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Original Research Article

Cross-Sectional Study of Distribution of Candida Species and Their Anti-Fungal Susceptibility Pattern among Vulvovaginal Candidiasis Patients in a Tertiary Care Hospital

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Conflict of interest: Nil

Abstract

Introduction: Vulvovaginal candidiasis (VVC) is one of the most common infection which women experience at least once in their lifetime, caused by different species of Candida. Thus, this study determines the distribution of Candida species and its antifungal susceptibility pattern in a tertiary care hospital.

Material and Methods: This is a cross sectional observational study done on the isolates of Candida obtained from the cases of Vulvovaginal candidiasis, from December 2022 to December 2023 and processed with standards using Germ tube test, CHROM agar, Corn meal agar and VITEK 2 Compact System.

Results: A total of 436 swabs were received during the study period of which 160 were found to be culture positive for candida. Thus, the prevalence was 36.7% with highest prevalence in the age group of 25-34 years. Candida albicans was the commonest species isolated and resistance to fluconazole was found to be 26.7% and 14.7% susceptible dose dependent to fluconazole, among the strains of Candida albicans. Candida glabrata and Candida krusei are intrinsically resistant to fluconazole.

Conclusion: Candida albicans was the most common species with highest prevalence of 75.6% followed by non albicans candida (24.4%). Among the NAC (24.4%), Candida tropicalis (10.6%) was the predominant species followed by Candida glabrata (8.7%). Emergence of fluconazole resistant Candida albicans and other Non albicans candida (NAC) can lead to treatment failure causing recurrent vulvovaginitis. Thus, speciation and its antifungal susceptibility testing can prevent the recurrence and resistance in future in cases of VVC.

Keywords: Vulvovaginal Candidiasis, Candida, CHROM, Corn Meal Agar, VITEK.

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Introduction

Candida is a yeast like fungus and is a commensal of human oral, gastrointestinal and genitourinary tract. It becomes pathogenic and causes infections when the host's resistance is lowered locally or systemically. [1] Most common infection is mucocutaneous candidiasis which can be divided into non-genital and genitourinary disease. [2]

The most frequent among genitourinary candidiasis is vulvovaginitis (VVC) in women. It is estimated that around 75% of all women, experience at least one episode of VVC during child bearing years, of whom about half have at least one recurrence. [3]

VVC is diagnosed with vaginal complaints of pruritis, irritation, soreness leading to dyspareunia and dysuria. Despite Candida albicans, being the most common cause of vulvovaginal candidiasis, the frequency of vulvovaginal candidiasis caused by other Non albicans candida (NAC) species is gradually increasing. [3,4] Resistance to azole antifungal therapy in Candida albicans and Non albicans candida species has become more common. Little is known regarding the distribution and in vitro antifungal susceptibility profile of candida, isolated from cases of VVC. In line with this, early and accurate identification of candida species and its

rapid antifungal susceptibility testing is of utmost priority. [4]

Material and Methods:

The present study was conducted in the Department of Microbiology, Topiwala National Medical College and B.Y.L Nair Charitable hospital, Mumbai, Maharashtra. It is a Cross sectional observational study, and the study period was from December 2022 to December 2023. A total of 436 vaginal swabs were received in the laboratory. Among these 160 were culture positive for candida. The 160 culture positive isolates were included in the study. All the isolates received were processed by standard methods and were recorded along with the demographic details. There was no direct interaction with the subject of the study hence waiver of consent was taken.

