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ORIGINAL ARTICLE

STUDY OF CHARACTERIZATION & ANTIFUNGAL SUSCEPTIBILITY TESTING OF CLINICALLY SIGNIFICANT CANDIDA SPECIES

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ABSTRACT: BACKGROUND: Over the last two decades Candida has accounted for the most serious opportunistic infections especially in the immuno-compromised individuals. Candida species have emerged as important causes of invasive infections & the rates of resistance to standard antifungal therapies are on the rise. Awareness regarding fungal infections has compelled the clinicians and laboratories to lay more emphasis on the detection of fungi; as speciation and antifungal tests are not routinely done. Over the past decade significant progress has been made with standardization of the methods for antifungal susceptibility testing, correlation between in-vitro results & patient outcome. **OBJECTIVES:** The aim of this study was to isolate, identify & determine the susceptibility pattern of clinically significant Candida species and study the spectrum of Non-albicans Candida species, thus contributing to overall reduction in the cost of treatment and duration of hospital stay. **METHODS:** The study was carried out at department of microbiology MVJ Medical Hospital Bangalore for one year from Aug 2010 – July 2011. 50 Candida species which were isolated from various clinical specimens were included in the study. They were identified by using various media & identification methods. Antifungal susceptibility testing was done on Yeast nitrogen base agar by disk diffusion method & analyzed. **RESULTS:** Non-albicans Candida (NAC) emerged as the commonest species with [39(22%)] causing fungal infection followed by Candida albicans [11(22%)]. Among the NAC isolates Candida tropicalis was predominant followed by Candida krusei, Candida glabrata & Candida guilliermondi. **CONCLUSION:** Studying the speciation & susceptibility patterns of Candida will help us understand the etio-pathology and might assist in better patient care.

KEYWORDS: Candida, Characterization, Sugar assimilation / fermentation, Antifungal susceptibility.

INTRODUCTION: Candida species are ubiquitous in distribution & cause various localized, invasive and disseminated diseases in normal and compromised individuals. Due to the commensal nature of Candida, they usually cause endogenous infections. Candida species are increasingly being identified as opportunistic pathogens in recent years.

NAC has been observed to replace Candida albicans at most of the clinical sites of infection. These species are assuming importance due to their decreased resistance to commonly used antifungal agents & their potential to emerge as opportunistic nosocomial pathogens.[1, 2] The patients at extremes of age and with co-morbidities are at highest risk for invasive candidiasis. The co-infection of HIV with TB has increased the incidence of opportunistic fungal infections.[3, 4, 5]

The increased frequency of non-albicans candida infection coupled with high levels of resistance to common antifungal drugs – are a cause of concern in prophylaxis and treatment of fungal infections. An early identification of Candida species provides an important help in selection of appropriate antifungal treatment.[6, 7]

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MATERIALS & METHODS: 50 Candida species isolated from various clinical specimens of MVJ Medical College Bangalore, over a period of one year from Aug 2010 – July 2011 were included in the study. The samples were studied, isolated & identified to the species level using standard laboratory protocol. The preliminary identification was done by microscopy and isolation on SDA. The isolates were further speciated by germ tube test, corn meal agar morphology – chlamydospore formation & sugar assimilation / fermentation tests.[8, 9, 10] Antifungal susceptibility testing by disk diffusion method was done on yeast nitrogen base agar for 4 antifungal agents [fluconazole, ketoconazole, itraconazole & amphotericin B] and analyzed. Antifungal susceptibility testing was done in accordance with CLSI document M44-A for yeasts. [10, 11]

RESULTS: Among 50 Candida isolates, Candida albicans were 11 (22%) and NAC 39 (78%). The different species of Candida isolated were as shown in Table I. The age and sex distribution in Candida infections were shown in Table II and III respectively. The extremes of age were affected and incidence was seen predominantly in males.

Associated infections always have an immuno-suppressed/immuno-compromised factor contributing to it. Table IV shows candidiasis from different infections where the incidence of pulmonary infections were higher. Sample-wise distribution of Candida species is shown in table V.

Table VI shows antifungal susceptibility pattern to various antifungal agents.

Species of Candida	Isolated No	%
Candida albicans	11	22%
Candida tropicalis	20	40%
Candida krusei	8	16%
Candida glabrata	4	8%
Candida guillermondi	3	6%
Candida parapsilosis	1	2%
Candida kefyr	3	6%

Table 1: Different species of Candida isolated

	1 -10	11 -20	21 -30	31 -40	41 -50	50+
C. albicans	3	-	1	1	3	3
NAC	4	-	3	5	8	20
Total	**7**	**-**	**4**	**6**	**11**	**23**

Table 2: Age Distribution – Candida species

Sex	%
Male	36 (72%)
Female	14 (28%)

Table 3: Sex Distribution – Candida species

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Associated Infections	No.(%)
Tuberculosis / Pneumonia	26 (52%)
Diabetes mellitus	06 (12%)
HIV infection	07 (14%)
Pregnancy	01 (2%)
Nosocomial	03 (6%)
Others	07 (14%)
Total	**50**

