The present study was based on the epidemiological picture of Candida albicans and non-albicans Candida encountered in different systemic and mucosal infections including HIV/AIDS in North East India. The introduction of chemotherapeutic and antibiotic agents as well as appearance of HIV infection and several other factors like diabetes, old age, etc., has led to emergence of several opportunistic pathogens and Candida species, probably the most important pathogen causing majority of infection. Candida species isolated from different clinical samples including patients with HIV/AIDS were subjected to species level identification using standard yeast identification protocol. Antifungal sensitivity test was done by Kirby-Bauer disc diffusion method. Out of 113 Candida species, 72.56% non-albicans Candida and 27.43% C. albicans were isolated. In this study, among non-albicans Candida, C. glabrata was 32% followed by C. tropicalis 30% which were isolated. Non-albicans Candida was found to be significant over C. albicans (P = 0.086) at ten percent level of significance. The present study support the need of species level identification and periodic surveillance of the antifungal susceptibility as it would provide selection of appropriate antifungal drug.

INTRODUCTION

Candida is one of the frequently encountered fungal opportunistic pathogen and associated with vast spectrum of human infection. Candida colonizes healthy intact skin. There is an increasing drift of disease by non-albicans Candida (NAC) (Kothavade et al., 2010). NAC species are currently the significant pathogens most frequently recovered from adults and children in tertiary care medical centers (Pappas et al., 2003; Narain 2003) and there is numerous reports on increase of Candida infection from India as well (Basu et al., 2003). The genus Candida is a heterogeneous group and many these species are found to be associated with human infection other than Candida albicans. Candida shows unique morphological forms like budding and blastoconidia which grow pseudohyphal form to true hyphae. A study by Capoor et al. (2005) showed that the combination of suppressed host defense and exposure of multiple risk factors are responsible for the Candida infection (Narain, 2003; Hachem et al., 2008). Fluconazole is the antifungal agent which is most commonly used for prophylaxis as it can be orally administered and is comparatively cheaper than other antifungal agents. Fluconazole is the drug of choice whereas amphotericinB is given intra-venous in critical patients. Early empirical (treatment which is based on symptoms and clinical experience rather than on a thorough knowledge of the cause of the disorder) therapy
MATERIALS AND METHODS

The samples of the present study were collected from the suspected patients of candidiasis of various age groups attending different departments of the government hospital, Silchar, Assam between 2008-2010. The samples were collected with due permission from concerned authorities. Among 500 clinically suspected cases of indoor and as well as outdoor patients from different wards (ICUs, medicine, surgery, paediatrics, obstetrics and gynaecology), a total of 113 Candida species were isolated from different clinical specimens and 20 oropharyngeal swabs were taken from HIV/AIDS patients, in which twelve show Candida growth in microscopy as well as in culture. Details of the patients were recorded. These 113 cases where Candida species were isolated as a significant cause of infection were found to be associated with many risk factors like prolonged hospital stay, long term antibiotic therapy, pregnancy, premature delivery, old age, long term use of indwelling catheter, underlying diseases like diabetes and malignancy. Distribution of positive samples among different age groups and sex is shown in Table 3. Samples were cultured on Sabouraud’s Dextrose Agar with chloramphenicol (SDAc) and incubated at 37 and 25°C (Chander, 2009). These were further identified to species level. For all strains, Gram stain, lactophenol cotton blue mount were done, germ tube test and Dalmu plate culture for Chlamydomospor was performed. Germ tube test was carried out with 0.5 ml of pooled human serum and for chlamydomospor formation, corn meal agar containing 1% Tween 80 was used. Dalmu plate cultures were studied after 3 to 5 days of incubation under low power objective (10x) first and then high power objective (40x). Final confirmations of strains were done by sugar assimilation and sugar fermentation test. Antifungal susceptibility was done by Kirby–Bauer disc diffusion method on Mueller-Hinton agar supplemented with 2% glucose and 0.5 µg/ml methylene blue dye (Kamat et al., 2009) and incubated at 35°C for 48 h. Zone of inhibition was read after 24 h of incubation. Readymade antifungal disc (HiMedia) of amphotericinB (20 mcg), fluconazole (10 mcg), itriconazole (10 m cg) and voriconazole (1 mcg) were used.

Statistical analysis

The results were analyzed using simple statistical test. The significance of the results obtained was statistically evaluated using appropriate tests, that is, Chi-square, paired t- test and correlation test.

