ISOLATION AND SPECIES IDENTIFICATION OF CANDIDA ISOLATED FROM PATIENTS OF VULVOVAGINAL CANDIDIASIS IN A TERTIARY CARE HOSPITAL

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ABSTRACT

Introduction: An increase in the predisposing conditions in recent years has resulted in an increasing incidence of Candida infections. The accurate species identification of Candida is important for the treatment, as not all species respond to the same treatment and also because of the problem of anti-fungal resistance in certain species. Therefore, the species level identification of the Candida isolates, along with their anti-fungal susceptibility patterns can greatly influence the treatment options for the clinician.

Objective:
- To detect prevalence of Candida in patients with vaginal discharge.
- To identify species of Candida isolates.

Methods: This study included 410 women with abnormal vaginal discharge. Two high vaginal swabs were collected and sent immediately to the laboratory. All vaginal samples were stained with gram's stain & oval budding yeast cells were identified as Candida. Second swab was inoculated on Sabouraud's Dextrose agar and Candida isolates (n=122) were identified at species level by battery of various tests like GTT, Corn meal agar, Carbohydrate fermentation, Carbohydrate assimilation and CHROM agar inoculation.

Results: Out of 410 samples Candida were isolated in 122 samples. Amongst the 122 Candida isolates highest was C. albicans 66.39%, followed by C. glabrata 15.65%, C. tropicalis 9.85%, C. parapsilosis 4.91%, C. krusei 2.4% and least was C. guilliermondii 0.8%. In pregnancy and OCP users maximum isolates were of C. albicans (78.04% & 55.55%, respectively) were as in diabetic patients C. glabrata (47.05%) was predominant isolate, while in HIV positive patients all isolates were C. albicans (100%).

Conclusions: In our study the prevalence of VVC is 29.75%. Commonest species found was C. albicans 66.39%. VVC is more common in females with associated risk factors like pregnancy, diabetes, HIV and OCP users. Commonest species in pregnancy, OCP users and HIV patients was C. albicans while C. glabrata was more common in diabetic patients.

Key Words: Vulvovaginal candidiasis (VVC), Candida Spp., Germ tube test (GTT), CHROM agar

INTRODUCTION

Candida albicans, the most frequent cause of candidiasis, can account for up to 75% of the yeasts recovered from clinical specimens. Although the frequency of isolation of non-albicans Candida (NAC) species is increasing gradually, C. albicans is the most common cause of candidiasis. Other species of Candida such as C. glabrata, C. krusei and C. tropicalis are emerging as important opportunistic pathogens and this transition had a significant clinical impact due to decreased susceptibility of these non-albicans yeasts to antifungal agents. Identification to the species level of yeasts cultured from various clinical specimen is increasingly necessary for clinical laboratories. Generally yeast identification procedures start with a germ tube test in clinical laboratories. It is a rapid method to differentiate C. albicans and C. dublieniensis from other Candida species. Up to 5% of the strains of C. albicans may be germ tube negative, and false
Accurate species identification is important for the treatment of the \textit{Candida} infections, as the non-albicans species of \textit{Candida} continue to be increasingly documented and as not all the species respond to the same treatment.\cite{15,16} The increase in the predisposing conditions in recent years has resulted in a concurrent increase in the number of patients who suffer from candidiasis.\cite{15,16} Hence, this study was undertaken to identify species of Candida isolated from the clinical cases of VVC.

**MATERIAL & METHODS**

The present study was carried out in the Department of Microbiology, SMIMER medical college, Surat during the period of July 2010 to October 2011. Total 410 OPD patients who were attending department of Obstetrics & Gynaecology with complain of abnormal vaginal discharge were included in the study. A detailed history of patients was taken. All procedures were done under aseptic precautions and with standard protocol.

**1 Collection of sample**

The amount, colour, character and smell of vaginal discharge were noted. The discharge was then collected by two sterile swabs from upper part of the posterior fornix and lateral vaginal walls.

**2 Processing of Specimen**

First swab was used for preparation of smear for direct Gram’s staining.

Second swab was inoculated onto Sabouraud’s dextrose agar supplemented with 0.05g/L Chloramphenicol. Culture plate was incubated at 37°C for 48 hours, cultures were examined for pasty, creamy and smooth white colonies of yeasts. Colonies were confirmed by gram's stain examination for oval budding yeast cells & further species identification done by battery of test.