Table 4: Candida and its associated infections

Samples	No. (%)
Sputum	26 (52%)
Faeces	09 (18%)
Pus	04 (8%)
Skin scrapings	03 (6%)
Ear swab	03 (6%)
Throat swab	01 (2%)
Urine	03 (6%)
Vaginal swab	01 (2%)
Total	**50**

Table 5: Candida species – Sample wise

Antifungal disk	C. albicans (11)	C tropicalis (20)	C krusei (8)	C glabrata (4)	C guillermondi (3)	C parapsilosis (1)	C kefyr (3)
Amphotericin B	S – 100%	S – 100%	S – 100%	S – 100%	S – 100%	S – 100%	S – 100%
Ketoconazole	S – 100%	S – 100%	I – 50%	S – 100%	S – 100%	S – 100%	S – 100%
Itraconazole	S – 100%	S – 60%	I – 30%	S – 40%	S – 70%	S – 60%	I – 50%
Fluconazole	S – 100%	S – 92%	R	S – 100%	S – 100%	S – 96%	S – 96%

Table 6: Susceptibility pattern of antifungal drugs

S – Sensitive I – Intermediate Sensitive R – Resistant

DISCUSSION: In the present study among NAC (78%), C. tropicalis emerged as the predominant isolate. In some of the other studies the % of C. albicans was higher than NAC which is in contrast to the present study. (Germian G & Pelletier R et al).[12] In our study, tuberculosis & pneumonia were the commonest pulmonary infections associated with candidiasis which was in accordance with other studies.(Bhramadatan K N et al).[13] Oropharyngeal candidiasis (OPC) continues to be a common opportunistic infection in patients with HIV & is predictive of increasing immuno-suppression. The increasing use of fluconazole to treat HIV patients with OPC has resulted in a change in the prevalence of different candida species and the emergence of azole resistance.[3]

In the present study, the prevalence of candiduria caused by Candida species was 6% and women were affected predominantly. The higher prevalence in female patients may reflect vaginal candidiasis as the yeasts may ascend from genitor-urinary tract to urinary tract. The licensed antifungal agents [fluconazole, ketoconazole & Amphotericin B] were highly susceptible (96-100%) against most of the Candida species regardless of the age group. Itraconazole was less active than all of the other azoles against all species with the exception of C. albicans where its sensitivity was 100% in our study as observed in MA Pfaller1& RN Jones et al.[14]

CONCLUSION: Considerable increase in the incidence of candidiasis has been observed especially in critical care settings. NAC's are gradually gaining importance and replacing C. albicans as etiological agents. Early isolation & identification of candida and study of susceptibility pattern of antifungal drugs can be useful towards better management of candidiasis.

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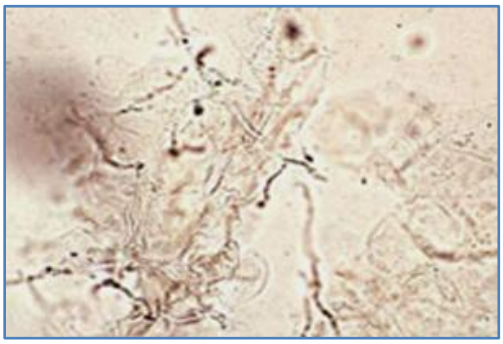
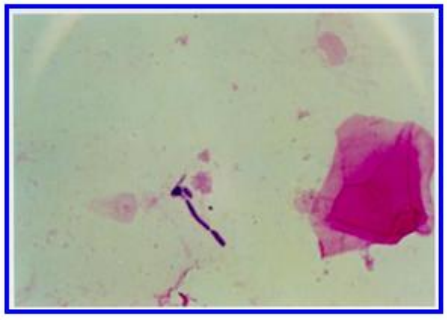
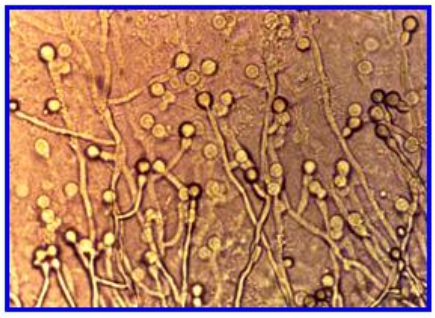


KOH wet mount showing fungal elements





Gram stain – Pus cells & Pseudohyphae.
Chlaymydospores on Corn meal agar [CMA]

CMA – Blastospores(singly/small groups)
with long pseudohyphae of Candida tropicalis

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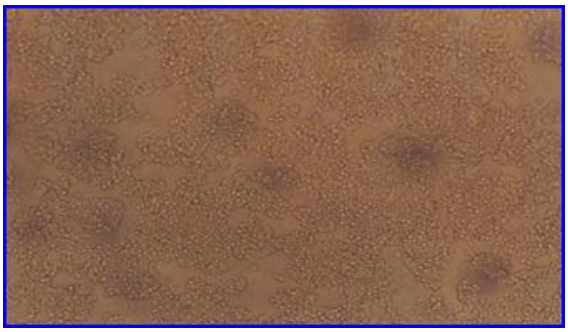
CMA – Clusters of blastospores along short hyphae of Candida guillermondi

CMA – Giant hyphae with blastospores at the end seen in Candida krusei



CMA – blastospores without hyphae of Candida glabrata

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