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Research Article

ISOLATION AND SPECIATION OF *CANDIDA* FROM LEUCORRHOEA SAMPLES OF REPRODUCTIVE AGE WOMEN WITH ANTIFUNGAL SUSCEPTIBILITY OF THE ISOLATES

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ABSTRACT

Objectives: (1) To isolate and identify *Candida* species from leucorrhoea samples of women aged 15–45 years. (2) To determine the distribution of *Candida* species. (3) To evaluate the antifungal susceptibility of the isolates.

Methods: A cross-sectional, descriptive study was conducted from October 2018 to January 2020 at King George Hospital and Andhra Medical College, Visakhapatnam. 259 high vaginal swab samples were collected from women with leucorrhoea symptoms. Direct microscopy and culture on Sabouraud's dextrose agar were used for *Candida* isolation. Species identification involved standard biochemical tests, including the germ tube test, cornmeal agar, HICHROME agar, and sugar assimilation/fermentation tests. Antifungal susceptibility was assessed using the disc diffusion method for six antifungals: amphotericin B, clotrimazole, fluconazole, itraconazole, ketoconazole, and nystatin.

Results: Out of 259 samples, 100 *Candida* isolates (38.6% prevalence) were obtained. The most affected age group was 31–35 years (34%), followed by 21–25 years (27%). Non-albicans *Candida* (NAC) species (59%) were more prevalent than *Candida albicans* (41%). Among NAC species, *Candida glabrata* (23%) and *Candida tropicalis* (19%) predominated. All *Candida* isolates were 100% susceptible to amphotericin B and nystatin. *C. albicans* demonstrated the highest resistance to fluconazole (26.9%). Among NAC species, *Candida krusei* exhibited 100% resistance to fluconazole and significant resistance to other azoles.

Conclusion: The study reveals a rising trend in NAC infections with higher antifungal resistance. Amphotericin B and nystatin remain the most effective treatments. Early species-specific identification and antifungal susceptibility testing are essential to guide appropriate therapy and curb emerging antifungal resistance. Further research is required to establish regional *Candida* epidemiology and optimize empirical treatment guidelines.

Keywords: *Candida*, Leucorrhoea, Antifungal susceptibility, Non-albicans *Candida*, Vulvovaginal candidiasis.

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INTRODUCTION

Leucorrhoea is defined as the excessive discharge of a white substance from the vagina, causing social, sexual, and psychological distress [1]. Vulvovaginal candidiasis (VVC) is a common cause of leucorrhoea, characterized by inflammation of the vulva and vagina due to *Candida* species [2]. Approximately 50–72% of women experience VVC during their lifetime, with a significant number encountering recurrent infections [3].

Candida species, which are part of the normal flora of the skin, mouth, and genital tract, become opportunistic pathogens under conditions that compromise the immune system. Predisposing factors for candidiasis include pregnancy, diabetes, antibiotic use, immunosuppressive therapy, and the presence of medical devices such as catheters [4-6].

There are one hundred and Sixty-three anamorphic species of the genus *Candida* with telomorphs in at least thirteen genera. Nearly twenty of these species were considered to be significant pathogens causing various infections in human beings, and out of these seven are well-known opportunistic pathogens.

The most common pathogenic species of this genus is *Candida albicans*. The genus Torulopsis have been merged with the genus *Candida* in 1978 and named *Candida glabrata*. *Candida stellatoidia* is now included within *C. albicans*, and *C. pseudotropicalis* is renamed as *Candida keyfr*. The telomorphic stage of *Candida* can be identified based on newer molecular techniques [4].

The extensive use of antimycotic drugs, particularly azoles, for prolonged therapeutic courses led to a change in the relative increase in the proportion of non-albicans species such as *Candida tropicalis* or *C. glabrata* and decrease in *C. albicans* as an etiological agent for VVC [7].

Identification of *Candida* species is vital for diagnosis and treatment, especially given the rise of non-albicans *Candida* (NAC) species and increasing antifungal resistance. Traditional identification methods include microscopy, culture on specialized media such as cornmeal agar (CMA) and HICHROME *Candida* Agar, and biochemical assays [8].

