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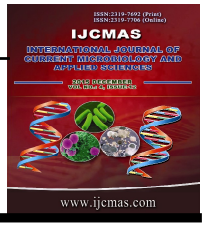

Original Research Article

Isolation and Speciation of *Candida* from Vulvovaginitis and their Antifungal Susceptibility

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Keywords

Vulvo Vaginal Candidiasis, Candida nonalbicans, *C. parapsilosis*, Antifungal susceptibility , Amphotercin -B

A B S T R A C T

Candidiasis is the commonest vaginal infection affecting about 75% women during reproductive age group. *Candida albicans* is the most common species causing Candidiasis but there has been an increase in Candida non albicans as reported in various studies. Isolation and speciation of Candida from cases of vulvovaginitis and their antifungal susceptibility. Materials and methods: This study group includes 100 high vaginal swabs from patients with vulvovaginitis attending OPD, KGH, Visakhapatnam. Standard mycological tests for candida isolation , speciation and antifungal susceptibility were done. Out of 100 samples,90 were culture positive for Candida. The most commonly isolated species was *C.parapsilosis* (33.3%) followed by *C.albicans* (22.2%), *C.glabrata* (20%), *C.dubliniensis* (14.4%), *C.krusei* (6.6%) and *C.tropicalis* (3.3%). Most of the Candida species were sensitive to Amphotericin- B, Ketoconazole, Clotrimazole and nystatin and were relatively resistant to Fluconazole and Itraconazole. In this study, there is an increase in infections with Candida non-albicans species and emergence of azole resistant *Candida albicans* and non albicans species specify the need of species identification and antifungal susceptibility. Antifungal susceptibility of Candida species prior to initiation of therapy is necessary to prevent the emergence and spread of drug resistance.

Introduction

Vaginitis is one of the common reasons for the gynaecology consultation among women of child bearing age group. Various studies have shown that 75% of healthy adult women will suffer with at least one episode of *Candida* infection during life time and 5% will have recurrent episodes [6,16,12]. Until recently the problem of vaginal candidiasis was often ignored or treated as an insignificant problem among female population[18]. In addition, many predisposing factors are associated with vaginitis such as reduced immunity, prolonged antibiotic therapy, use of contraceptives, pregnancy, diabetes, tissue transplant and use of immunosuppressive agents[5].

Numerous studies showed that *Candida albicans* is the common pathogen in 80-90% of cases but Candida non-albicans species are gaining importance as pathogens over the past few decades[10,18].

Non-albicans species most commonly encountered are *C. tropicalis*, *C. parapsilosis*, *C. glabrata* and *C. krusei*[(14)]. Accurate species identification is important for the treatment of *Candida* infections, as

the non-albicans species of *Candida* continue to be increasingly documented.

Of the different conditions caused by *Candida* in genital areas, Vulvo Vaginal Candidiasis [VVC] is the most common[1,3]. Diagnosis helps in preventing the patient to progress to chronic and complicated VVC. Antifungal susceptibility testing helps to prevent chronic VVC by right drug administration. The present study was conducted to determine the various species of *Candida* causing VVC and to highlight the importance of *Candida non-albicans* as a causative agent.

The main objectives of this study to isolate and speciate *Candida* in patients with Vulvo Vaginal Candidiasis. And also to determine their antifungal susceptibility pattern by disk diffusion method.

Materials and Method

This study was carried out in the Department of Microbiology, Andhra Medical College, Visakhapatnam during January to July 2015. The study group consists of 100 women with complaints of excessive vaginal discharge and pruritus, attending Gynaecology Out-Patient Department(OPD), King George Hospital (KGH), Visakhapatnam.

Two high vaginal swabs were collected from each patient, one for direct microscopy, another for culture. Direct microscopic examination of KOH mount (Figure 1) and Gram stained smear (Figure 2) were done and examined for the presence of budding yeast cells and pseudohyphae.

Samples were inoculated on Sabouraud's Dextrose Agar (SDA) with gentamicin and were incubated at 25^0C and 37^0C for 48 – 72 hrs. Colonies appeared within 1-3 days as creamy white, smooth pasty with a yeasty odour (Figure 3). Standard mycological tests were used to identify the isolates [13]. *Candida* isolates were identified by microscopic examination of Gram stained smear (Figure 4) and germ tube formation. Rapid method of identifying *C. albicans* is by its ability to form germ tubes within two hours when incubated in human serum at 37^0C (Reynolds-Braude phenomenon)[7] was done (Figure 5).

