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Species distribution and antifungal susceptibility pattern *Candida species* isolated from patients with vulvovaginal candidiasis by Vitek 2 compact system, in Western Maharashtra.

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Abstract:
Background: *Vulvovaginal candidiasis (VVC) is the most common cause of fungal infection in reproductive age group. It is of special concern due to its chronic course and frequent recurrence due to inappropriate or incomplete treatment. Due to emergence of antifungal drug resistance, traditional practice of empirical treatment does not work in all patients. With this background present study was conducted to determine the prevalence, species distribution and antifungal susceptibility pattern of Candida species causing VVC.* ***Material and methods:*** *241 vaginal swabs were collected from clinically suspected cases of VVC. Speciation and antifungal susceptibility testing (AFST) was performed using fully automated Vitek 2 compact system.* ***Results:*** *Prevalence of VVC in present study was found to be 12 %(29/241). Candida albicans was the most common Candida species isolated from from VVC. Other species isolated were C. lusitaniae, C. glabrata and C.parapsilosis. Candida albicans showed 100% sensitivity for caspofungin and micafungin followed by 96% sensitivity to amphotericin B and fluconazole. C. albicans as well as and Non albicans candida showed 17.4% reduced sensitivity to voriconazole.* ***Conclusion:*** *Local data about the most common pathogens causing VVC and their antifungal susceptibility pattern should be available for clinicians to decide appropriate therapy for patient. With increasing need for antifungal susceptibility testing, Vitek 2 compact acts as rapid and accurate tool.*

Keywords: *Vulvovaginal candidiasis, AFST, Vitek 2 compact, automation, C.albicans, non-albicans candida*

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I. Introduction

Candida belongs to the commensal flora in oral cavity, skin and genitourinary tract in humans. In susceptible hosts it acts as opportunistic pathogens. It is a leading etiological agent of fungal infections in human beings. Candida species causes variety of human infections ranging from cutaneous infections to life threatening systemic infection.[1] The most common clinical manifestation of candidiasis is vulvovaginal candidiasis (VVC) which is a sexually transmitted disease in females specifically in reproductive age group.[2] *Candida species* are broadly divided in to two groups namely *Candida albicans* and nonalbicans *Candida species*. *Candida albicans* is the most common among all species of Candida causing vaginitis.[3] About 50-70% women worldwide are affected by vulvovaginal candidiasis, and about 40% of them have recurrent episodes.[4,5] In few patients (5%), vulvovaginal candidiasis becomes refractory to treatment and takes chronic course.[6,7]

Occurrence of vulvovaginal candidiasis is commonly associated with predisposing factors like socio demographic factors, personal hygiene, sexual activity, diabetes mellitus, oral contraceptive pills, immunosuppression, and hormonal changes as seen in pregnancy. [8, 9] Increased incidence of vulvovaginal candidiasis is observed with increased use of antibiotics. Antibiotics eliminate the vaginal lactobacilli which are healthy bacteria protecting the vaginal epithelium. As result of this *Candida species* can adhere more to the vaginal mucosa and there is increased growth and colonization of this organism in the genital tract leading to infection. [8, 10]

Fluconazole, voriconazole, nystatin, ketoconazole, caspofungin, clotrimazole, flucytocine are the different antifungal agents used for treatment of candidiasis. Empirical treatment for vulvovaginal candidiasis essentially involves antifungals, mainly azoles. Diagnosis and treatment of VVC is done mainly based on clinical findings. Treatment based on microbial culture and other laboratory based tests is still low. Due to such

a high and inappropriate use of the antifungal agents has resulted in emergence of drug resistance. Other concern about this antifungal treatment is cost and safety. [11, 12]

There is increased requirement for antifungal susceptibility testing by clinicians due to emergence of drug resistance. Conventional microbiological tests for identification and speciation of *Candida species* are time consuming and tedious to perform in routine diagnostic laboratory. Vitek 2 compact system is a promising alternative to conventional methods for rapid and accurate identification and antifungal susceptibility testing (AFST) of *Candida species.* Vitek 2 compact system gives susceptibility in the form of minimum inhibitory concentrations, which are very useful for treatment purpose to decide the dose and duration drug. [13, 14] This system is based on the principle of spectrophotometry, which detects metabolic changes and yeast growth in micro wells provided in the yeast ID and AST cards. The results obtained are compared with extensive database and then final identification and antimicrobial sensitivity is given. [15, 14]

