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SPECIATION OF CANDIDA & ITS ANTIFUNGAL SUSCEPTIBILITY PROFILE FROM PATIENTS WITH VAGINITIS ATTENDING STD CLINIC AT A TERITIARY CARE HOSPITAL

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ABSTRACT

Background: Candidiasis is the most common vaginal infection affecting approximately 75% of reproductive age group women. Rapid identification of yeast isolates to species level is essential to optimize antifungal treatment. **Aim**: To identify the species of *Candida* from patients of vaginitis and to study antifungal susceptibility pattern of isolated candida species. **Methods:** Vaginal swabs were collected from women in the reproductive age groups who attended STD clinic with clinically diagnosed vaginitis during the months of June 2019 to December 2019. The swabs were inoculated onto SDA. 91 candida were isolated. Isolated colonies of candida were inoculated onto CHROM agar & species were identified by color of the colonies produced as per manufacturer chart. Colonies also inoculated onto corn meal agar for chlamydospore formation. Antifungal susceptibility was done by disc diffusion method. **Results:** A total of 150 women of reproductive age group who were clinically diagnosed to have Vaginal Candidiasis were included in the study. Out of 150 vaginitis patients, 91 were positive for *Candida* species. All the isolates were speciated comprising 4 species. *C. albicans* - 42 (46.1%), *C. glabrata* - 41 (45.1%), *C. Krusei* - 5 (5.5%) and *C. tropicalis* - 3 (3.3%). Antifungal susceptibility testing result of all *Candida* isolates - 100% susceptible to amphotericin B, nystatine, flucytocine, voriconazole, ketoconazole. All the Candida isolates except *C. krusei* were sensitive to fluconazole. *C. krusei* showed 100% resistance to fluconazole. **Discussion:** In the present study, *C. albicans* is most common species (46.1%) followed by *C. glabrata*. *C. albicans* adhere to vaginal epithelial cells in significant higher number than other *Candida* species this could explain relative higher frequency of *C. albicans* in vaginal Candidiasis. **Conclusion:** Presumptive identification followed by confirmation of *Candida* species helps to initiate early appropriate antifungal treatment there by reducing the morbidity and mortality.

KEYWORDS: Vulvovaginal Candidiasis, Vaginitis, Non albicans candida, Antifungal susceptibility.

INTRODUCTION

Vaginal candidiasis is second to bacterial vaginosis as the most common mucosal infections that affect large number of otherwise healthy women of child bearing age.[1,2] Upto75% of reproductive age women are infected with vaginal candidiasis at least once and about half of these women experience recurrence.[3-5] ln addition to discomfort and the cost associated with medication and health care visits, several studies have suggested that vaginal candidiasis may increase a woman's risk for contacting other sexually transmitted diseases such as human immunodeficiency virus (HIV).[6] Vaginal candidiasis if untreated can lead to chorioamnionitis with subsequent abortion, prematurity, congenital infections of the neonate in pregnant woman and pelvic inflammatory disease resulting in infertility in women of childbearing age.[7] *Candida albicans* is the common pathogen in 80-90% of cases but non-albicans *candida* species are gaining importance as a pathogen over the past few decades.[5] Vulvovaginal candidiasis is characterised by vulval pruritus, dysuria, swelling or redness, vulval edema, fissures, excoriation or thick curdy discharge. Vaginal pH is usually normal.[5]

The present study was undertaken to determine the prevalence of the various *candida* species among vaginal candidiasis and to determine the antifungal susceptibility pattern of the isolates. This data will provide important information in developing effective strategies for prevention and possible treatment option for vaginal candidiasis.

AIM OF THE STUDY: Speciation of *Candida* and its Antifungal susceptibility profile among patients with vaginitis.

OBJECTIVES OF THE STUDY

- To identify the species of *candida* from patients of vaginitis.
- To study the Antifungal susceptibility pattern of isolated *Candida* species.

MATERIAL AND METHODS

It is a cross-sectional study conducted at a tertiary care centre in Hyderabad during the months of June 2019 to December 2019. The necessary institutional ethics committee clearance was obtained. A total of 150 women of reproductive age group participated in the study after obtaining written informed consent.

Inclusion criteria

- Females in the reproductive age group (15- 40 years).
- Patients complaining of vulval pruritis, dysuria, curdy vaginal discharge, dyspareunia, low back ache and pain in the lower abdomen or clinically diagnosed as vaginal Candidiasis.
- Patients giving consent.