**3 Tests for Identification of Candida Species**

**A) Germ Tube Test (GTT)-**

The suspected Candida colonies were inoculated into 0.5 ml human serum in a small test tube. Reading were taken after 2-3 hrs of incubation at 37°C. A drop of suspension was examined on slide under microscope for germ tube production-true hyphal structure.

**B) Test for Chlamydospore formation –**

Pure culture of \textit{Candida} species was inoculated on Corn Meal agar & Dalmau plate culture was done. Inoculated plates were incubated at 30°C for 24-48 hrs. At the end of incubation, examined microscopically through the cover slip and observe for the presence of chlamydomspore.

**C) Carbohydrate Assimilation tests-**

Sugar Assimilation test was performed using following sugars.

Glucose, Sucrose, Maltose, Lactose, Cellobiose, Raffinose and Trehalose.

**Procedure-**

2-3 colonies were taken and suspension was prepared in normal saline. Lawn culture was done on Yeast Nitrogen Base (YNB, Sugar free medium). Carbohydrate discs were placed onto the surface of plate and incubated at 37°C. Results were read after 20-24 hours. In case of insufficient growth plates were further incubated till 48 hours. Utilization of particular carbohydrate was determined by presence of growth around the disk.

**D) Carbohydrate fermentation tests-**

Carbohydrate fermentation was performed for glucose, sucrose, maltose and lactose.

**Procedure-**

1-2 colonies of 1 mm diameter was inoculated into sugar fermentation tubes containing the appropriate sugars and Durham’s tubes. Inoculated tubes were incubated at 24°C for up to 1 week. Tubes were examined at 48 hours intervals for acid production (yellow colour) and gas formation (in Durham’s tubes). Production of gas indicates fermentation while only acid formation may indicate that the sugar has been assimilated. The reactions were read as A/G for each sugar separately.
E) CHROM agar Candida Differential Medium- 

Suspected Candida colonies from SDA agar was inoculated onto CHROMagar. The plates were incubated at 30°C for 48-72 hrs. The different Candida species shows following colours of colonies:

<table>
<thead>
<tr>
<th>Candida spp.</th>
<th>Color of Colonies</th>
<th>Candida spp.</th>
<th>Color of Colonies</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. albicans</td>
<td>Apple green colonies; consistent</td>
<td>C. krusei</td>
<td>Large, flat, spreading, pale pink with matt surfaces</td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>Dull blue, to purple color that diffused into surrounding agar with pale pink edges</td>
<td>C. parapsilosis</td>
<td>White to pale pink</td>
</tr>
<tr>
<td>C. glabrata</td>
<td>White large glossy pale pink to violet colonies</td>
<td>C. guilliermondii</td>
<td>Small pink to purple</td>
</tr>
</tbody>
</table>

Results: Total 122 Candida species were isolated out of 410 vaginal swab specimens.

Out of 122 isolates 78 were germ tube test positive while 44 isolates showed negative germ tube test.

Inoculation on CHROMagar shows following results:

C. albicans 81/122 (66.39%), C. glabrata 91/122 (74.96%), C. tropicalis 6/122 (4.91%), C. krusei 3/122 (2.40%), C. guilliermondii 1/122 (0.80%)

(Table-1, Graph-1, Fig-1). We have considered CHROM agar as gold standard test.

Inoculation on Corn meal agar had shown following results:


Carbohydrate fermentation test results:


Carbohydrate assimilation test had shown following results:

C. albicans 78/122, C. glabrata 17/122, C. tropicalis 11/122, C. parapsilosis 5/122, C. krusei 3/122, C. guilliermondii 1/122 (Table-1, Fig-2).

The species isolates amongst the pregnant patients were: C. albicans 78.04% (n=32), C. glabrata 9.70% (n=4), C. tropicalis 2.4% (n=1), C. parapsilosis 2.4% (n=1), C. krusei 7.3% (n=3), C. guilliermondii 0% (n=0) (Graph-2).

The species isolates amongst the OCP users were: C. albicans 23.52% (n=4), C. glabrata 47.05% (n=8), C. tropicalis 17.64% (n=3), C. parapsilosis 5.88% (n=1), C. krusei 0% (n=0), C. guilliermondii 5.88% (n=1) (Graph-2).

The species isolates amongst the diabetic patients were: C. albicans 55.55% (n=10), C. glabrata 5.50% (n=1), C. tropicalis 33.33% (n=6), C. parapsilosis 5.5% (n=1), C. krusei 0% (n=0), C. guilliermondii 0% (n=0) (Graph-2).

The species isolated from PLWH patients were all C. albicans 100% (n=7) (Graph-2).