The study aims to assess the incidence of *Candida* infections, identify *Candida* species, and analyze their antifungal susceptibility patterns among women of reproductive age with leucorrhoea.

Aims and objectives

The aims of the present study were:

1. To isolate *Candida* from leucorrhoea samples of reproductive-age women (15–45 years)
2. To identify and speciate the genus *Candida* isolated from leucorrhoea samples

3. To study the distribution of different species of *Candida* among the isolates
4. To study the antifungal susceptibility of different *Candida* isolates.

METHODS

This cross-sectional, descriptive study was done at King George Hospital and Andhra Medical College, Visakhapatnam from October 2018 to January 2020. Approval of the Institutional Ethical Committee was taken before the initiation of work. 259 High Vaginal swab samples were taken from leucorrhoea patients.

Inclusion criteria

All sexually active women between 15 and 45 years of age with excessive vaginal discharge are included who were not on any anti-fungal treatment for the previous 48 h and who have consented or for whom a legal guardian has consented to participate in the study.

Exclusion criteria

Patients who are currently on treatment with antifungals, patients who had genital prolapse and malignancy of the genital tract.

Methodology

Two high vaginal swabs are collected and placed immediately into a sterile plastic container which is labeled and sent to the laboratory.

Then direct microscopy was done using 10% KOH mount preparation, Gram's staining, and were examined under microscope for the presence of budding yeast cells and pseudohyphae (Figs. 1 and 2).

The sample was inoculated on the blood agar plate and incubated at 37°C on two Sabouraud's dextrose agar slants and were incubated one each at 25°C and 37°C. The plates and slants were examined at 24 h, 48 h and 72 h for growth (Fig. 3 and 4). Any growth was studied by smear examination using Gram's stain method for the presence of budding yeast cells (Fig. 5).

All the cultures showing budding yeast cell growth were confirmed by standard identification tests such as the Germ tube test, the Urease test. All the isolates showing urease negative results were tested using the following tests for speciation of *Candida*.

1. CMA inoculation for studying morphology (Fig. 6)
2. Hichrome *Candida* agar inoculation (Table 1 and Fig. 7)
3. Sugar assimilation tests (Fig. 8)
4. Sugar fermentation tests.

Table 1: Species differentiation of *Candida* on CHROM agar

Candida species	Colour of colonies on chrom agar
Candida species	Colour on chrom agar
Candida albicans	Light green
Candida tropicalis	Steel blue
Candida krusei	Pale pink
Candida parapsilosis	White
Candida glabrata	Purple/pink
Candida dubliniensis	Dark green
Candida guillermondii	Pale pink/cream

Antifungal susceptibility testing was done for all the *Candida* isolates using the disc diffusion method.

RESULTS

Out of 259 samples, 100 *Candida* species were isolated, indicating a prevalence rate of 38.6%. *Candida* species were most frequently isolated from women aged 31–35 years (34%), followed by the 21–25 year age group (27%) (Table 2).

Out of 100 *Candida* isolates 41% were *C. albicans* and 59% were NAC (Fig. 9). Further species distribution of NAC is shown in Fig. 10.

Pregnancy followed by antibiotic usage are the risk factors associated with VVC in this study (Fig. 11).

Antifungal susceptibility pattern of all the isolates is shown in Table 3.

All 41 isolates of *C. albicans* were sensitive to amphotericin B and nystatin.

- Among the NAC except for *C. keyfr*, all other isolates were 100% susceptible to amphotericin B and nystatin
- Ketoconazole was the next effective drug with 84% of susceptibility, followed by Itraconazole at 76% and Clotrimazole 67%
- Antifungal drug fluconazole, which was commonly used, showed only sensitivity to 54% and 46% resistance
- The predominant isolate in NAC group, *C. glabrata* was showing 100% susceptibility to Amphotericin B and Nystatin. It was showing 82.6% susceptibility to Ketoconazole, followed by itraconazole (69.5%) and clotrimazole (65.2%). It was showing 52.2% susceptibility to Fluconazole and 47.8% resistance.

C. tropicalis was showing 100% susceptibility to amphotericin B and nystatin. It was showing 84.2% susceptibility to ketoconazole, followed by itraconazole(57.8%) and clotrimazole(52.6%). It was showing 31.5% susceptibility to fluconazole and 68.5% resistance. Most species of *C. tropicalis* in the present study were showing resistant to fluconazole.