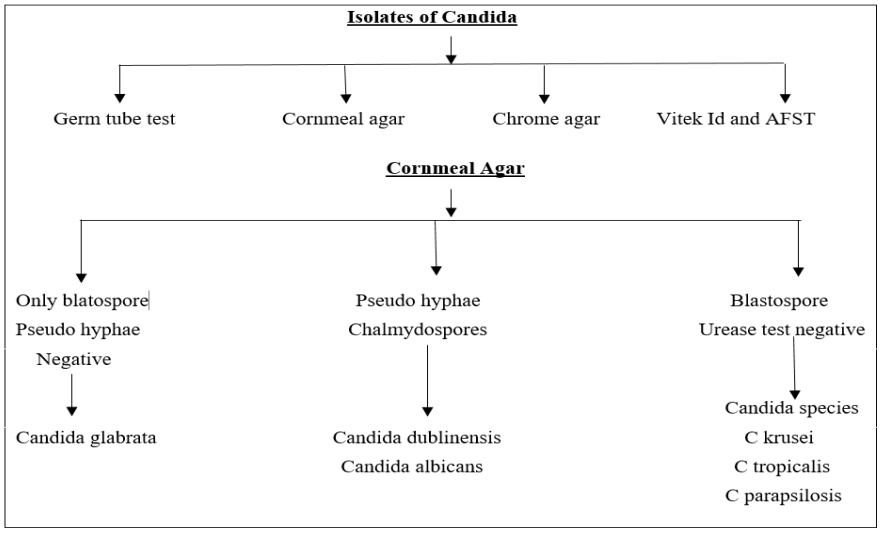

Chart 1: Isolates of *Candida*

Results:

Table 1: Age distribution of females with Vulvovaginitis

Age group of females (years)	No. of isolates	% of isolates
20 – 24	27	16.9
25 – 34	**77**	**48.1**
35 – 44	40	25.0
45 – 60	16	10.0
Total	160	100.0

In Table 1, Amongst the 160 isolates studied, majority of females had their age between 25 – 34 years with highest prevalence of 48.1%. Females of age between 35 – 44 years had the prevalence of 25%. Prevalence was seen to decline with increasing age.

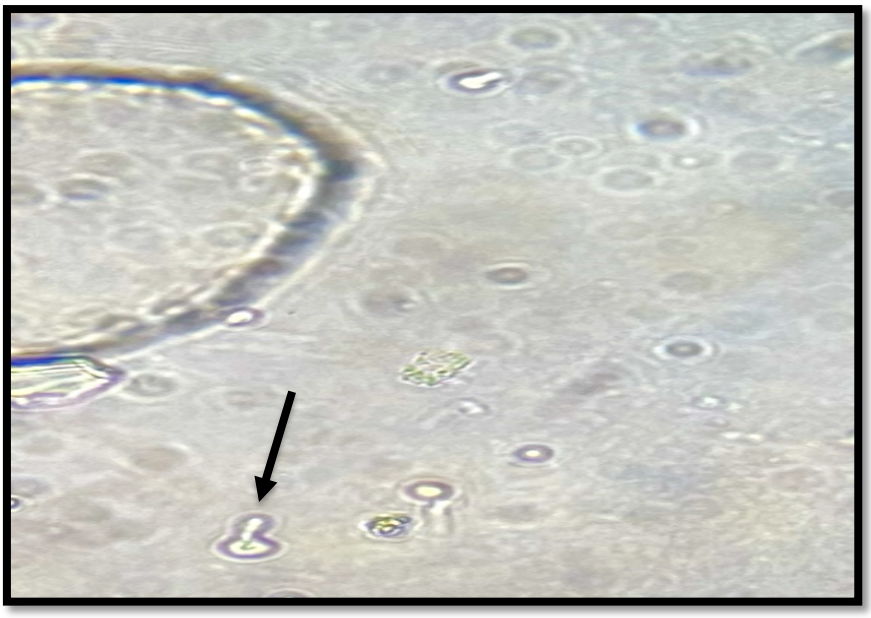

Figure 1: Germ tube test

In Fig 1, Germ tube seen in the form of a tube like projection, arising from the yeast cell, with parallel walls and without any constriction at the point of origin or attachment to the yeast cell.

