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Prevalence and Antifungal Susceptibility of *Candida albicans* in Patna, India

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**Abstract**

Candida species are the most common cause of opportunistic fungal infection worldwide. Candida is the major fungal pathogen of humans, causing diseases ranging from superficial mucosal infections to disseminated, systemic infections that are often life threatening. A striking feature of its pathogenicity is ability to grow in yeast, pseudohyphal and hyphal forms. The hyphal form has an important role in causing disease by invading epithelial cells and causing tissue damage. Among Candida spp., *Candida albicans* is the most common infectious agent. This dimorphic yeast is a commensal that colonizes skin, the gastrointestinal and the reproductive tracts. Non-*C. albicans* species are also emerging pathogens and can also colonize human mucocutaneous surfaces. The pathogenesis and prognosis of candidial infections are affected by the host immune status and also differ greatly according to disease presentations. This study aimed at determining the prevalence and antifungal susceptibility pattern of *Candida albicans* in Patna and how they relate to patient’s demographic data.

**Keywords**
*Candida albicans*, antifungal susceptibility test, Amphotericin B, nosocomial infection

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**Introduction**

With changes in the medical and surgical management of patients over the past three decades, fungi have emerged as a major cause of human disease. Of the disseminated mycoses, candidiasis remains the most prevalent, with *Candida albicans* causing more invasive infections than any other fungus (Elizabeth, 2009). Candidiasis or thrush is a fungal infection (mycosis) of any of the *Candida* species, of which *C. albicans* is the most common, also referred to as yeast infection. Candidiasis is also technically known as candidosis, moniliasis and oidiomycosis (William et al., 2006). *Candida* spp. are the most common cause of fungal infections leading to a range of life-threatening invasive to non-life-threatening mucocutaneous diseases (Achkar and Fries, 2010). *Candida* infections can present in myriad ways. This dimorphic yeast is a commensal that colonizes skin, the gastrointestinal and the reproductive tracts. Consequently, they are also from the setting of candidiasis, albeit at a lower frequency. The pathogenesis and prognosis of candidial infections are affected by the host immune...
status and also differ greatly according to disease presentations. Therefore, diagnosis, management and treatment choices vary and need to be considered in the overall setting of the affected human host.

Due to the recognition of the increasing importance of Candida spp. as an opportunistic pathogen, an effective method is required to type the organism. Analysis of the proteins of Candida spp. can be used as a sensitive method of characterizing the yeast on the basis of their protein band profile. Keeping these facts in mind, the present study was undertaken to find out the prevalence and Antifungal susceptibility pattern of Candida albicans in superficial and deep seated infections.

Materials and Methods

The present study was conducted in the Department of Microbiology, Nalanda Medical College, Patna from mid September to mid February 2016. The cases selected for the study included symptomatic high-risk patients in whom no other aerobic pathogen was identified, and Candida spp. were isolated repeatedly in pure culture, and these infections were associated with clinical signs and symptoms of infection. Twenty nine isolates of C. albicans from various clinical specimens were obtained from a total of 253 patients. The isolates were identified by standard mycological methods that included colony morphology on Sabouraud’s dextrose agar (SDA), germ tube test (GTT), morphology on corn meal agar (CMA) and sugar fermentation/assimilation profile (Milne, 1996). Further antifungal susceptibility testing was performed.

Antifungal Susceptibility Testing

Susceptibility testing to nystatin (100 units), clotrimazole (10μg), fluconazole (10μg) and amphotericin-B (100units) (all from HiMedia Laboratories, Mumbai, India) was performed by the disc diffusion method (Chakrabarti et al., 1995). Candida kefyr strain Y/16 was used as control. The medium used was yeast nitrogen base glucose (YNBG) agar (HiMedia Laboratories, Mumbai, India). For susceptibility testing of azoles, 1.5% l-asparagine (HiMedia Laboratories, Mumbai, India) was added to the YNBG agar. Results were interpreted as either sensitive or resistant. Antifungal susceptibility testing to voriconazole (Pfizer Inc.) was done by broth microdilution and Alamar blue colorimetric microdilution method. These were also used to re-test the fluconazole resistant isolates identified by the disc diffusion method. broth microdilution was performed in accordance with the guidelines in NCCLS document M27-A (1997). Quality control isolates of C. albicans (ATCC 10231), C. parapsilosis (ATCC 22019) and C. krusei (ATCC 6258) were included with every test. Endpoints were determined visually by observing a colour change from blue (indicating no growth) to pink (indicating growth).

