Candida species are opportunistic yeasts affecting the genitourinary tracts. A one year study was carried out at a multi-speciality medical college hospital to understand the frequency of vaginal candidiasis and to determine the susceptibility profile of the Candida species to antifungal agents. A total of 250 High vaginal swabs were cultured out of which 121 (48.4%) were positive for Candida species. The study showed a higher incidence of vaginal candidiasis. Pregnancy has been the most frequently implicated risk factor (29.75%). C. albicans (35.5%) was the predominant species isolated followed by non albicans species like C. tropicalis (26.4%), C. glabrata (20.6%), C. krusei (15.7%), and C. dubliniensis (1.6%). Susceptibility tests revealed that resistance to amphotericin B and flucytosine is infrequent in the isolates of Candida albicans. The overall susceptibility rate for fluconazole, for C. albicans were 76%, and resistance were 16.27% followed by itraconazole resistance (13.95) and voriconazole 9.30% respectively. In the azole groups, highest resistance is observed against fluconazole (53.84%) followed by itraconazole (32%), voriconazole (21.79%), fluconazole (26.92%) and amphotericin (12.8%) among the non albicans isolates. Epidemiological profile of genitourinary candidiasis varies globally depending upon socioeconomic and health factors. In our setting we found an increase in non albicans Candida infections and the emergence of azole resistant C. albicans and non albicans species in vaginal candidiasis cases.

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Keywords- Vulvovaginal Candidiasis, Opportunistic Fungal Infections, Candida Species, Antifungal Susceptibility Testing.

1.1 Introduction
Vaginal candidiasis is one of the most common vaginal infections in women, in the fertile period, and also the most frequent and most important fungal disease. Until recently, the problem of vaginal candidiasis was often ignored or treated as an insignificant problem among the female population. [1]. In addition, many psychological and emotional stress related problems are associated with vaginitis such as; reduced immunity, prolonged antibiotic therapy, use of contraceptives, malnutrition, pregnancy, diabetes, obesity, tissue transplant, use of immunosuppressive agents, neutropenia etc. [2]. Presence of indwelling central venous catheters and prior haemodialysis has also been identified as a risk factor. Sexual intercourse with an infected person is also a mode of spread of genital candidiasis [3]. There is a balance between Candida, normal bacterial flora, and immune defence mechanisms. When this balance is disturbed, colonization is replaced by infection. It is possible that there are multiple mechanisms by which Candida can cause cell damage and lead to direct invasion of hyphae in epithelial tissues. During vaginal candidiasis, vagina is in the normal pH range (pH 4-4.5), as opposed to mixed infections (bacterial, Trichomonas), where pH levels rises [4].

Numerous studies around the world showed that Candida albicans is responsible for the largest number of symptomatic episodes of vaginal candidiasis. Percentage of non-albicans was high in the recent decades and varied from 85 to 90%. Non-albicans species are most commonly represented by C. tropicalis, C. glabrata, and C. krusei [5]. Accurate species identification is important for the
treatment of the Candida infections, as the non-albicans species of Candida continue to be increasingly documented. Hence, the objective of the study was to document the frequency of vulvovaginal candidiasis, speciation of the isolates of candida and determine the susceptibility profile of the Candida species to antifungal agent’s amphotericin B, fluconazole, itraconazole, voriconazole and fluycytosine. 

1.2 Materials and Methods

The study was carried out as part of my PhD thesis with permission from the Institutional Ethical Committee (IEC) for a period of one year from August, 2011 to August, 2012 at the multi-specialty Trivancore Medical College Hospital, Kerala.

1.2.1 Inclusion Criteria and sample size

Married, pregnant and sexually active women between 18-45yrs of age who presented with self-reported symptoms of vaginal discharge, genital burning or burning during micturition during the study period were included. All the standard operating procedures were followed and total 250 High vaginal swabs (HVS) are collected from patients attending with genitourinary symptoms with their consent. Out of which 121 were positive for Candida species from women clinically diagnosed of vaginal candidiasis. The women were grouped according to their age into 3 categories; (18 – 25), (26 – 35), (36 - 45) groups.