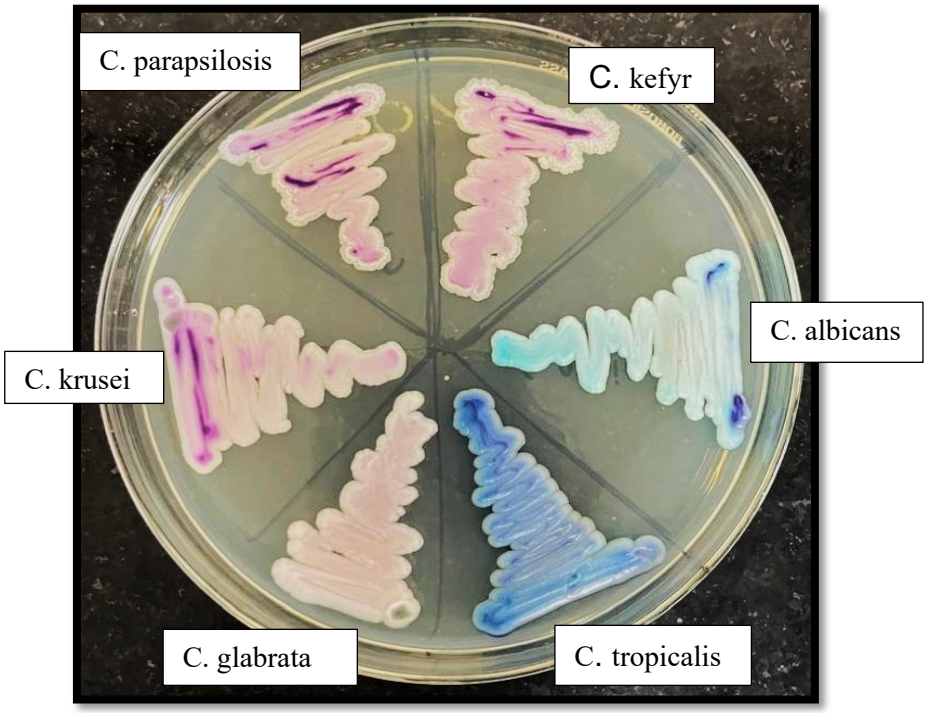

Figure 2: Chrome agar

In Fig 2, Speciation was done using CHROM agar**,** Candida albicans – Light green, Candida tropicalis – Metallic blue, Candida krusei – Rose pink/purple, Candida glabrata – white, Candida parapsilosis – Light pink, Candida kefyr - Pink

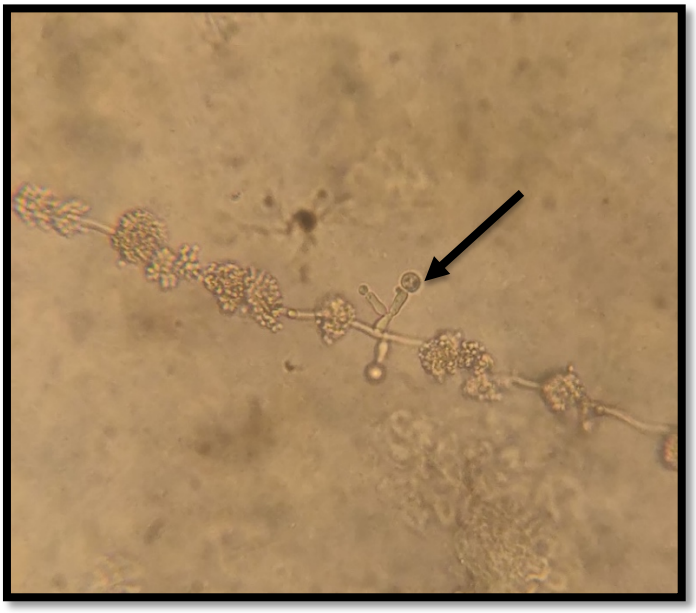

Figure 3: Candida albicans on Corn meal agar

In Fig 3, Speciation using Corn meal agar showing, Candida albicans as blastospores in clusters along Pseudo hyphae with large thick walled chlamydospores at the ends of hyphae.

Figure 4: Candida krusei on Corn meal agar

Fig 4, Speciation using Corn meal agar showing Candida krusei as blastospores with cross matchstick appearance along pseudo hyphae**.**

Table 2: Identification of candida isolates identified by conventional methods and VITEK 2 compact system:

	Candida isolates by Conventional method					
	Candida albicans		**Non-albicans Candida**		**Total**	
Candida isolates by VITEK2 compact system	**N**	**%**	**N**	**%**	**N**	**%**
Candida albicans	116	95.9	0	0.0	116	72.5
Non-albicans Candida	5	4.1	39	100.0	44	27.5
Total	**121**	**100.0**	**39**	**100.0**	**160**	**100.0**
Chi-Square value = 135.958, DF = 1, Cohen-Kappa Value = 0.919, P-value = 0.001* **						
P-value by Chi-Square test. P-value<0.05 is considered to be statistically significant. ***P-value<0.001.						