**DISCUSSION**

Despite therapeutic advances, vulvovaginal Candidiasis remains a common problem worldwide, affecting all strata of society. Candidal infections are more dynamic than other diseases prevailing in the community. Their epidemiological profile varies from country to country and from one region to another within a country depending upon demographic, socio-economic and health factors.

There is always a need of a medium which helps not only in the isolation but also in the identification of the agent at the species level. CHROMagar is a differential medium being widely used to differentiate Candida species. It facilitates the detection and identification of yeasts from mixed cultures and can provide results in 24 to 48 hrs sooner than standard isolation and identification procedures. It is superior to SDA in terms of suppressing the bacterial growth. A major advantage of CHROMagar is the ability to detect mixed cultures of yeasts in clinical specimens. Although CHROMagar appears to be quite accurate in identifying the most common Candida species, it is not proposed as a substitute for standard identification protocols.

Sensitivity of GTT in our study is 96.2% which is quite comparable with J.E. Hoppe et al (98.9%) and Arthur E. Crist et al (94.7%).

As shown in table 2 Candida albicans was the major pathogen causing VVC in various studies. Candida albicans produces protease, phosphates and carbohydrates which enhance its attachment to human epithelium. So, C. albicans is more adhesive than other non-C. albicans species (This could be considered as one of the likely reasons that this species are predominant rather than non-C. albicans species).

In present study the incidence of C. albicans was 66.39% which is quite comparable with the studies of K. kikani et al (66%) Vijaya et al (66%), Latha Ragunathan et al (65%), K
Dota et al (65%)\textsuperscript{13} and Emam et al\textsuperscript{8} (68.9%) (Table-2).

As per Table 2 the incidence of non-albicans species is very similar to findings of Ritcher et al\textsuperscript{8}, Latthi Raghunathan et al\textsuperscript{8} and Emam et al.\textsuperscript{8} C. glabrata found second common spp. after C. albicans in most of the studies.

In this study the incidence of C. glabrata is 47.1% in diabetic patients while it was 39% in Goswami et al\textsuperscript{15} and 61.3% in Ray et al\textsuperscript{4} in diabetic patients (Table-3).

Incidence of vaginal candidiasis is remarkably higher during pregnancy due to physiological changes.\textsuperscript{19} Various study has reported incidence of symptomatic vaginal candidiasis high in pregnancy and increases during the course of gestation.\textsuperscript{18} C. albicans was most frequently isolated also in pregnancy 78.04% in this study. Ovaisogie F.E et al\textsuperscript{8} has reported 61.5% and Dias L.B et al\textsuperscript{17} has reported 92.3% C. albicans in pregnant patients (Table-3).

**CONCLUSION**

In our study most common species was *Candida albicans* in cases of VVC. *Candida glabrata* was the most common species in diabetic patients and *Candida albicans* was the most common in HIV.

In India there is an increase in prevalence of non-albicans *Candida* spp. due to increase in immunocompromised patients. This also specifies the need of species identification and antifungal susceptibility as a part of the laboratory diagnosis of vaginal candidiasis.

CHROM agar is very good medium for species identification of Candida isolates, which give result in short time compared to other methods of species differentiation. This will ultimately help clinician to choose appropriate antifungal in cases of VVC.

**Abbreviations:**

VVC - Vulvovaginal Candidiasis
SDA - Sabouraud’s Dextrose agar
NAC - Non albicans Candida
DM - Diabetes Mellitus
HIV - Human Immunodeficiency Virus
PLWH - Patient Living With HIV
AG - acid, gas production
GTT - Germ Tube Test
CHROM Agar - Chromogenic agar
OCP - Oral Contraceptive Pill

**REFERENCES**


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We acknowledge the immense help received from the scholars whose articles are cited and included in references of this manuscript. We are also grateful to authors/editors/publishers of all those articles, journals and books from where the literature for this article has been reviewed and discussed.
Table 1: Results of Various Methods for species identification