1.2.2 Exclusion criteria

Women of age group below 18 and above 45 years, catheterised patients, patients with cervical malignancies, women in menopause are not included in the study. Vaginal swabs showing the evidence of bacterial and protozoal infections are also excluded.

1.2.3 Collection of High Vaginal Swabs

High vaginal swabs were collected by using speculum insert into the vagina to separate the vaginal walls. Wipe away the excess cervical mucus with a tissue. Using a sterile long(22cm) rigid cotton swab inserted carefully into the uterine cervix rotated gently and put into the tube, then properly labelled and transported to the laboratory.[6]

1.2.4 Processing of High Vaginal Swabs

The swabs were inoculated on Sabouraud’s dextrose agar supplemented with 0.06 mg/ml Gentamicin, without Cyclohexamide (SDA) (Hi-Media Laboratories, Mumbai, India) and incubated at 37 °C and room temperature. The smear was stained by Gram’s Method for microscopic examination. 

1.2.5 Species identification procedure

Samples were inoculated on SDA without Cyclohexamide and incubated at 37°C. Plates were examined after 48 hours incubation. Candida species were identified based on colony morphology, germ tube testing, chlamydospores on corn meal agar, pigmenations on the CHROM agar, and carbohydrate assimilation tests using dextrose, maltose, sucrose, lactose, raffinose, mellibiose, xylose, and trehalose. [6,7]

1.2.6 Antifungal Susceptibility testing

All the isolates were subjected for antifungal susceptibility testing for amphotericin B, fluconazole, itraconazole, voriconazole, fluycytosine by disc diffusion method with M44-A Clinical Laboratory Standards(CLSI) guidelines[8].

1.2.7 Disk diffusion method

Mueller Hinton Agar (MHA) supplemented with 2% glucose and 0.5 µg/ ml methylene blue dye was used. The agar surface was inoculated by using a swab dipped in a Candida cell suspension adjusted to the turbidity of a 0.5 McFarland standard (10⁶ cells/ml). Antifungal discs were dispensed on agar surface and the plates were incubated aerobically at 35°C and read at 24 h. Zone diameter endpoints were read manually with callipers and interpreted according to standard sizes. The interpretive categories include susceptible (S), resistant (R) and susceptible dose dependent (SDD). By maintaining blood levels with higher doses of antifungal, an isolate with an SDD endpoint may be successfully treated with a given azole [8].

1.3 Results

The present study included 250 female patients who presented with genital manifestations, suspicious of candidial infection. The study revealed an overall isolation rate of 48.4% (121/250) candidiasis from high vaginal swabs. All the 121 positive isolates of Candida species were processed for speciation and Antifungal sensitivity testing. Age wise distribution showed 49.58% of pr

### Table 1. Age wise distribution of patients from which Candida species isolated

<table>
<thead>
<tr>
<th>Age wise</th>
<th>C. albicans</th>
<th>C. tropicalis</th>
<th>C. glabrata</th>
<th>C. krusei</th>
<th>C. dubliensis</th>
<th>Percentage age wise</th>
</tr>
</thead>
<tbody>
<tr>
<td>18-25</td>
<td>13</td>
<td>11</td>
<td>10</td>
<td>9</td>
<td>0</td>
<td>35.53</td>
</tr>
<tr>
<td>26-35</td>
<td>23</td>
<td>16</td>
<td>11</td>
<td>8</td>
<td>2</td>
<td>49.58</td>
</tr>
<tr>
<td>36-45</td>
<td>7</td>
<td>5</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td>14.87</td>
</tr>
<tr>
<td>Total</td>
<td>43</td>
<td>32</td>
<td>25</td>
<td>19</td>
<td>2</td>
<td>48.4</td>
</tr>
</tbody>
</table>