Knowledge of local species distribution and antifungal susceptibility pattern of Candida species based on lab data help the physician to decide rational and effective empirical antifungal treatment for vulvovaginal candidiasis. With this background, the present study was conducted,

1. To estimate the prevalence of vulvovaginal candidiasis.
2. To determine the different species and antifungal susceptibility pattern of different Candida species involved in causation of VVC by using Vitek 2 compact automated system. .

II. Material And Methods

A retrospective laboratory based observational study was conducted over a period of one year from January 2019- December 2019 in Department of Microbiology of a clinical diagnostic centre situated in Pune district, Maharashtra. Patient details like demographic data, laboratory findings and clinical details were obtained from electronic medical records for analysis.

Vaginal swabs sent for microbial culture from female patients visiting Gynaecology OPDs having signs and symptoms suggestive of vulvovaginitis and having vaginal discharge were included in the study. Vaginal discharge was collected by physician using sterile cotton swab and kept in a sterile container. Samples were immediately transported to the diagnostic Microbiology laboratory at 2-8 degree C for further processing. After receiving samples (vaginal swab) in the laboratory they were immediately processed. Samples were inoculated on blood agar and MacConckey agar and Sabourauds Dextrose agar incubated at 37 degree C for 24 hours aerobically. From the vaginal swab a gram stain smear was also prepared. On gram stain, presence of pus cells and yeast cells was noted. The plates were examined for growth after 24rs of incubation. Any growth obtained was correlated with gram stain findings. If there was no growth after 24hrs of incubation plates were further incubated for next 24 hrs, before reporting them as no growth. [15]

Identification and antifungal susceptibility testing of Candida isolates:

Growth obtained after conventional culture method were processed on Vitek 2 compact fully automated system by Biomerieux France for identification and AFST using YST-21343 and AST-YS01 cards. Suspension of pure culture growth was made in saline suspension provided by biomerieux to get a turbidity equivalent to 1.8-2 McFarland standards with the help of DensiChek turbidity meter (Biomerieux, France). Two different tubes, one for identification and one for AFST were needed for a single isolate. After preparation of saline suspension of required density in first tube, 280 ul of suspension was transferred to second tube from first tube. Yeast ID and AST cards were then put in first and second tube respectively. Tubes and cards are kept in cassettes and then those cassettes were transferred inside the Vitek-2 instrument. The suspension aspiration, sealing of cards, incubation takes place automatically. Cards were incubated at 37 °C for 18-24hrs. Optical density was measured from each micro well of cards every 15 mins. Based on the readings of op were noted by with optical density readings taken automatically at every 15 min. Identification and AFST was interpreted and established based on these readings.[16] Data analysis in the present study was performed by using Microsoft excel.

III. Results

Over a period of one year total 241 samples from patients with signs and symptoms of vulvovaginitis were received and processed in the laboratory. Age range of patients included in the present study was 19-41 years. Maximum patients of aerobic vaginitis belonged to age group of 25-35 years(79.3%) , followed by 15-25 years(13.8%) as shown in **Figure 1**.