Exclusion criteria

- Patients diagnosed as bacterial vaginitis and mixed bacterial and fungal vaginitis will not be taken into consideration.
- Patients not giving consent.

METHODOLOGY

After obtaining written informed consent, a detailed clinical, personal and sexual history of the patient was taken. External and internal local examination of patients was done. Amount, colour, characteristic and smell of vaginal discharge were observed. Two swabs were collected from the lateral walls of vagina by sterile cotton tipped swabs. One of them was used to prepare a direct wet mount and to perform Gram staining. The other swab was inoculated on slants of Sabourad's dextrose agar with added gentamicin (0.06ug/ml) and plates of Hi Chrome candida differential Agar (Himedia laboratories). The plates and slants were inoculated aerobically at 37°C for a period of 24-48 hours. The colonies on SDA were subjected to Germ tube test and Chlamydospore formation on Corn meal agar. The colour of the colonies produced on Hichrome Candida differential agar was interpreted and species identified as per the manufacturer chart. Antifungal susceptibility done by disc diffusion method on Muller Hinton Agar supplemented with 2% glucose and 0.5% ug/ml methylene blue as per CLSI guidelines M44-A document. Antifungal agents tested were Amphotericin B 20ug, nystatin 50ug, flucytosine 5ug, voriconazole 1ug, ketoconazole 30ug and fluconazole 25ug.

RESULTS

A total of 150 women were included in the study. Maximum patients (50.6%) were in the age group 25-40 [Table 1].

Out of 150 vaginitis patients, 91 were positive for *Candida* species. Age distribution of confirmed cases of vaginal discharge was, less than 19 years age group -5 (5.5%), 19–24 years age group - 20 (21.97%) cases, 25–40 years age group - 61 (67.03%) cases, and more 40 years age group- 5 (5.5%) cases [Table 1].

Out of the total *Candida* sps, *C.albicans* was the commonest with 42 isolates (46.1 %) followed in order by *C.glabrata with* 41 isolates (45.1%). The other species isolated were *C.krusei* -5 (5.5%), and *C.tropicalis* -3(3.3%) [Table 2,3]. The overall prevalence of non–albicans *candida* sps in the study was 49 (53.85%). The important observation in the study was a striking increase of non albicans *Candida* sps with 53.85%.

Colors of the Candida species produced on Hi-chrome Candida differential agar were *C.albicans* produced bluish green colonies, *C.tropicalis* metallic blue, *C.glabrata* pink to lilac, & *C.krusei* pink colored colonies [Table 4][Figure- 1].

Microscopic appearance of the *Candida* species [Figure- 2].

Antifungal susceptibility testing result of all Candida isolates showed 100% susceptible to amphotericin B, nystatine, flucytosine, voriconazole, ketoconazole, fluconazole except C.krusei isolates which showed 100% resistance to fluconazole [Figure- 3].

Table 1: Age Distribution.

Age group	No. of samples collected	No. of Candida positive samples
<19 years	23 (15.3%)	5 (5.5%)
19–24 years	42 (28%)	20 (21.97%)
25–40 years	76 (50.6%)	61 (67.03%)
>40 years	9 (6.1%)	5 (5.5%)
Total	150 (100%)	91 (100%)

Table 2: Distribution of Candida Species.

Candida albicans	42 (46.15%)
Non- albicans species	49 (53.85%)

Table 3: Distribution of Total Candida Species.

Candida species	Number	Percentage
Candida albicans	42	46.1%
Candida glabrata	41	45.1%
Candida krusei	5	5.5%
Candida tropicalis	3	3.3%
Total	91	100%

Table 4: Different Colours of Candida Species on Hichrome Agar.