| Total Percentage | 35.5 | 26.4 | 20.6 | 15.7 | 1.6 |

We analyzed the predisposing factors of vaginal candidiasis from positive isolates, pregnancy has been the most frequently associated risk factor (29.75%) followed by use of antibiotics (19.83%),Intrauterine devices(14%)(Table 2) Antifungal susceptibility testing was performed by disk diffusion method (GM-MHA) CLSI M-44 A) and it showed C. albicans were 100% susceptible to amphotericin B and fluycytosine. Whereas 85% of Candida albicans
The highest frequency of findings show factor associated with vaginal candidiasis in our study. Pregnancy (29.75%) was the most common predisposing factor. Urinary bladder catheterization (9.9%) and stay in ICU (7.4%) were the next most common factors. Immunosuppression (10.7%), Diabetic mellitus (8.26%), and History of antibiotics (6.97%) were also significant. Lower frequency results (14.87%) were obtained in the age group of (26-35) years, followed by age group of (18-25). And the reports of Sehgal [15] and Srujana et al [13] also showed the age group of above 40 years as second most frequent risk factor in our study. Antibiotic usage (19.83%) was the second most frequent risk factor in our study. Antibiotic usage will cause the elimination and alteration of normal bacterial flora and allows the colonization of Candida. The other major risk factor was the use of Intrauterine devices, (14%) of patients had IUDs in our study. So pregnancy, use of chemotherapeutic agents and IUDs had been identified as risk factors associated with vaginal candidiasis in our study. These risk factors associated in our study correlated well with the studies of Mirela et al [18] and Lundstrom et al [19]. Numerous worldwide studies showed that Candida albicans are responsible for the greatest number of symptoms associated with the vaginal candidiasis. Our findings have also showed 48.7% (59) of C. albicans was identified from 121 positive cases. Similar findings are reported from the studies of Mahmoudi et al [5], Uma et al [20], S.R. Fan et al [21], Ahamed et al [22].

Our study showed the increase in frequency of non-albicans species as potential causes of vaginal candidiasis. It is important to emphasize that in the past three decades there has been an increasing percentage of infections caused by non-albicans species of Candida, particularly, C. tropicalis, C. glabrata, and C. krusei and it is resistant to conventional therapy. C. glabrata was the most common species among the vaginal isolates (56, 50.4%) followed by C. albicans (39, 35.1%) in the study of Srujana et al [13]. The overall prevalence of non albicans species in our study.

### Table 2. Predisposing factors for Vaginal Candidiasis

<table>
<thead>
<tr>
<th>Predisposing factors</th>
<th>Number of patients</th>
<th>Percentage of isolation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnancy</td>
<td>36</td>
<td>29.75</td>
</tr>
<tr>
<td>History of antibiotics</td>
<td>24</td>
<td>19.83</td>
</tr>
<tr>
<td>Intrauterine devices</td>
<td>17</td>
<td>14.00</td>
</tr>
<tr>
<td>Immunosuppression</td>
<td>13</td>
<td>10.7</td>
</tr>
<tr>
<td>Urinary catheters</td>
<td>12</td>
<td>09.9</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>10</td>
<td>08.26</td>
</tr>
<tr>
<td>Intensive care unit</td>
<td>9</td>
<td>07.4</td>
</tr>
</tbody>
</table>

### Table 3. Antifungal susceptibility pattern by disc diffusion method

<table>
<thead>
<tr>
<th>Antifungals</th>
<th>No (n)</th>
<th>Amphotericin B</th>
<th>Fluconazole</th>
<th>Itraconazole</th>
<th>Voriconazole</th>
<th>Flucytosine</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>C. albicans</strong></td>
<td>43</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S%</td>
<td>SDD %</td>
<td>R%</td>
<td>S%</td>
<td>SDD %</td>
<td>R%</td>
<td>S%</td>
</tr>
<tr>
<td>100</td>
<td>0</td>
<td>0</td>
<td>76</td>
<td>6.97</td>
<td>16.27</td>
<td>79</td>
</tr>
<tr>
<td><strong>C. tropicalis</strong></td>
<td>32</td>
<td>87.5</td>
<td>0</td>
<td>12.5</td>
<td>62</td>
<td>12.5</td>
</tr>
<tr>
<td><strong>C. glabrata</strong></td>
<td>25</td>
<td>80</td>
<td>8</td>
<td>12</td>
<td>32</td>
<td>8</td>
</tr>
<tr>
<td><strong>C. krusei</strong></td>
<td>19</td>
<td>91.8</td>
<td>5.2</td>
<td>15.78</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td><strong>C. dubliniensis</strong></td>
<td>02</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>