Strains of *Candida* isolated were inoculated on Corn Meal agar (CMA) by Dalmau culture plate(Figure6) technique and were incubated at 25^0C and were observed for chlamydospore production (Figure 7,8).

For species identification, the isolates from SDA were inoculated on to *Candida* chromagar and incubated overnight at 37^0C in dark for 48 hours. Only pigmented colonies were considered for species identification (Figure 9,10).

C.albicans - Light green
C.parapsilosis - Cream to pale pink
C.tropicalis - Blue
C.glabrata - Pink to purple
C.krusei - Pink
C.dubliniensis - Dark green

Antifungal susceptibility testing was done by modified Kirby-Bauer disk diffusion method as per CLSI guidelines M44-A[21]. Inoculum was prepared from the yeast grown on SDA for 24 hours and was adjusted to match the turbidity of 0.5 Mc Farland standards. Sterile applicator swab was moistened in that cell suspension and inoculated on the surface of Mueller-Hinton agar supplemented with 2% glucose and 0.5µg/ml methylene blue.

Antifungal discs were placed and incubated in Biological Oxygen Demand (BOD) for 24

hrs and the zones of inhibition were observed(Figure.11).

Antifungal discs used were

Amphotericin-B - 20µg
Itraconazole - 10µg
Fluconazole - 10µg
Ketoconazole - 10µg
Clotrimazole - 10µg
Nystatin - 100units/disc
Standard zone size interpretation

For azoles is

- Susceptible – when ≥17mm diameter
- Intermediate – in between 14mm-16mm diameter
- Resistant - when ≤13mm diameter.

For Amphotericin B and Nystatin is

- Susceptible - when ≥15mm diameter
- Intermediate – in between 13mm-14mm diameter
- Resistant - when ≤12mm diameter

Results and Discussion

During the study period, total 100 women with complaints of excessive vaginal discharge with pruiritis were included in the study. The demographic data of the cases were noted and represented in table1

In study group, most of the patients were in the age group of 26-35 yrs (45%), followed by 36-45 yrs (25%), 18-25 yrs (18%), 46-55 yrs (8%) and above 56 yrs (4%) (Table 1). Out 100 cases, 25(27.7%) were diabeteic, 20 (22%) were pregnanat , 17(18%) patients have the history of prolonged antibiotic usage, 15(16%) patients were with IUCDs, and 11(12%) patients were on oral contraceptive pills (table 2).

Out of 100 samples, 90 (90%) were culture positive for *Candida*, 10 (10%) were negative for *Candida spp* (Table 3)

Among all *Candida* isolates, 20 (22.2%) were *Candida albicans* and 70 (77.7%) were *Candida non albicans*(Table 4).

The most common species isolated was *C.parapsilosis* (33.3%), followed by *C.albicans* (22.2%), *C.glabrata* (20%), *C.dubliniensis* (14.4%), *C.krusei* (6.6%) and *C.tropicalis* (3.3%) (Table 5).

Most of the *Candida* species were sensitive to Amphotericin- B, Ketoconazole, Clotrimazole and Nystatin with zones ≥13mm and were relatively resistant to Fluconazole and Itraconazole with zones ≤12mm(Table 6).

Candida species are usually component of normal flora of vagina. Changes in bacterial flora of vagina, acidity of vaginal fluid and hormonal variation are generally necessary for *Candida* to induce pathological changes associated with clinical symptoms.

Vulvovagional candidiasis(VVC) is one of the most common presenting complaints in child bearing age and sexually active period. The rise in VVC infection could be due to several factors like preganacy, prlonged use of antibiotics, oral contraceptive pills, antifungal drugs, HIV, diabetes mellitus, and STD.

In the present study, the common age group affected was 26-35 yrs, which corresponds to the fertility period, followed by 36-45 yrs. Similar findings were reported by Dharmik P.G et al 2012[4], and kandati jithendra et al 2015 [8].