RESULTS

From different clinical samples, 113 Candida sp. were isolated (Table 1). All the 113 Candida sp. were found with microscopy and culture positive on both blood agar and SDA for Candida and were only considered and subjected to the tests for further identification. The distribution of C. albicans and NAC is shown in (Figure 1). Here, in this study, among NAC, Candida glabrata was 32% followed by Candida tropicalis (30%) which was isolated and the other non-albicans Candida species are summarized in Table 2. It is also seen that NAC is in

<table>
<thead>
<tr>
<th>Sources of clinical isolates</th>
<th>Total no. of sample</th>
<th>Total no. of positive Candida isolate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>100</td>
<td>19</td>
</tr>
<tr>
<td>Urine</td>
<td>100</td>
<td>53</td>
</tr>
<tr>
<td>HVS</td>
<td>100</td>
<td>21</td>
</tr>
<tr>
<td>CSF</td>
<td>100</td>
<td>05</td>
</tr>
<tr>
<td>Sputum</td>
<td>100</td>
<td>15</td>
</tr>
<tr>
<td>Total</td>
<td>500</td>
<td>113</td>
</tr>
</tbody>
</table>

HVS- High vaginal swab, CSF- cerebrospinal fluid.

Table 1. Distribution of Candida isolates in different clinical samples.

Table 2. Distribution of different isolates in patient population.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Total number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. albicans</td>
<td>31 (27.43)</td>
</tr>
<tr>
<td>C. glabrata</td>
<td>26 (23.00)</td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>25 (22.12)</td>
</tr>
<tr>
<td>C. guilliermondii</td>
<td>17(15.04)</td>
</tr>
<tr>
<td>C. parapsilosis</td>
<td>14 (12.38)</td>
</tr>
<tr>
<td>Total</td>
<td>113 (100%)</td>
</tr>
</tbody>
</table>

C. albicans 27%
C. tropicalis 27%
Non albicans candida 73%

Figure 1. Distribution of C. albicans and Non-albicans Candida.
maximum number in both sexes (Table 3). Out of 20 oropharyngeal swabs from HIV/AIDS patients, Candida species grew in twelve cases. C. tropicalis is isolated as prevalent strain in oropharyngeal candidiasis in HIV positive patients (Table 4). The interpretive criteria of susceptibility and resistance of antifungals is shown in Table 6.

The susceptibility pattern showed that of the 113 isolates, 36% were resistant to fluconazole, 24 and 21% were resistant to itraconazole and voriconazole, respectively, where no resistance was seen for amphotericinB (Table 7).

**DISCUSSION**

Candida infections in immunocompromised patients often shows severe picture and are found to be associated with strains which are resistant to conventional antifungal therapy. The prior colonization precedes infection in most of the cases of candidiasis (Vazquez et al., 1993). A variety of local and systemic host factors and exogenous factors have been described to increase the prevalence of Candida infection (Hachem et al., 2008; Shaheen and Taha, 2006). Among all the Candida sp., C. albicans exhibited 27.43% positivity and the rest were non-albicans Candida, this finding is in concordance with the reports of other authors (Capoor et al., 2005; Pfaller, 1996, Saha et al., 2008). The predominant non-albicans Candida was C. glabrata (26/82) followed by C. tropicalis (25/82) and these are found to be associated with hospital acquired infection (Pfaller, 1996) and many reports showed the prevalent isolates as C. tropicalis (Kothavade et al., 2010; Capoor et al., 2005; Pfaller 1996; Kashid et al., 2011). In this study, there is single species difference between C. glabrata and C. tropicalis. Other non-albicans Candida species were Candida guilliermondii and Candida parapsilosis. In all the clinical samples in this study, non-albicans Candida is found in maximum number (Table 4). By excluding 21 high vaginal swab samples (Table 5), it was observed that, the frequency of candidiasis is more (Figure 2) among females (51/113) as compared to males (41/113), but statistically by Chi square test, it has been seen that the incidence of Candida species distribution is independent on sex ($\chi^2 = 0.141$ and the critical value of $\chi^2 = 3.84$ at
very high resistance to fluconazole for all candidal isolates although the amphotericinB susceptibility is high (Adhikary et al., 2011). The isolates recovered from HIV positive individual also showed a prevalence of non-
albicans Candida (Table 4) and found more resistant to fluconazole. Although the prevalence study of non-
albicans Candida and C. albicans was done by paired t-
test (p<0.10) and correlation test (p<0.05), in both methods, prevalence of non-
albicans Candida was found to be significant over C. albicans at ten and five percent level of significant, respectively. The increased prevalence of non-
albicans species was found to be replacing C. albicans and this finding is correlation with a study by Jha et al. (2006).

**Conclusion**

In view of several studies and an epidemiological investigation of infection with Candida in this north-east part of India, significant increase in association with non albicans Candida was shown. The lower socioeconomic condition, lack of awareness on hygiene, seasonal temperature and humidity of this geographical area also contributed much to the significant association of Candida species causing infection. Unlike antibiotic susceptibility test, antifungal susceptibility test is not commonly used. Antifungal susceptibility test is still an unexploited method in many Indian routine clinical microbiology laboratories. This study also focuses on species level identification and the use of antifungal susceptibility test in routine clinical laboratory. The rational use of antifungal agents in hospitals may minimize the resistance against drugs. This simple and flexible technique contributes a useful aid in management of critical patients.
REFERENCES