Antifungal disc | Susceptible Zone (mm) | Susceptible dose dependent Zone (mm) | Resistant Zone (mm)
--- | --- | --- | ---
Amphotericin-B | >15 | 10-14 | <10
Flucytosone | >19 | 15-18 | <15
Itraconazole | 14 | 12-13 | <13
Voriconazole | 17 | 16-14 | <13
Flucytosine | >20 | 12-19 | <19

were sensitive to flucytosine, 91% of the isolates were sensitive to voriconazole. The highest resistance of 40% was seen in case of fluconazole (Table 3).

### 1.4 Discussion

Vaginal candidiasis is an extremely common infection in 60-70% women during their reproductive age at least once in their lives [9, 10]. The present study had revealed the highest incidence rate of candidiasis 48.4% (121/250), which is similar to the study of Akortha et al [11] Saldanha et al [12] and Srujana et al [13]. The highest frequency of (49.58%) vaginal candidiasis was observed in the age group of (26-35) years, followed by age group of (18-25). Lower frequency results (14.87%) were obtained in the age group of above 40 years in our study.

The study of Ako et al [14] supports that vaginal candidiasis occurs most frequently in the age group (20-25). And the reports of Sehgal [15] also showed the age group 21-30 years had the highest incidence of vaginal candidiasis. So women of childbearing age groups are more vulnerable to vaginal candidiasis.

We analyzed the predisposing factors of vaginal candidiasis from positive candida isolates, pregnancy has been the most frequently associated risk factor (29.75%) followed by use of antibiotics (19.83%), Intrauterine devices (14%), Immunosuppression (10.7%), Diabetic mellitus (8.26%), Urinary bladder catheterization (9.9%) and stay in ICU (7.4%) Pregnancy (29.75%) was the most common predisposing factor associated with vaginal candidiasis in our study. Our findings show agreement with studies of Sobel [9] and Okungbova et al [16], Ahmet et al [17] that high level of reproductive hormones and increase glycogen content of vagina favours candidiasis in pregnancy.

History of antibiotics, corticosteroids usage (19.83%) was the second most frequent risk factor in our study. Antibiotic usage will cause the elimination and alteration of normal bacterial flora and allows the colonization of Candida. The other major risk factor was the use of Intrauterine devices, (14%) of patients had IUDs in our study. So pregnancy, use of chemotherapeutic agents and IUDs had been identified as risk factors associated with vaginal candidiasis in our study. These risk factors associated in our study correlated well with the studies of Mirela et al [18] and Lundstrom et al [19]. Numerous worldwide studies showed that Candida albicans are responsible for the greatest number of symptoms associated with the vaginal candidiasis. Our findings have also showed 48.7% (59) of C. albicans was identified from 121 positive cases. Similar findings are reported from the studies of Mahmoudi et al [5], Uma et al [20], S.R. Fan et al [21], Ahamed et al [22]. Our study showed the increase in frequency of non-albicans species as potential causes of vaginal candidiasis. It is important to emphasize that in the past three decades there has been an increasing percentage of infections caused by non-albicans species of Candida, particularly, C. tropicalis, C. glabrata, and C. krusei and it is resistant to conventional therapy. C. glabrata was the most common species among the vaginal isolates (56, 50.4%) followed by C. albicans (39, 35.1%) in the study of Srujana et al [13]. The overall prevalence of non albicans species in our study.
was (64.4%) which is more than that of \textit{C albicans} (35.5%). Most frequently isolated non albicans in our study have been \textit{C. tropicalis} (26.4%), \textit{C. glabrata} (20.6%), \textit{C. krusei} (15.7%) and \textit{C. dubliniensis} (1.6%). Kauffman [23] reported \textit{C. albicans} was present in 51.8% and \textit{C. glabrata} was present in 15.6% of the patients with candidiasis, in their study performed in 751 patients. Reports from other workers like Saldanha et al [12], Mirela et al [17], Corsello et al [24], and Bankar et al [25] showed similar observations.