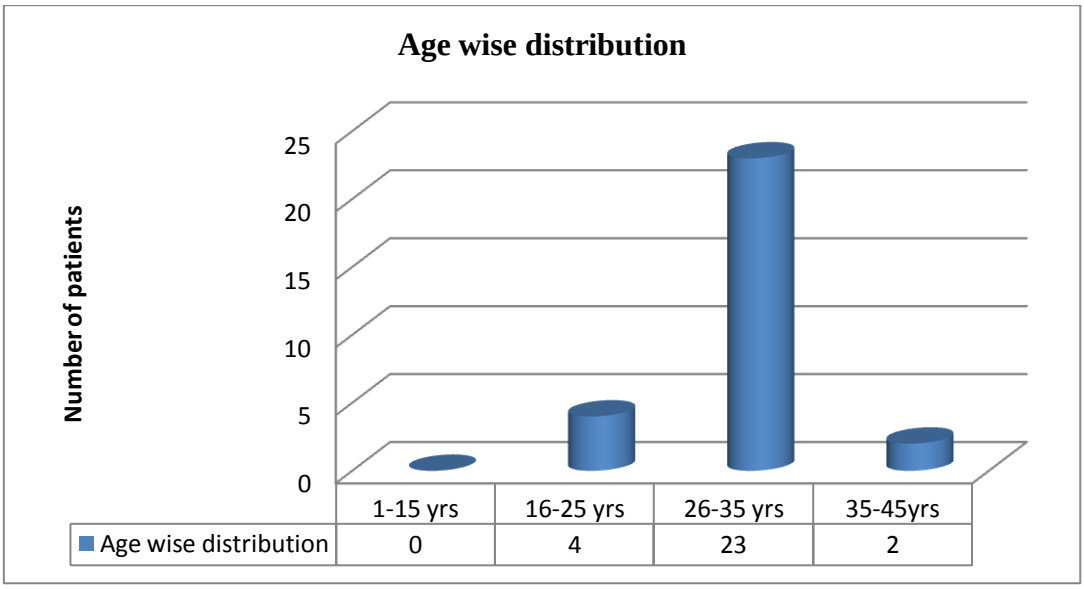

Figure 1: Age wise distribution of cases of VVC

Out of 241 vaginal samples received for culture, 29 samples grew *Candida species* resulting in 12% prevalence of aerobic vaginitis in present study. In present study, *Candida albicans* (86.2%) was the most common species isolated. Other non-albicans *Candida species* isolated were *C. lusitaniae, C. glabrata, C.parapsilosis.* **(Table 1, Figure 2)**

Table 1: Species distribution of Candida species in vulvovaginitis

Species	Number	Percentage
C.albicans	25	86.2%
C.lusitaniae	2	6.9%
C.parapsilosis	1	3.4%
C.glabrata	1	3.4%