- *Candida dubliniensis*isolates in the present study showed 100% susceptibility to amphotericin B followed by 85.7% susceptibility with nystatin and ketoconazole
- *Candida krusei* isolates in the present study showed 100% susceptibility to Amphotericin B and Nystatin. *C. krusei* isolates showed 100% resistance to Fluconazole. *C. krusei* isolates showed 83.4% resistance to Ketoconazole and Clotrimazole. *C. krusei* isolates showed 66.6% resistance to Itraconazole
- In the present study, *Candida parapsilosis* isolates were 2 which were 100% sensitive to amphotericin B, fluconazole, itraconazole, ketoconazole, nystatin. 50% of them were susceptible to clotrimazole
- In the present study, *C. keyfr* isolate was one that is resistant to Amphotericin B, clotrimazole, fluconazole, ketoconazole and sensitive to itraconazole and nystatin.

In the present study, *C. keyfr* isolate was one that is sensitive to all antifungal drugs tested.

Fig. 11 shows the comparison of the anti-fungal susceptibility pattern of *C. albicans* and NAC species In the present study, the highest resistance

Table 2: Age-wise distribution of samples (n=259) and *Candida* species isolated (n=100)

S. No.	Age(in years)	No. of samples	Percentage	No. of *Candida* species isolated	Percentage
1	15–20	11	4.2	3	3
2	21–25	65	25.1	27	27
3	26–30	41	15.8	21	21
4	31–35	78	30.2	34	34
5	36–40	31	11.9	9	9
6	41–45	33	12.8	6	6
	Total	259	100	100	100

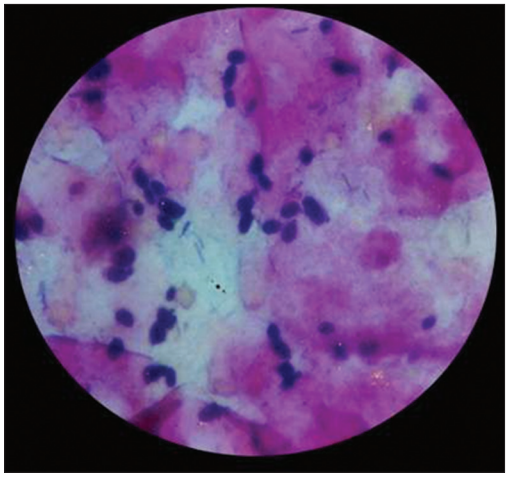

Fig. 1: Direct smear showing budding yeast cells of *Candida* with vaginal epithelial cells

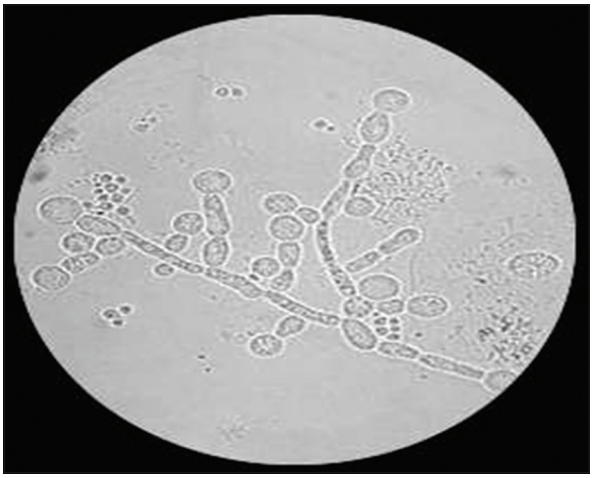

Fig. 2: Potassium hydroxide mount showing budding yeast cells of *Candida* along with pseudohyphae

Fig. 3: Blood agar plate showing growth of *Candida*

Fig. 4: Sabouraud dextrose agar slant showing cream-colored colonies of *Candida*