We speculate this increasing detection of non-albicans species are probably related to the widespread and inappropriate use of antifungal therapies (self medication, topical use, long-term treatments and repeated candidial episodes). Hence, the reliable and rapid identification method of \textit{Candida} species is a fundamental goal of microbiology laboratories.

In vitro antifungal susceptibility testing is becoming important because of the emergence of new non albicans strains and the increased inherent and acquired resistance to azoles and amphotericin-B. So agar-based antifungal susceptibility testing is an attractive alternative to the microdilution method. It is easy to perform and inexpensive for routine laboratories. CLSI M44-A disc diffusion testing with glucose methylene blue Muller Hinton agar is a very convenient method for antifungal susceptibility testing [26].

Antifungal susceptibility testing in our study revealed that none of the \textit{Candida albicans} isolates tested were resistant to amphotericin-B and fluucytosine. For fluconazole, the overall susceptibility rate for \textit{C. albicans} were 76%, susceptible dose dependent were 6.9% and resistance were detected in 16.27% followed by itraconazole (13.95) and voriconazole resistance were 9.30% respectively. 12.5% of \textit{C.tropicalis} isolates were resistant to amphotericin B, fluconazole resistance was 25%, followed by itraconazole (21.87%) voriconazole and fluucytosine (18.75%). \textit{C.glabrata} isolates showed (12%) resistance to amphotericin B, (48%) resistance to fluconazole, (40%) resistance to itraconazole, (28%) to voriconazole and 6.25% resistance to fluucytosine. \textit{C.krusei} isolates were (15.78%) resistance to amphotericin B, 15.78% resistance to fluconazole and (42%) resistant to itraconazole and voriconazole (21%). Our findings correlate with the study of Saldanha et al [12] and Noake et al[27] which showed \textit{Candida} species are more susceptible to amphotericin-B (92%) and fluucytosine (88%) and 60% resistance to azoles.\textit{(Table 3)}

Most non-albicans \textit{Candida} species in our study had higher azole resistance and infections they cause are often difficult to treat. \textit{C. glabrata} showed higher resistance to fluconazole and itraconazole in our study. A similar study by Sobel [9] showed higher resistance. In \textit{C. krusei}, this is intrinsically resistant to azoles and was not tested in our study [28]. One of the possible explanations for more frequent isolation of non-albicans species from vulvovaginitis patients may be the increased use of topical azole agents. So the present study emphasizes the need for testing the antifungal susceptibility tests for the \textit{Candida} isolates to control the spread of new resistant strains in the future.

\textbf{1.5. Conclusions}

Vaginal candidiasis is predominant during pregnancy. The epidemiological profile of vaginal candidiasis varies from country to country as well as within the country depending upon so many factors like socioeconomic and health factors. Whereas in India there is an increase in infections with non-albicans \textit{Candida} spp and the emergence of azole resistant \textit{C. albicans} and non albicans species also specify the need of species identification and antifungal susceptibility as a part of the laboratory diagnosis of vaginal candidiasis. The emergence of fluconazole resistance in non albicans \textit{Candida} may caution against its use as a prophylactic agent in hospitals. However, \textit{C. krusei} exhibit intrinsic resistance to fluconazole. Effective antifungal treatment is an important criterion in treating the candidial infections. Therefore, screening programme is essential to monitor the antimicrobial resistance. The epidemiological status of candidiasis plays an important role in the control strategies. This study also provides the baseline information on the prevalence and antifungal susceptibility pattern of \textit{Candida} isolates in our region.

\textbf{1.5 References}


Source of support: Nil; Conflict of interest: None declared