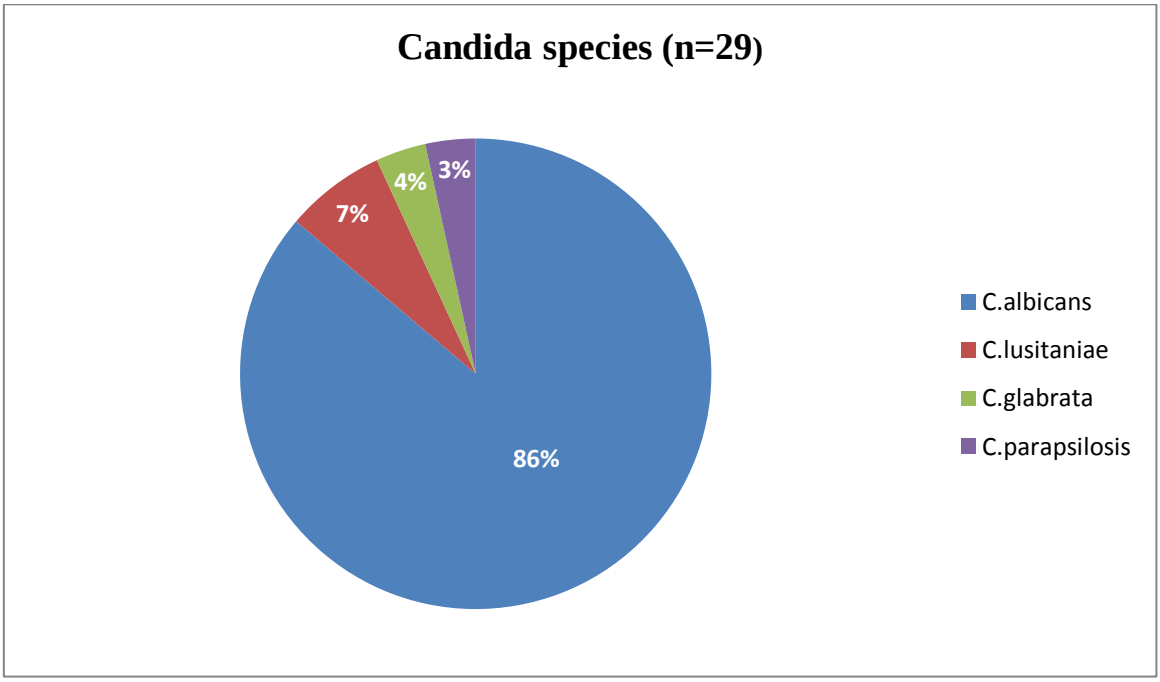

Figure 2: Distribution of Candida species in VVC

C. albicans showed 100% sensitivity to Caspofungin and Micafungin followed by 96% sensitivity to Amphotericin B and Fluconazole. Non albicans *Candida species* showed highest sensitivity to Amphotericin B, Caspofungin and Micafungin. **(Table 2, Figure 3)**

Table 2: Antifungal susceptibility pattern of *Candida species*

Antifungal drugs	Sensitivity	C.albicans (n=25) (%)	*C.lusitaniae (n=2) (%)*	C. parapsilosis *(n=1)(%)*	C.glabrata *(n=1)(%)*
Amphotericin B	S	24 (96)	2(100)	1(100)	1(100)
	IR	0	0	0	0
	R	1(4)	0	0	0
Caspofungin	S	25(100)	2(100)	1(100)	1(100)
	IR	0	0	0	0
	R	0	0	0	0
Flucytosine	S	23(92)	2(100)	1(100)	1(100)
	IR	0	0	0	0
	R	2	0	0	0
Fluconazole	S	24(96)	2(100)	0	0
	IR	0	0	0	0
	R	1(4)	0	1(100)	1(100)
Micafungin	S	25(100)	2(100)	1(100)	1(100)
	IR	0	0	0	0
	R	0	0	0	0
Voriconazole	S	21(84)	2(100)	0	0
	IR	4(16)	0	0	1(100)
	R	0	0	1(100)	0

Note: S=sensitive, IR=intermediate resistant, R=resistant

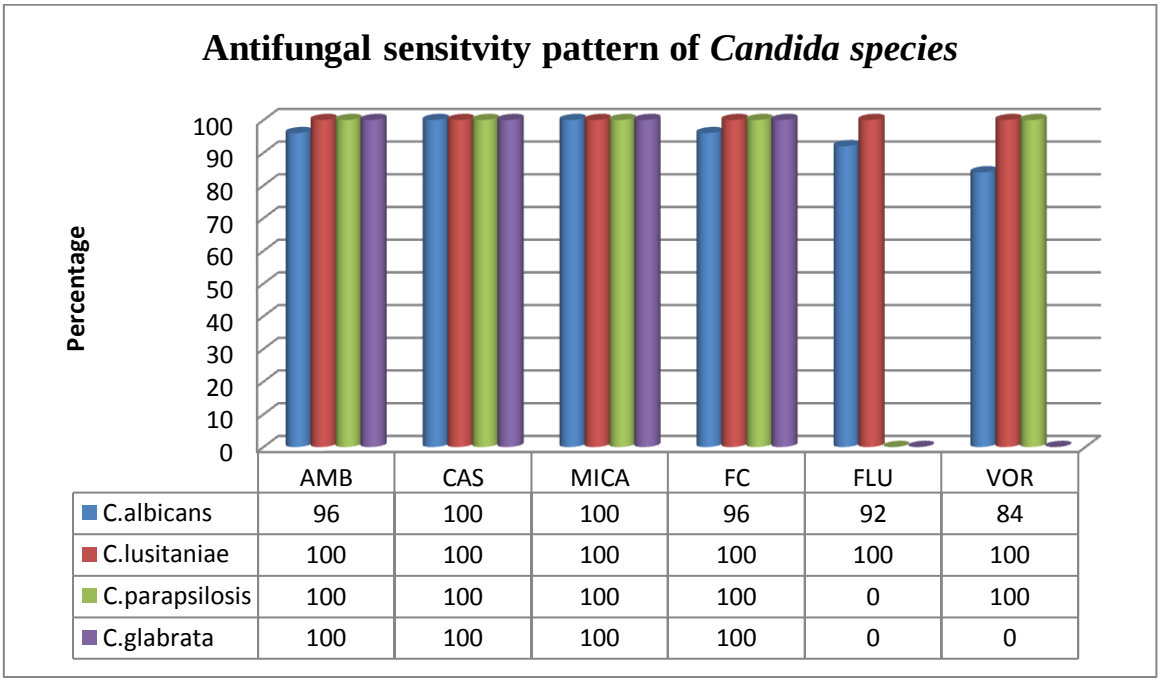

Figure 2: Antifungal sensitivity pattern shown by different Candida species.