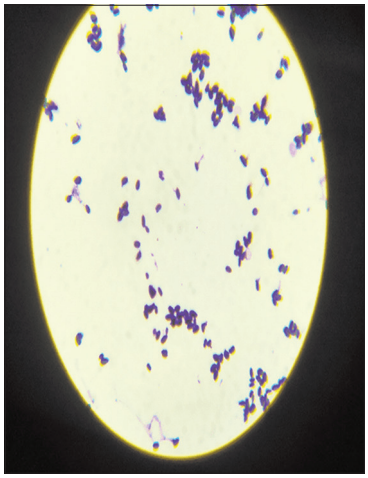

Fig. 5: Gram's staining of colony showing budding yeast cells of *Candida*

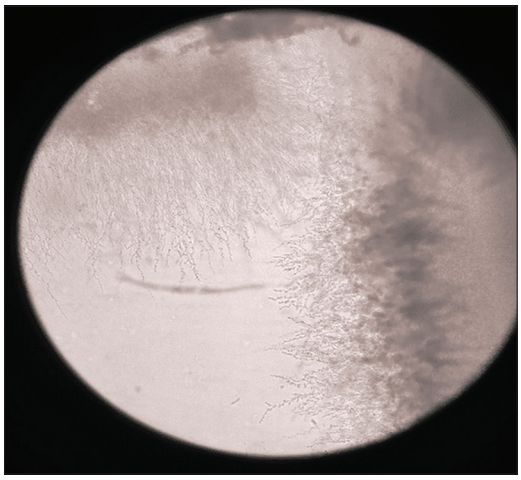

Fig. 6: *Candida tropicalis* showing Pine tree appearance of blastoconidia on cornmeal agar

of *C. albicans* was 26.9% to fluconazole. All the *C. albicans* isolates were 100% susceptible to Amphotericin B and Nystatin. The effective drugs were Ketoconazole, Itraconazole, and Clotrimazole in that order of decreasing susceptibility.

NAC showed 98.3% susceptibility to amphotericin B and nystatin. NAC also showed the highest resistance to fluconazole, which is 59.4% followed by clotrimazole 47.5%, itraconazole 35.6%, and ketoconazole 23.8% (Fig. 12).

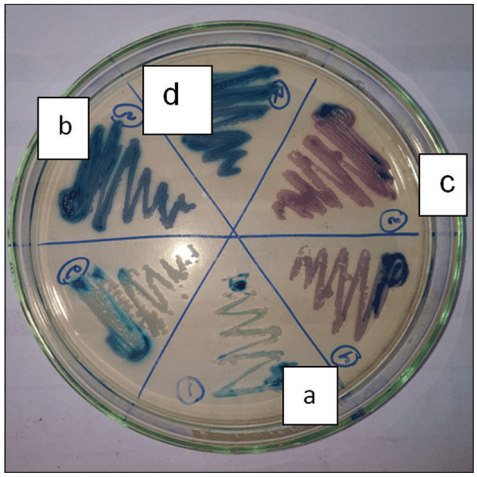

Fig. 7: CHROM agar plate showing different species of *Candida.* (a) *Candida albicans*, (b) *Candida dubliniensis*, (c) *Candida glabrata*, (d) *Candida tropicalis*