Candida albicans	Bluish green
Candida glabrata	Pink to lilac
Candida krusei	Pink
Candida tropicalis	Metallic green

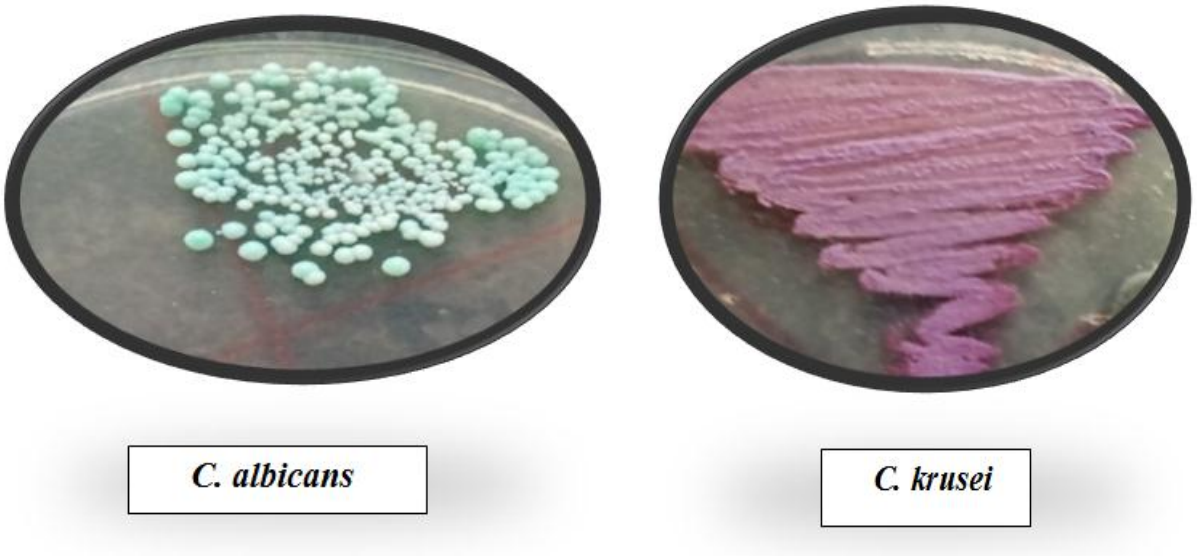

Figure - 1: Showing *Candida* species on HICHROME agar.

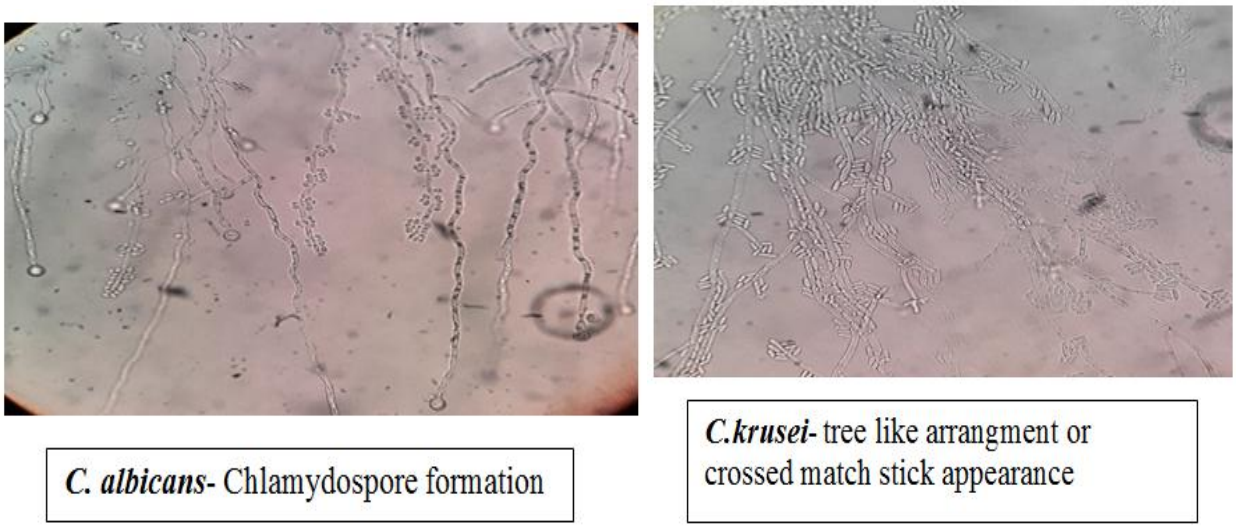

Figure -2: Showing microscopic appearance of *Candida* species.