Distribution of detection of overall candida isolates by VITEK 2 compact system is statistically significantly associated with the detection by conventional method, with Cohen-Kappa value of 0.919 higher to perfect level of agreement (P-value<0.05).

Table 3: Antifungal susceptibility of Candida albicans and Non albicans Candida (NAC) species by VITEK 2 compact system

Candida	Status	Fluconazole	
		n	%
Candida albicans	S	68	58.6
116	SDD	17	14.7
	R	31	26.7
	I	0	0
Non albicans candida	S	26	59
44	SDD	1	2.3
	R	1	2.3
	I	0	0

Antifungal susceptibility testing done on Automated VITEK 2 Compact system showed the above susceptibility pattern, in which isolates of Candida albicans were resistant to fluconazole 31 (26.7%), and susceptible dose dependent 17 (14.7%) Among the Non albicans candida, resistance noted to fluconazole was 1(2.3%) and Susceptible dose dependent 1(2.3%).

Discussion

Vulvovaginal candidiasis (VVC) is the most common infection of lower genital tract in women of the reproductive age group. [5] In the present study prevalence of VVC was 36.7%. Study done by Salvi et al [7] showed prevalence of (31.6%), and Kumar et al [8] showed (33.14%). But higher prevalence was seen in studies done by Bitew et al [4] (41.4%), Bashir et al [6] (41.8%), Ghaddar et al [17] (44.8%), Babin et al [9] (48.4%) and Samal et al [5] (48.4%). The differences in prevalence could be due to multiple factors, including socio-demographic characteristics, immune status of the patients, treating patients with broad spectrum antibiotics, immune suppressive drugs and hormonal influences. However, Raghunathan et al [18] (22.2%), Kalaiarasan et al [16] (23.7%) reported lower prevalence than the present study. Factors like improvement in personal hygiene, or in the economic status which resulted from education, safe sex practices, healthy lifestyle, limiting use of antibiotics and increasing awareness help in reducing the risk of vulvovaginal candidiasis.

The highest prevalence has been found in the age group of 25-34 years (48.1%), followed by 35-44 (25%). The study done by Kumar et al [8] showed highest prevalence in the age group of 21-30 years, Bashir et al [6] (26-30 years), Kalaiarasan et al [16] (21-30 years) and Samal et al [5] (24-34 years).Such high prevalence in the reproductive age group can be due to lower levels of protective cervical antibodies, increased sexual activity and influence of reproductive hormones which helps in maintaining the acidic pH and enhancing the yeast adherence to vaginal epithelial cells. Postmenopausal women appear to be more resistant to candida colonization, therefore prevalence of vulvovaginal candidiasis is seen to decline with increasing age.

The preliminary identification of Candida albicans was made by use of Germ tube test. In the present study, 75.6% (121 / 160) of the isolates were positive for Germ tube test, while 24.4% were negative for germ tube test. Candida tropicalis can produce germ tube like structures, which may be confused with true germ tubes after an extended incubation period. Therefore, further speciation using CHROM and Corn meal agar was important for identification of Candida species.

On CHROM agar 121 isolates (75.6%) were identified as Candida albicans, 17 isolates (10.6%) as Candida tropicalis and Candida glabrata (n=14, 8.7%). Some of the other Non albicans candida (8 isolates, 5.1%) gave light pink to purple pink colour on CHROM agar. The species like Candida parapsilosis, Candida krusei and Candida kefyr could not be identified using CHROM agar. Thus, the subjective identification and low discriminative power of CHROM agar restricts its use for the identification of some Non albicans candida. Study by Bharathi et al [19] also encountered such limitation with CHROM agar.

Based on the findings of Corn meal agar, conventional identification of our study found Candida albicans (75.6%) as the predominant species followed by, Candida tropicalis (10.6%), Candida glabrata (8.7%), Candida parapsilosis (3.1%), Candida krusei (1.3%) and Candida kefyr (0.6%).