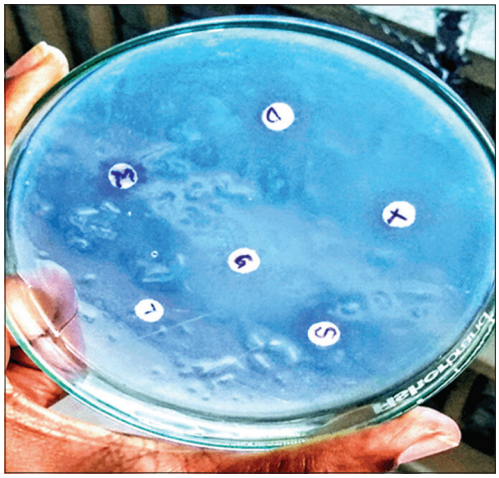

Fig. 8: Sugar assimilation reactions of *Candida tropicalis*

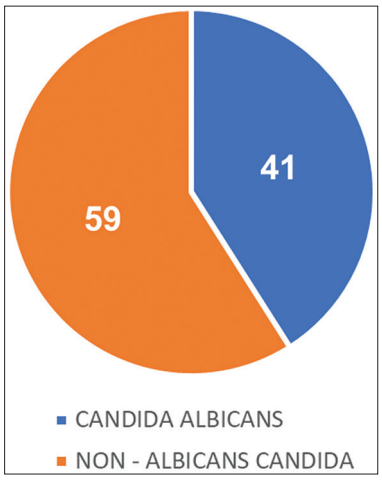

Fig. 9: Pie diagram showing species

DISCUSSION

The prevalence of *Candida* infection (38.6%) in this study aligns with other research studies reporting rates between 17% and 42%. This figure is comparable to the isolation rate of 35% found by Krishnasamy *et al*. [9]. and 41.4% by Bitew and Abebaw [10].

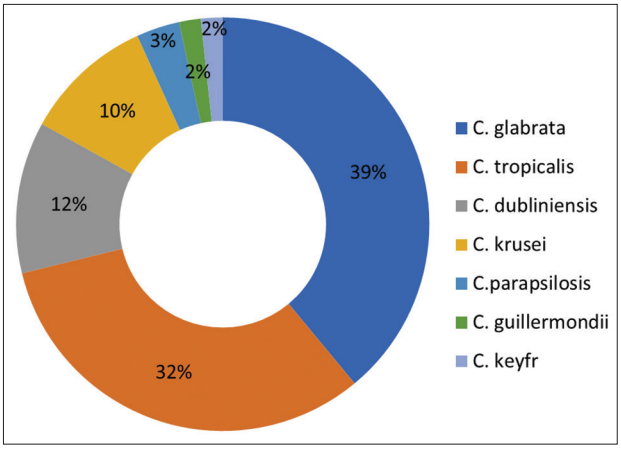

Fig. 10: Pie diagram showing species distribution of *Candida* isolates (n=100) distribution of non albicans *Candida* isolates (n=100)

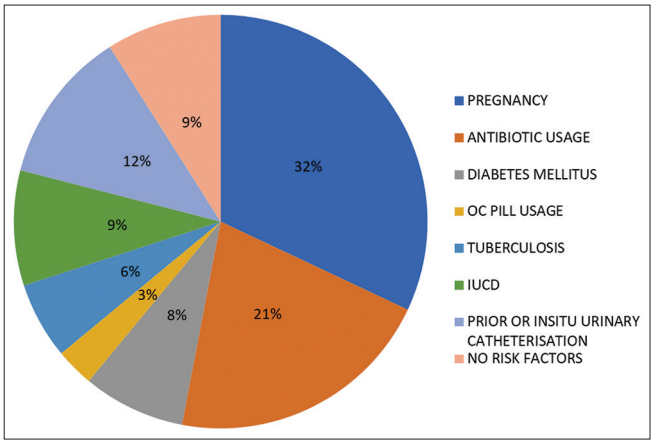

Fig. 11: Pie diagram showing risk factors associated with *Candida* isolated patients (n=100)

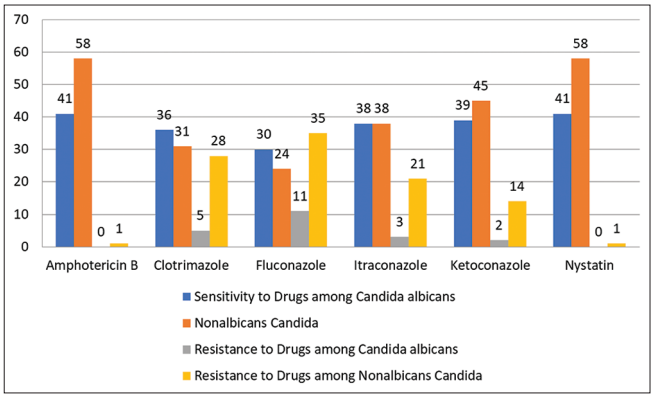

Fig. 12: Bar diagram showing antifungal susceptibility pattern of *Candida albicans* and non-albicans *Candida* (n=100)

The highest isolation rate occurred in women aged 31–35 years, which may be attributed to hormonal fluctuations and increased antibiotic usage during reproductive years.

The highest incidence occurred in the 31–35 age group (34%), consistent with Panda *et al*. (34% in the same age group) [11] and Ragunathan *et al*. (45.5% in the 26–35 group) [12].

