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Full Length Research Paper

Trend of *Candida* infection and antifungal resistance in a tertiary care hospital of north east India

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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.

Key words: Fungi, yeast, *Candida albicans*, Non-*albicans Candida,* opportunistic pathogen.

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

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

Sources of clinical isolates	Total no. of sample	Total no. of positive *Candida* isolate
Blood	100	19
Urine	100	53
HVS	100	21
CSF	100	05
Sputum	100	15
Total	500	113

HVS- High vaginal swab, CSF- cerebrospinal fluid.

for Candidiasis in high-risk patients has the potential to reduce morbidity and mortality by preventing further progression of infection. However, selection of appropriate empiric therapy is complicated by increasing prevalence of NAC species (Davis et al., 2007). Therefore, species level identification is compulsory for proper selection of drugs. The present study was based on the epidemiological picture of *C. albicans* and NAC in different infections including HIV/AIDS, isolated in remote state of north east India. Extensive literature survey shows no previous study on prevalence of *Candida* in the same study population.

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 gynecology), 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 Chlamydospore was performed. Germ tube test was carried out with 0.5 ml of pooled human serum and for chlamydospore 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.

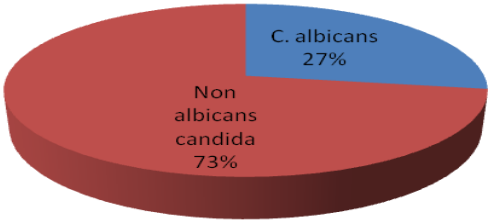

Figure 1. Distribution of *C. albicans* and Non-*albicans Candida*.

Table 2. Distribution of different isolates in patient population.

Isolate	Total number (%)
C. albicans	31 (27.43)
C. glabrata	26 (23.00)
C. tropicalis	25 (22.12)
C. guilliermondii	17(15.04)
C. parapsilosis	14 (12.38)
Total	113 (100%)

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 3. Distribution of *C. albicans* and non-*albicans Candida* in male and female.

Age in years	*C. albicans*		Non-*albicans Candida*		Total
	Male	Female	Male	Female	
0-10	3	3	4	8	18
11-20	1	3	4	2	10
21-30	2	4	3	20	29
31-40	1	3	2	9	15
41-50	2	2	4	0	8
51-60	2	2	6	6	16
>60	1	2	6	8	17
Total	12	19	29	53	113

Table 4. Distribution pattern of *Candida* species in HIV positive cases.

Isolate	Total number (%)
C. albicans	2 (16.66)
C. tropicalis	7 (58.33)
C. parapsilosis	3 (25.00)
Total	12 (100%)

Table 5. Distribution of *C. albicans* and NAC in different clinical samples.

Sources of clinical isolates	*C. albicans* (%) n=31	Non-*albicans Candida* (%) n=82
Blood	6(31.57)	13(68.42)
Urine	13(24.52)	40(75.47)
HVS	5(23.80)	16(76.19)
CSF	2(40)	3(60)
Sputum	5(33.33)	10(66.66)
Total	31(27.43)	82(72.56)

HVS- High vaginal swab, CSF- cerebrospinal fluid.

Table 6. Interpretive criteria of susceptibility and resistance of antifungals.

	Zone diameter in mm		
	Sensitive	Dose dependent	Resistance
Fluconazole	≥19	15-18	≤14
Itraconazole	>16	10-15	<9
Voriconazole	>17	14-16	<13
AmphotericineB	>15	10-14	<9

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

Table 7. Susceptibility of *Candida* species to antifungal drugs.

Antifungal drug		*C. albicans* (n=31) (%)	*C. glabrata* (n=26) (%)	*C. tropicalis* (n=25) (%)	*C. guilliermondi* (n=17) (%)	*C. parapsilosis* (n=14) (%)	Total (n=113) (%)
Fluconazole	S	8(25.8)	11(42.3)	7(28)	6(35.2)	8(57.1)	35.3
	SSD	11(35.4)	4(15.3)	11(44)	5(29.4)	1(7.1)	28.3
	R	12(38.7)	11(42.3)	7(28)	6(35.2)	5(35.7)	36.2
Itriconazole	S	17(54.8)	11(42.3)	10(40)	9(52.9)	8(57.1)	48.6
	SSD	8(25.8)	7(26.9)	10(40)	3(17.6)	3(21.4)	27.4
	R	6(19.3)	8(30.7)	5(20)	5(29.4)	3(21.4)	23.8
Voriconazole	S	17(54.8)	14(53.8)	12(48)	11 (64.7)	9(64.2)	55.7
	SSD	6(19.3)	8(30.7)	7(28)	3(17.6)	2(14.2)	23.0
	R	8(25.8)	4(15.3)	6(24)	3(17.6)	3(21.4)	21.2
AmphotericinB	S	29(93.5)	23(88.4)	23(92)	16(94.1)	12(85.71)	91.1
	SSD	2(6.4)	3(11.5)	2(8)	1(5.8)	2(14.2)	8.8
	R	0(0)	0(0)	0(0)	0(0)	0(0)	0

S = sensitive, SSD = sensitive dose dependent, R= resistance.

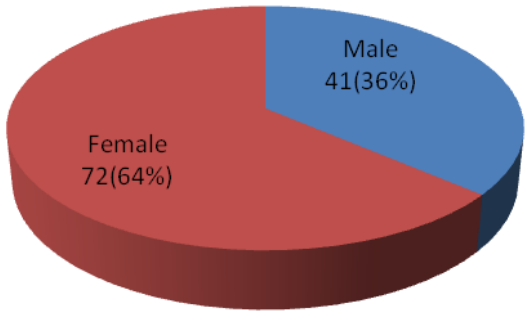

Figure 2. Gender wise occurrence of *Candida* species.

five percent level of significance with one degree of freedom). The susceptibility pattern of all *Candida* isolates showed that 91% were sensitive to amphotericinB, 65% to voriconazole followed by 49% to itraconazole and to 35% fluconazole (Table 7). The extended prophylactic use of fluconazole in suspected cases would be a probable cause of high resistance pattern to fluconazole in our institute. Another established fact is that antifungal drug response *in vitro* may be dose dependent which is expressed as susceptible dose dependent (SDD), that is, although susceptible *in vitro* but resistance failure may be seen *in vivo* at the usual dose. In such situations, increase in dose of drug above the usual dose often results in clinical cure (Saha et al., 2008). Wide spread use of fluconazole in various clinical conditions is the major cause of NAC dominance over *C. albicans* (Kothavade et al., 2010). Western data have shown that *Candida* species are reliably susceptible to polynes, azole and echinocandins. But Indian studies shown a 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.

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