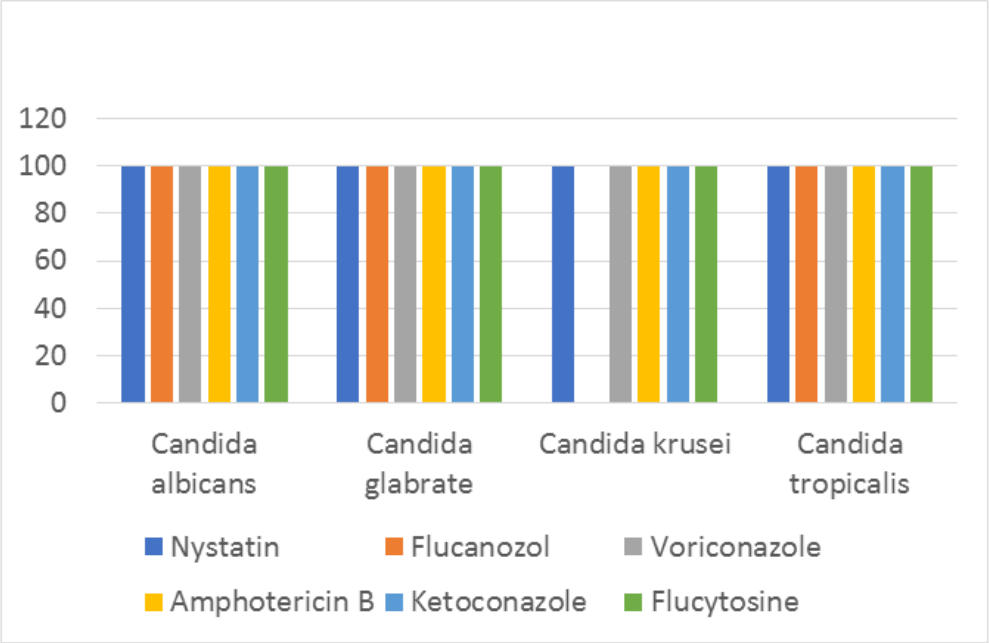

Figure - 3: Diagram showing antifungal susceptibility of isolates.

DISCUSSION

Major percentage (90%) of etiology of vaginal discharge is due to Bacterial vaginosis, candidiasis and trichomoniasis. In the present study, vaginal candidiasis was found in 91 (60.8%) symptomatic women with the highest incidence of vaginal candidiasis seen in 25–40 years age group which is similar to other studies.[1,8] Culture is the most sensitive and the gold standard method for identification and speciation of Candida.[7] In the present study, *C.albicans* is the most common species (46.1%) which concurs with many studies in India and abroad (Paulitschet al., Geiger et al.,).[11,12] Recent studies conducted by Deorukhhar and Saini (Deorukhkar et al.,)[9] states non albicans *Candida* as emerging pathogens in cases of VVC. Studies conducted by Paulitsch et al.,[11] documented the increased isolation of non albicans *Candida, C.kefyr* from recurrent and chronic VVC. Present study documented 53.85% of non albicans *candida* which is strikingly high and concurs with the study of Neerja jindal et al.,[13] who reported an overall 36.6% of non albicans Candida.

Thus in recent years there has been a speculating increase in number of non albicans *Candida* infections which is clearly represented in present study. Among non-albicans *Candida, C. glabrata* was the commonest isolate which correlates with the study of Mohanty et al.,[10] but is in contrast to Deorukhkaret al.,[9] where *C.tropicalis* was the commonest isolate. Study conducted by Swarajya Lakshmi et al.,[14] reported that most frequent *Candida* species were *Candida guillermondi* and *Candida tropicalis* which account for 24% and 16% of the total isolates.

Current trends show an increased prevalence of non-albicans *Candida* which was significantly higher in present study. The possible reason for this may be the indiscriminate use of antifungals which eliminates more sensitive *C. albicans* and selects azole resistant non-albicans Candida.[8] It has been stated, that variety of vaginal candidiasis caused by resistant *Candida* strains, susceptibility testing was rarely indicated. However, in the present study, *C. krusei* isolates are found to be resistant to fluconazole. Hence, there is a need for routine antifungal susceptibility testing of all *Candida* isolates. In the present study, *C. albicans* and majority of non-albicans *Candida* species are susceptible to voriconazole and fluconazole.

CONCLUSION

Changing trends in the antifungal susceptibility testing towards fluconazole recommends routine antifungal susceptibility testing of *Candida* isolates in clinical microbiology laboratories. Presumptive identification followed by confirmation of *Candida* species helps to initiate early appropriate antifungal treatment there by reducing the morbidity and mortality.

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