In our study, major predisposing factor implicated in causation of VVC was diabetes(27.7%) followed by prgnancy (22%), prolonged usage of antibiotics,

IUCDS and usage of oral contraceptive pills which coincides with studies of Kandati jithendra et al 2015 [8] who reported diabetes as major predisposing factor and Velvizhi et al 2014[20], who reported pregnancy (25%) as second most common predisposing factor associated with VVC, Sobel et al 2007 [19] Okungbova et al 2003 [15] and Baris A et al 2011 [2] that high level of reproductive harmones and increase glycogen content of vagina favours candidiasis in pregnancy.

Table.1 Age Wise Distribution of Cases

Age In Years	Number	Percentage
18 – 25 yrs	18	18%
26 – 35 yrs	45	45%
36- 45 yrs	25	25%
46– 55 yrs	8	8%
>56yrs	4	4%
Total	100	100%

Table.2 Risk Factors Associated with VVC

Risk factors	Number (percentage)
Diabetes	25 (27.7%)
Pregnancy	20 (22%)
Antibiotic usage	17 (18%)
IUCDS	15 (16%)
Oral contraceptive pills	11 (12%)

Table.3 Cuture Positivity from Total No of Cases 100

Total no of cases	Culture positive for *Candida spp*: no (%)	Culture negative for *candida spp* : no (%)
100	90 (90%)	10 (10%)

Table.4 Distribution of *C albicans* and *non albicans* from Total Isolates No 90

C albicans (%)	*C. non albicans* (%)
20 (22.2%)	70 (77.7%)

Table 5 Speciation of Candida Isolates

Species	(Number percentage)
C.Parapsilosis	30 (33.3%)
C.albicans	20 (22.2%)
C.glabrata	18 (20%)
C dubilniensis	13 (14.4%)
C krusei	6 (6.6%)
C tropicalis	3 (3.3%)

Table.6 Antifungal Sensitivity Pattern of C albicans and C Non Albicans Species

Antifungal drugs	*C.albicans* (sensitivity %)	*C.non-albicans* (sensitivity%)
Amphotercin B	80	92
Itraconazole	56	62
Fluconazole	60	58
Ketotconazole	89	76
Clotrimazole	78	69
Nystatin	76	72

Figure.1 KOH Mount Showing Budding Yeast Cells, Pseudohyphae and Epithelial Cells

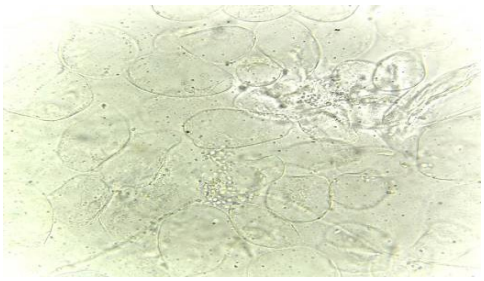


Figure.2 Direct Gram's Stained Smear Showing Gram Positive Budding Yeast Cells, Pseudohyphae and Epithelial Cells

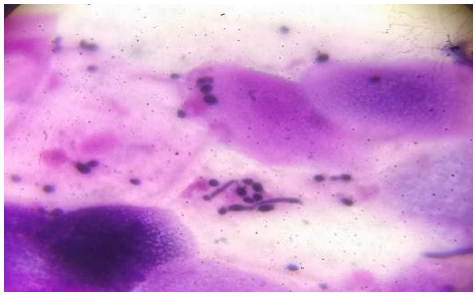


Figure.3 SDA Showing Smooth, Creamy White Pasty Colonies

Figure.4 Grams Stained Smear from Growth on SDA

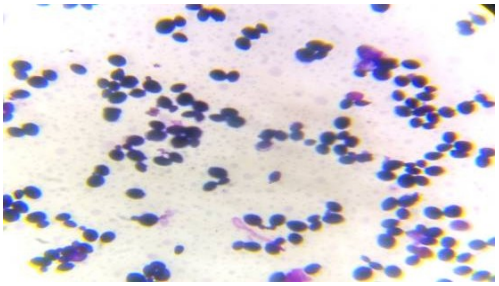


Figure.5 Germ Tube Test in *C.albicans* (Reynolds- Braude Phenomenon)