Notably, the present study recorded a higher prevalence of NAC species (59%) than *C. albicans* (41%), reinforcing the shifting epidemiology

Table 3: Antifungal susceptibility pattern of the isolates of *Candida* (n=100)

Species of *Candida*	Amphotericin B		Clotrimazole		Fluconazole		Itraconazole		Ketoconazole		Nystatin	
	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)
Candida albicans (n=41)	41 (100)	--	36 (87.8)	5 (12.2)	30 (73.1)	11 (26.9)	38 (92.6)	3 (7.4)	39 (95.10)	2 (4.90)	41 (100)	--
Candida glabrata (n=23)	23 (100)	--	15 (65.2)	8 (34.8)	12 (52.2)	11 (47.8)	16 (69.5)	7 (30.5)	19 (82.6)	4 (17.4)	23 (100)	--
Candida tropicalis (n=19)	19 (100)	--	10 (52.6)	9 (47.4)	6 (31.5)	13 (68.5)	11 (57.8)	8 (42.2)	16 (84.2)	3 (15.8)	19 (100)	--
Candida dubliniensis (n=7)	7 (100)	--	3 (42.8)	4 (57.2)	3 (42.8)	4 (57.2)	5 (71.4)	2 (28.6)	6 (85.7)	11 (14.3)	6 (85.7)	1 (14.3)
Candida krusei (n=6)	6 (100)	--	1 (16.6)	5 (83.4)	--	6 (100)	2 (33.4)	4 (66.6)	1 (16.6)	5 (83.4)	6 (100)	--
Candida parapsilosis (n=2)	2 (100)	--	1 (50)	1 (50)	2 (100)	--	2 (100)	0	2 (100)	0	2 (100)	--
Candida kefyr (n=1)	--	1 (100%)	--	1 (100)	--	1 (100)	1 (100)	0	0	1 (100)	1 (100)	--
Candida guillermondii (n=1)	1 (100)	--	1 (100)	--	1 (100)	--	1 (100)	--	1 (100)	--	1 (100)	--
Total percentage	99 (99)	1 (1%)	67 (67)	33 (33)	54 (54)	46 (46)	76 (76)	24 (24)	84 (84)	16 (16)	99 (99)	1 1

S-Sensitive; R-Resistant

toward NAC as also reported by studies such as Kalaiarasan *et al*. (82.4% NAC) [13] and Lakshmi *et al*. (77.78% NAC) [14].

Among NAC species, *C. glabrata* (23%) and *C. tropicalis* (19%) were predominant, paralleling findings from Verghese *et al*. (38% *C. glabrata*) [15] and Fathima and Rajendran (22.2% *C. tropicalis*) [16], Vijaya *et al*. [17] and Mahadevaiah *et al*. [18].

Speciation of *Candida* by CHROM agar based on color differentiation offered a rapid, convenient, and reliable method for the identification of clinically significant *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 [19] Studies by Vijaya *et al*. [20] concluded that primary isolation and provisional identification of clinically important species of *Candida* was possible with CHROM agar.

In the present study, the Germ tube test was positive in 41 isolates of *C. albicans* and 7 isolates of *C. dubliniensis* which were correlating with the studies mentioned above.

Species distribution of *Candida* isolates in the present study was compared to Richter *et al*. [21], Varghese *et al*. [15], Mohanty *et al.* [22], Garg *et al*. [23], Shukla *et al*. [24], and Deepthi *et al* [25].

All the above studies showed *C. albicans* as the predominant isolate, and the present study correlated with these studies.

In the present study, *Candida* isolates were predominant among pregnant women, followed by women with antibiotic usage. Ragunathan *et al*. [12], Parameshwaran *et al* [26], Rajeshkumar *et al* [27]. Studies showed Pregnancy was the most common predisposing factor associated with vaginal candidiasis followed by the history of antibiotics and corticosteroids usage in their studies.

Anti-fungal susceptibility testing using the disk diffusion method for yeasts was established in 2003 according to the Clinical and Laboratory Standards Institute (CLSI) document M44-A, and since then, many studies were undertaken to find the resistance development patterns among *Candida* species.

In vitro, drug sensitivity testing has been improved over the past few years because of the increased incidence of primary and acquired resistance to azoles.