Statistical Analysis

The ‘Z’ test for proportion was used to compare the data. A p-value of < 0.05 was taken as indicative of statistical significance, and a p-value of < 0.01 was considered highly significant.

Results and Discussion

In the present study, 29 isolates of C. albicans (11.46%) were obtained from 253 high-risk patients, implying an isolation rate of 11.46%. The age of patients in the study group ranged from newborns to >61 years. There was a slight female preponderance in the study population (M:F ratio= 0.9:1). The maximum number of C. albicans isolates
were from urine (35.6%), and cervical swabs (20.5%). Other specimens in the study included BAL fluid, CSF, pus, sputum etc. (Fig.1). The antifungal susceptibility pattern of the isolates showed that all (100%) isolates were sensitive to nystatin, amphotericin-B and voriconazole in the concentrations tested. Fluconazole resistance was observed in 7 (24.13%) isolates (Table 1).

**Table 1** Susceptibility Pattern of Isolates

<table>
<thead>
<tr>
<th>Antifungal agent</th>
<th>Disc potency</th>
<th>No.of strains</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Clotrimazole</td>
<td>10µg</td>
<td>27(93.11)</td>
<td>2(6.89)</td>
<td></td>
</tr>
<tr>
<td>Nystatin</td>
<td>100 units</td>
<td>29(100)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Fluconazole</td>
<td>10µg</td>
<td>22(85.87)</td>
<td>7(24.13)</td>
<td></td>
</tr>
<tr>
<td>Amphotericin-B</td>
<td>100 units</td>
<td>29(100)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Voriconazole</td>
<td>-</td>
<td>29(100)</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

**Fig.1** Isolation of C. albicans from Various Clinical Specimens

A significant increase in the incidence of fungal infections, especially those due to yeasts, particularly *Candida* species is being seen in recent times. This is mostly due to rising numbers of immunosuppressed patients and widespread use of broad-spectrum antibiotics and steroids. *Candida albicans* is considered the most pathogenic member of the genus *Candida* and is the species most commonly isolated from clinical materials, although infections with other species of *Candida* have been described in recent times (Pfaller, 1996; Fadda *et al.*, 2008). Due to the recognized importance of these agents as nosocomial and opportunistic pathogens, it is important to have a simple, effective and relevant typing method for the characterization of
these yeasts. They have further concluded that these techniques could provide additional criteria for serologic and immunological studies of C. Albicans. Antifungal susceptibility testing of our isolates revealed that all isolates were sensitive to amphotericin-B, nystatin and voriconazole. However, significant resistance (24.13%) was observed for fluconazole. Our findings are in accordance with those of Fadda et al. (2008) who also found that there was decreased susceptibility to the older azoles among Candida albicans isolates. Similar susceptibility of C. Albicans isolates was also reported by Mokaddas et al. (2007).

In conclusion, the increase of Candida infections might be related to several factors such as the:

(1) Human Immunodeficiency Virus; (ii) neutropenic persons due to anticancer treatments; (iii) the abusive use of extended spectrum antibiotics; (iv) metabolic disorders such as diabetes mellitus. The need of the hour is to understand every aspect of Candidal infections. Early and specific diagnosis is crucial and the decision to treat a patient with these unusual infections is often based on little clinical and microbiological information. Treatment decisions need careful consideration of the epidemiological factors and the immune status of the population at risk. This method can also be used in an outbreak situation or to compare different isolates to establish identity or non-identity.

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References


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