
<i>C.glabrata</i>	10
<i>C.krusei</i>	NIL
TOTAL	22



**Figure 9. Biofilm Formation By Tube Method**

**DISCUSSION:**

*Candida* species are usually a part of normal flora of the female genital tract but can become pathogenic and cause Candidiasis when the patient's immunity is lowered. Usage of antibiotics that can eliminate the other normal flora like *Lactobacilli* that can lead to an imbalance in the vaginal pH thus infecting millions of women every year.

Although *Candida albicans* is still considered the causative agent, non-albicans *Candida* species, especially *Candida glabrata*, are increasingly recognized as the causative agent of VVC in the western population as stated in a study done by Bruna Gonçalves<sup>3</sup>. In India *Candida tropicalis* species is seen increasingly among the non albicans species as seen in studies done by Namarata Kalia et

al<sup>9</sup> and Lakshmi Krishnasamy et al<sup>2</sup> from North and South India respectively. The total number of non-*Candida albicans* species detected in our study was 33 isolates (53.23%) which is similar to the study done by Kanishka et al which was 58%<sup>10</sup> and in contrast to a study done by Panda et al which was 39.2%<sup>11</sup>.

It is usually an imbalance between the vaginal colonization of *Candida* as well as the local environment of the host that normally leads to the occurrence of VVC after several physiological or non-physiological events, as stated in a study done by Bruna Gonçalves et al<sup>3</sup>.

Different host-related and behavior factors have been identified as predisposing factors. The host-related risk factors include pregnancy or on hormone replacement therapy, having uncontrolled diabetic disease, being immunosuppressed, exposure to antibiotics or glucocorticoids, and being genetically prone to them. Oral contraceptives, intrauterine devices, spermicides, condoms, hygiene behavior, clothing and sexual behavior are some of the factors<sup>3</sup>.

Reed et al.<sup>12</sup> in his study highlighted that, despite the growing list of identified risk factors for vulvovaginal candidiasis (VVC), the complex interaction between host factors and microbial characteristics in both the onset and recurrence of the infection remains poorly understood.

In our study, a total of 448 vaginal swabs were received in the microbiology laboratory from patients between 16-78 years of age with symptoms of vulvovaginal candidiasis i.e. chronic odorless curdy white discharge, itching, dysuria, dyspareunia of the lower genital tract were the most common presentations. This period of study was done from June 2023 to May 2024.

Among the 62 positive isolates, the most common age group that presented with vulvovaginitis was between 20 to 40 years which is similar to a study done by Krishnaswami et al in 2018 from South India<sup>2</sup>.

All the samples were subjected to Gram stain and culture in Sabouraud Dextrose Agar followed by Hicrome agar and Tetrazolium Reduction medium (TRM). Germ tube test was done to check for the

presence of germ tube formation to confirm *C. albicans* species phenotypically.

Identification is more reliable by molecular procedures such as PCR-RFLP by using species-specific primers. They are uncomplicated, fast and precise. The primary advantage lies in their high sensitivity and specificity. The phenotypic techniques give a clue of the genus that is to be later substantiated by the molecular techniques which will aid in the improved diagnosis leading to good prognosis of patient<sup>13</sup>.

The most common species in our study was found to be *Candida albicans* (46.77%) which is in concordance with a study by Namarata Kalia et al in the year 2015 (47%)<sup>9</sup> Krishnaswamy et al in the year 2018 (44%)<sup>2</sup>, from North and South India respectively and a study by Rudresh et al in 2024 (46.3%)<sup>14</sup>. However, study from Krishnaswami et al [2] and Namarata Kalia et al<sup>9</sup> had *C. tropicalis* as the most common non albicans *Candida* unlike our study in which *C. Glabrata* (32.2%) was the most common among the non-*Candida albicans*. The findings is similar to a study done by Panda et al (31.8%) [11] and Rudresh et al (36.1%) [14].

Speciation of *Candida* is crucial for treatment because of resistance that is seen in the organism recently. The reasons are mainly either one *Candida* species can intrinsically be resistant to a particular antifungal agent, or the organism could have developed secondary resistance. For instance, *Candida krusei* intrinsic resistance to fluconazole is already known [9].

In recent times a lot of *Candida* species are showing resistance to the antifungal drugs that were once susceptible. Failure to perform *Candida* speciation and initiating treatment with a resistant antifungal agent can result in an unfavorable prognosis for the patient. Poor prognosis of the patient can lead to recurrent vulvovaginal candidiasis. Recurrent vulvovaginal candidiasis caused by non albicans species such as *C. glabrata*, *C. tropicalis*, *C. krusei* need a longer course of treatment and hence speciation and antifungal susceptibility testing is important as stated in a study by Hubertine et al [15].

In this study antifungal susceptibility testing was done by microbroth dilution method following the CLSI M60 guidelines to identify the exact susceptibility pattern. A total of eight antifungal drugs were selected for the study that included amphotericin B, anidulafungin, caspofungin, micafungin, itraconazole, posaconazole, voriconazole and fluconazole.

In our study, all isolates were completely sensitive (100%) to voriconazole and micafungin, followed by caspofungin (94%), which is comparable to the findings of Bitew et al. [16]. Resistance was observed against fluconazole in 42 isolates which was 68% in contrast to the study conducted by Panda P S et al which was only 32.15% [11]. Resistance was also noted with amphotericin B (87%), anidulafungin (85%), and posaconazole (83%), while itraconazole showed moderate resistance (69%).

Fluconazole resistance was observed in all the species of *Candida glabrata* and *Candida krusei*, (intrinsically resistant) which is similar to a study done by Khan et al in the year 2018 [17].

Intrinsic resistance is known with *C. krusei* and hence any way this drug is not to be given to patients with *C. krusei* infection.

The relevance of biofilm forms a virulence factor for *Candida* and gives varied responses to antifungal agents, in our study we have detected the biofilm formation of *Candida* isolates from vaginal swabs by tube method using crystal violet staining, a similar study was done by Tapan et al [18].

Out of the 62 isolates tested for biofilm production, 22 produced mild biofilms and 40 did not produce. The biofilm production was then correlated with the antifungal susceptibility pattern obtained. Among the isolates which produce biofilms, resistance was seen to fluconazole, followed by itraconazole followed by posaconazole. Two isolates showed resistance to anidulafungin.

## CONCLUSION:

Based on this study, we observed that the most *Candida* species was found to be *Candida albicans*, the most common age group to be affected are women between 20 to 40 years of age. Molecular identification of *Candida* species by using species specific primers ITS1 and ITS4 is faster, more accurate, and offers greater sensitivity and specificity compared to conventional phenotypic methods, which can be used for faster identification by clinicians. The microbroth dilution method used in the study determines the minimum inhibitory concentration (MIC) of antifungal agents, providing essential information for clinicians to select appropriate therapy and improve patient outcomes. This must be followed before the prescription of any antifungal drugs to the patient as some candida species are intrinsically resistant to certain azoles. This can lead to poor prognosis of the patient.

Biofilm formation was also examined, as it is a key virulence factor that significantly hinders the

eradication of the organism. Given the rising antifungal resistance among *Candida* isolates, timely and accurate species identification along with antifungal susceptibility testing is crucial for effective patient management.

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