<table>
<thead>
<tr>
<th>Various Species</th>
<th>Chrome agar no.(%)</th>
<th>Corn Meal agar no. (%)</th>
<th>Not identified by Corn meal agar no. (%)</th>
<th>Carbohydrate Fermentation no. (%)</th>
<th>Not identified by Fermentation no. (%)</th>
<th>Carbohydrate Assimilation no. (%)</th>
<th>Not identified by Assimilation no. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C.albicans</td>
<td>81 (100%)</td>
<td>81 (100%)</td>
<td>-</td>
<td>77 (95.06%)</td>
<td>04 (4.94%)</td>
<td>78 (96.23%)</td>
<td>03 (3.77%)</td>
</tr>
<tr>
<td>C.glabrata</td>
<td>19 (100%)</td>
<td>17 (89.47%)</td>
<td>02 (10.53%)</td>
<td>17 (89.47%)</td>
<td>02 (10.53%)</td>
<td>17 (89.47%)</td>
<td>02 (10.53%)</td>
</tr>
<tr>
<td>C.tropicalis</td>
<td>12 (100%)</td>
<td>11 (91.66%)</td>
<td>01 (8.34%)</td>
<td>10 (83.33%)</td>
<td>02 (16.67%)</td>
<td>11 (91.66%)</td>
<td>01 (8.34%)</td>
</tr>
<tr>
<td>C.parapsilosis</td>
<td>6 (100%)</td>
<td>6 (100%)</td>
<td>-</td>
<td>5 (83.33%)</td>
<td>01 (16.67%)</td>
<td>5 (83.33%)</td>
<td>01 (16.67%)</td>
</tr>
<tr>
<td>C.krusei</td>
<td>3 (100%)</td>
<td>3 (100%)</td>
<td>-</td>
<td>3 (100%)</td>
<td>-</td>
<td>3 (100%)</td>
<td>-</td>
</tr>
<tr>
<td>C.guilliermondii</td>
<td>1 (100%)</td>
<td>1 (100%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1 (100%)</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>122 (100%)</td>
<td>119 (97.54%)</td>
<td>03 (2.46%)</td>
<td>112 (91.80%)</td>
<td>09 (8.2%)</td>
<td>115 (94.26%)</td>
<td>07 (5.74%)</td>
</tr>
</tbody>
</table>
**Table 2: Comparison of various previous studies on Candida**

<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>C. albicans 81</td>
<td>66.39</td>
<td>66</td>
<td>70</td>
<td>65</td>
<td>68.9</td>
<td>66</td>
<td>62</td>
<td>69.6</td>
<td>65</td>
</tr>
<tr>
<td>C. glabrata 19</td>
<td>15.65</td>
<td>14</td>
<td>18</td>
<td>22.5</td>
<td>13.4</td>
<td>1.9</td>
<td>26</td>
<td>8.7</td>
<td>-</td>
</tr>
<tr>
<td>C. tropicalis 12</td>
<td>9.85</td>
<td>11.2</td>
<td>8</td>
<td>7.5</td>
<td>8.9</td>
<td>26.4</td>
<td>9.1</td>
<td>6.55</td>
<td>-</td>
</tr>
<tr>
<td>C. parapsilosis 6</td>
<td>4.91</td>
<td>4.9</td>
<td>5</td>
<td>5</td>
<td>4.4</td>
<td>1.9</td>
<td>2.6</td>
<td>4.3</td>
<td>-</td>
</tr>
<tr>
<td>C. krusei 3</td>
<td>2.40</td>
<td>2.3</td>
<td>12</td>
<td>-</td>
<td>-</td>
<td>3.8</td>
<td>-</td>
<td>6.55</td>
<td>-</td>
</tr>
<tr>
<td>C. guilliermondii 1</td>
<td>0.80</td>
<td>1.3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>8.7</td>
<td>-</td>
</tr>
</tbody>
</table>

**Table 3: Percentage wise comparison of Candida in patients with predisposing factors**

<table>
<thead>
<tr>
<th>Candida Species</th>
<th>Diabetes mellitus</th>
<th>Pregnancy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Present study</td>
<td>Goswami et al</td>
</tr>
<tr>
<td>C. albicans</td>
<td>23.5</td>
<td>26</td>
</tr>
<tr>
<td>C. glabrata</td>
<td>47.1</td>
<td>39</td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>17.6</td>
<td>17</td>
</tr>
<tr>
<td>C. parapsilosis</td>
<td>5.88</td>
<td>-</td>
</tr>
<tr>
<td>C. krusei</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>C. guilliermondii</td>
<td>5.88</td>
<td>-</td>
</tr>
</tbody>
</table>

**Graph 1: Species distribution of Candida in vaginal discharge**
**Figure 1:** CHROM Agar indicating different colors of various species

**Graph 2:** % wise Species distribution of Candida in Risk factors

**Figure 2:** Cornmeal Agar Morphology of Different Species
A: *Candida albicans*: pseudohyphae with terminal chlamydo- spores; irregular or spherical clusters of blastospores at septa
B: *Candida tropicalis*: Long filamentous hyphae, blastospores borne singly along the hyphae, sometimes with small chlamydoconidia.