Abbreviations: AMB= amphotericin B; CAS=caspofungin, FLU= fluconazole, MICA= micafungin, VOR= voriconazole, FC= flucytosine.

IV. Discussion

In the reproductive age group the most common fungal infection in females is vulvovaginal candidiasis. Approximately 60-70% females experience at least one episode of VVC in their life time. [17,18] In present study, the most common age group affected was 26-35 years followed by 36-45 years, which coincides with the reproductive age group. Many other authors have shown high incidence of VVC in child bearing age, which is in comparison with present study.[2,19,20] There are various reasons for more incidence of VVC in reproductive age group which includes increased sexual activity, decreased levels of cervical protective antibodies, hormonal changes.[2]

Prevalence of vulvovaginal candidiasis in present study was found to be 12%. Which is in agreement with studies conducted by Ahmad et al [8] and Khan et al [21], but it was much lower than the prevalence shown by Kombade et al [1], Olowe et al [9] and Toua et al [22] Variation in the prevalence of VVC in different studies can be attributed to difference in study population, socio economic status of patients, associated co morbidities like immunosuppression, hormonal changes. [15]

The most common species isolated in cases of VVC was *C.albicans* in present study. Our finding is in agreement with many other studies in which predominant species was found to be *C.albicans* as shown in **Table 3.** However many other studies have shown recovery of non albicans Candida species specifically *C. glabrata* as predominant pathogen in VVC.[1,10,23,24,25] Rise in incidence on non albicans Candida species can be attributed to overuse of antifungal drugs which are active against C.albicans , but not against non-albicans Candida species, which creates a selective pressure for survival of resistant microorganism.[1]

Table 3: Studies showing distribution of Candida species

Studies	***C.albicans* (%)**	***C.lusitaniae* (%)**	***C.parapsilosis* (%)**	***C.glabrata* (%)**
Rati et al (2015)[2]	54.5	-	-	4.54
Bitew et al (2018)[15]	58.6	1.2	2.3	3.4
Khan et al(2018)[21]	41.7	-	-	14.8
Present study (2019)	86.2	6.9	3.4	3.4

In present study, antifungal susceptibility results showed 100% sensitivity to caspofungin and micafungin by both *C. albicans* and non-albicans *Candida species*. This finding is in agreement with study conducted by Bitew et al [15] in which he has shown highest sensitivity to Caspofungin and Micafungin. Amphotericin B was the next sensitive antifungal agent with 96% and 100% sensitivity in *C.albicans* and non albicans *Candida species* respectively which is agreement with studies conducted by Rati et al [2], Sara et al [26] and Quindos et al. [27] Irreversible fungicidal action after binding to the yeast cells is one of the reason for such high sensitivity to amphotericin B. *C. albicans* and single strain of C.glabrata isolated in present study showed resistance to fluconazole and reduced sensitivity to voriconazole. *C.glabrata* is known to have less sensitivity to azoles. Azoles are the mainstay of empirical treatment for VVC. High usage of these drugs could have been responsible for emergence of drug resistance for azole group of antifungals. [12, 28]

V. Conclusion

C.albicans was found to be the most common Candida species causing vulvovaginal candidiasis showing overall very less antifungal drug resistance. Most sensitive antifungals found in the present study were caspofungin and micafungin. Local prevalence, species distribution and antifungal susceptibility pattern of Candida species should be available to guide clinicians regarding empirical treatment for vulvovaginal candidiasis. Automation in clinical microbiology is need of the hour for rapid and accurate diagnosis of fungal infections as the conventional methods are very time consuming, difficult to perform and interpret.

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