Although topical clotrimazole, nystatin, and miconazole are usually prescribed for VVC, however a single dose of oral fluconazole is more acceptable in some patients both for prophylaxis and cure. On the other hand, resistance to fluconazole among less sensitive *Candida* species (especially non-albicans such as *C. glabrata*, *C. tropicalis* and *C. krusei*) has been increased during the last decades.

In this study, most of the *Candida* species were sensitive to amphotericin B, ketoconazole, clotrimazole, and nystatin and were relatively resistant to fluconazole and itraconazole which coincides with studies of Jithendra *et al*. [28] and Vignan *et al*. [29], Chakraborty *et al*. [30].

In the present study, all 41 isolates of *C. albicans* were sensitive to amphotericin B and Nystatin. Similarly, in a study done by Shaik *et al*. [31] all *C. albicans* isolates showed 100% sensitivity to amphotericin B and nystatin.

In the present study, 84% of tested isolates showed susceptibility to ketoconazole, which also correlates with the study done by Shaik *et al*. [31], which showed 90% susceptibility to Ketoconazole.

Among the azole group of antifungals, resistance to fluconazole was shown by *C. krusei* isolates with 100% resistance and 47.8% of *C. glabrata* isolates. These findings are similar to that reported by Sasikala *et al*. [3] and Bitew and Abebaw [10].

C. albicans showed 26.9% resistance to Fluconazole which is in concordance with Jayalakshmi *et al*. [32], Shaik *et al* [31].

More resistance to azole derivatives was seen in *C. albicans* [33-35]. This is because it is the most common species isolated and azole group is the most common antifungals used against them.

In the present study, *C. krusei* is reported to be intrinsically resistant to fluconazole (100%) and 83.4% resistance to ketoconazole, and 66.6% resistance to clotrimazole. These findings are similar to that reported by Sasikala *et al*. [3], Lavanya *et al*. [19] and Bitew and Abebaw [10].

In the present study, a higher rate of antifungal resistance was observed in NAC as compared to *C. albicans* [34,36].

Resistance to fluconazole in the present study was 46%, aligning with previous studies such as Khan *et al*. (62%) [37], Khadka *et al*. (36%) [38].

The study highlights the growing prevalence of NAC species with higher antifungal resistance, stressing the importance of species-specific identification and routine antifungal susceptibility testing to guide appropriate therapy.

Limitations of the present study

- *Candida* isolates were speciated considering it a pathogen since they were from symptomatic females. No specific tests were done to differentiate it from commensal and colonizer
- The post-therapeutic analysis of the patients was not done in this study. Hence the *in vivo* susceptibility to antifungal drugs could not be analyzed.
- The history of multiple sex partners could not be elicited. Hence, this study could not correlate the prevalence of candidiasis in women with multiple sex partners
- CLSI guidelines for antifungal susceptibility testing by disc diffusion method species only for fluconazole and voriconazole. In the present study, antifungal susceptibility with clotrimazole disc has been tried. A correlation study with minimum inhibitory concentration was not done in the present study.

CONCLUSION

The study highlights a rising trend of NAC infections over *C. albicans*, linked to increased predisposing conditions. Early identification and antifungal susceptibility testing are crucial for guiding appropriate treatment, reducing empirical anti-fungal use, and improving patient outcomes. There is a need for extensive research to understand local *Candida* species prevalence and develop guidelines for effective empirical therapy. Accurate diagnosis and antifungal testing are essential to predict treatment failures, guide clinical decisions, and support better patient care.

AUTHORS CONTRIBUTIONS

Dr. Yasoda Devi Kakaraparthi: Designed and implemented the study by collecting the samples and performing appropriate laboratory tests. Analyzed and interpreted the results. Wrote the original draft and finalized the manuscript.

Dr. Vijaya Kumar Punnapu: Provided critical feedback on the manuscript. Assisted in analyzing the data. Revised and edited the manuscript.

Dr. Swathi Kuna: Contributed to the development of methodology.

Dr. Lakshmi Mounika Addanki: Revised and edited the manuscript.

CONFLICTS OF INTEREST

Authors have no conflicts of interest.

AUTHOR FUNDING

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