Conventional methods of identification for candida are time consuming. Various commercially available methods which allow identification within several hours, have been developed and evaluated. In the present study use of Automated VITEK 2 Compact system for identification of Candida species and its comparison with the conventional

methods done showed some misidentification. It was mainly encountered with Candida albicans as it identified 116 (72.5%) isolates correctly as Candida albicans, 5 (3.12%) isolates were misidentified as Non albicans Candida in which, 3 (1.9%) isolates were given as Candida dubliniensis and 2 isolates (1.3%) as Candida lusitaniae. Similar findings were observed in the study done by Kaur et al. The measure of agreement between these two detection methods inferred that there is a higher to perfect level of agreement between Conventional and Automated VITEK 2 Compact method in our study.

In the present study, antifungal susceptibility testing was assessed for fluconazole, voriconazole, amphotericin B, Caspofungin, Micafungin and Flucytosine using Automated VITEK 2 compact system.

Resistance was noted to Fluconazole (26.7%) and (14.7%) susceptible dose dependent to fluconazole, among the strains of Candida albicans.

The study done by Babin et al [9] also detected resistance in Candida albicans to fluconazole (16.27%) and (6.9%) Susceptible dose dependent. ElFeky et al. [20] also reported resistance in Candida albicans to fluconazole (10.5%). However, the study done by Seck et al [14] and Bitew et al [4] showed no resistance to fluconazole, which is in contrast to our study.

Among the Non albicans Candida species, Candida glabrata and Candida krusei are intrinsically resistant to fluconazole. Candida tropicalis showed resistance to fluconazole in 5.9% isolates and Susceptible dose dependent in 5.9%. No resistance to fluconazole was reported by Candida parapsilosis, Candida lusitaniae and Candida kefyr in the present study. The study done by Bashir et al [6] also detected resistance of 17.2 % to Fluconazole in Non albicans candida. Babin et al [9] also reported resistance to azoles in Candida tropicalis, Candida glabrata, and krusei. Emergence of azole resistant Candida albicans in VVC was seen in the present study which is similar to studies done by Bashir et al [6] and Babin et al [9] However, Bitew et al [4] reported no resistance to azoles in their study.

According to the 2021 Centres for Disease Control and Prevention guidelines and NACO guidelines 2024, uncomplicated VVC should be treated with topical preparations of short duration. Oral treatment with fluconazole consists in administering a single dose of 150 mg of the drug. Moreover, in approximately 50% of patients treated with fluconazole, VVC recurs after 6 months. Thus, the American Society of Infectious Diseases recommends that patients should use topical and oral azoles concurrently. Treatment of non - albicans species VVC is challenging because they are very often resistant to azoles and nearly 50% of affected women might present minimal or no symptoms. Thus, the present study depicts increasing resistance to azoles seen in Candida albicans and some Non albicans candida isolates. The increasing resistance is probably due to inappropriate use of antifungal drugs (self-medication, topical use, long term treatment, syndromic management and repeated episodes) Thus, the present study signifies identification should always be followed by antifungal susceptibility testing as the emergence of resistant Candida isolates is increasing which can further lead to treatment failures causing recurrent Vulvovaginitis.

Conclusion

Vulvovaginal candidiasis is most prevalent in the women of reproductive age. Although time consuming, conventional methods of identification are still considered reference methods. Automated VITEK 2 compact system appeared to be an alternative method for rapid identification of species. Regarding the antifungal susceptibility, Candida albicans, the main species involved in VVC showed a decreased susceptibility rate to fluconazole. Gradually increasing azole resistance, lack of awareness and its injudicious use can cause recurrence. Increase in emergence of azole resistant Candida albicans and Non albicans candida in VVC emphasizes the need for speciation and antifungal susceptibility testing of candida isolates from cases of VVC to treat and effectively control the resistant strains in future.

Limitations

- Antifungal susceptibility of the misidentified isolates by VITEK 2 Compact system was given as per automated method.
- Mixed isolates were not included in the study

Disclosure

- Author contribution: All the authors contributed equally to the manuscript.
- Ethics approval :Yes

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