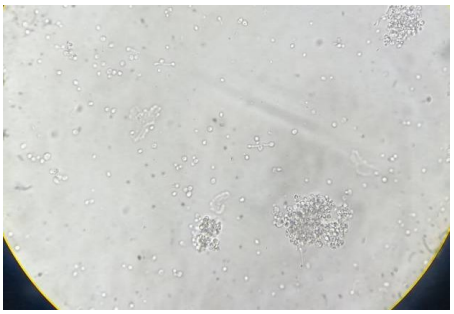


Figure.6 Dalmau Plate Culture for Chlamydospore Formation

Figure.7 Chlamydospoes of *C.Albicans*

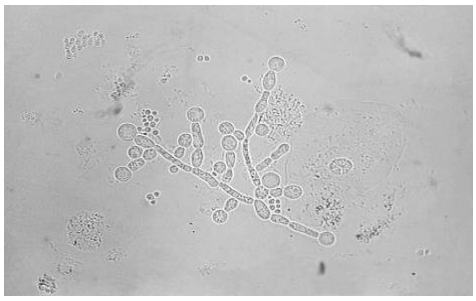


Figure.8 Chlamydospoes of *C.Parapsilosis*

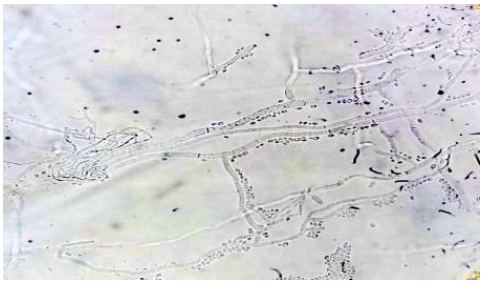


Figure.9, 10 *Candida* Species on Chrom Agar

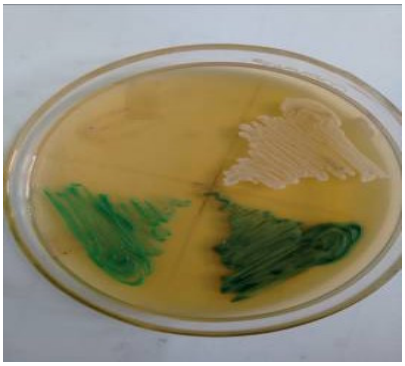
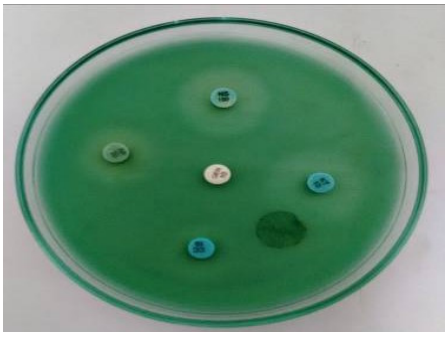


Figure.11 Antifungal Susceptibility Test for *Candida Species*



Among all *candida* isiolates in our study, increased in prevalence of *C nonalbicans* spp (77.7%) than *C. albicans* (22.2%) was noted, which coincides with studies of Velvezhi G et al in 2014 [20] and Sachin C Deorukhkar et al 2013 [17] and kandati jithendra et al 2015 [8]

Predominant isolate in our study was *C parapsilosis* (33.3%) followed by *C albicans* (22.2%), *C.glabrata*, *C.dubliniensis*, *C.Krusei*, *C.tropicalis* which coincides with study of Velvizhi G et al 2014 [20] who reported C parapsilosis as predominant pathogen where as Sachin C Deorukhkar et al 2013[17] reported *C.tropicalis* and *C. glabrata* as major pathogens but Latha Raghunadan et al 2014[11], Kandati Jithendra et al 2015[8] and Kauser Fatima et al 2014[9] reported *C albicans* as predominant isolate.

In this study most of the *Candida* species

were sensitive to Amphotericin- B, ketoconazole, Clotrimazole and Nyastatin and were relatively resistant to Fluconazole and Itraconazole which coincides with studies of kandati jithendra et al 2015 [8] and velvizhi G et al 2014 [20]

In conclusion, in this study, there is an increase in infections with *Candida non-albicans* species and emergence of azole resistant *Candida albicans* and *non albicans* species specify the need of species identification and antifungal susceptibility. Antifungal susceptibility of *Candida* species prior to initiation of therapy is necessary to prevent the emergence and spread of drug resistance.

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