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PREVALENCE AND ANTIFUNGAL SUSCEPTIBILITY OF CANDIDA SPECIES ISOLATED FROM TERTIARY CARE HOSPITAL IN NORTH EAST KARNATAKA

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ABSTRACT

Introduction: Candida is a yeast like fungus causing commonest fungal infections. Being endogenous in nature, in individuals with risk factors can lead to candidiasis. Candidiasis caused by various species, important being C.albicans. As there are chronic, treatment failure cases noted in these infections it is important to study the antifungal susceptibility pattern of candida. **Objectives:** Study of prevalence, speciation and antifungal susceptibility of Candida species from samples received by Microbiology Laboratory was the aim. **Methods:** 150 isolates were considered for the study. Candida was identified from clinical samples by wetmount, gram stain and culture on SDA. The organisms were further speciated by germ tube test, cornmeal agar morphology, sugar assimilation and fermentation tests. Antifungal sensitivity was performed by using disc diffusion method. **Results:** The most common isolate found to be Candida albicans (61.3%) followed by non albicans Candida mainly C.tropicalis (18%), C.glabrata 16(10.6%), C.guilliermondii 10(6.6%) and C.krusei 5 (3.3%). 90% of isolates were from mucocutaneous area. The common predisposing factors observed were pregnancy, HIV infection, diabetes, prolonged contact with water. Resistance to commonly used antifungal agents found to be Fluconazole – 17.3%, Ketoconazole- 11.3%, Nystatin -5.3%, and Amphotericin-B - 2%. **Conclusion:** C.albicans is the common cause of candidiasis followed by C.tropicalis, C.glabrata, C.krusei. Antifungal susceptibility testing need to be carried out before starting therapy because of varying susceptibility pattern in different species of the Candida.

Key words: Candida; candidiasis; C.albicans; Antifungal susceptibility.



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INTRODUCTION

Candida is yeast like fungus causing commonest fungal infections. Candida is normal inhabitant & changes in the internal and external environment induce the harmless saprophyte to become a true pathogen especially in immunocompromised individuals¹. These predisposing factors are ageing, pregnancy, AIDS, steroidal therapy, secondary to bacterial infections and wide spread use of certain medical and surgical practices.² Candidiasis is mainly caused by *C. albicans*, while there has been striking increase in the frequency with non albicans Candida species in last few years. There has been an increase in treatment failure, because of drug resistance. Change in drug susceptibility of different species of Candida and the introduction of newer antifungal agents has made the invitro susceptibility testing of antifungal agents more important which helps in rational use of the same.^{3,4}

MATERIALS AND METHODS

The present study was carried out in the department of Microbiology, M.R. Medical College, Gulbarga for a period of one year from March 2009 to February 2010. The 150 cases of laboratory diagnosed candida isolates constituted the study. Specimens collected aseptically were subjected for Direct examination by a) Wet Mount b) Gram's stain^{1,5,6}. For Culture Sabouraud's dextrose agar (SDA) with Chloramphenicol and gentamycin was used. The sample was

inoculated on SDA slopes and incubated at 25°C. The slopes were observed regularly daily for 2 days to 3 weeks.⁶ Colonies were identified with the colony characters and by gram's stain. Once the colonies were confirmed, speciation was done by the Germ tube test, Corn meal agar inoculation, Sugar Fermentation & Sugar Assimilation test. Antifungal susceptibility testing is done by Disc diffusion method. The antifungal agents used for disc diffusion method are Amphotericin B (10µg), Fluconazole (10µg), Nystatin (10µg) & Ketoconazole (10µg)^{1,7,8}

RESULTS

Total 150 isolates of Candida species isolates from various clinical specimens were speciated and their antifungal susceptibility was found. The prevalence of *Candida albicans* and non albicans candida are studied⁹. Age distribution of patients was from 3 months to 68 years. The highest incidence was seen in the age group 20-40 years comprising 53.3%. The Candida species isolated more from female (55%) than male (45%) patients in the ratio of 1:1.22 (M:F). The highest number of isolates was from cervical swab isolated from vulvovaginitis constituting 52 (34.6%). The other major samples were skin scrapings 24 (16%), oral swabs 23 (15.3%), nail scrapings 14 (9.3%), sputum 12 (8%) followed by urine 9 (6%), pus 5 (3.3%), blood 5 (3.3%), ear swabs 4 (2.6%) and stool 2 (1.3%) - (Table-1).

Table 1
Distribution of isolates among various clinical specimens

Clinical specimens	No. of Patients	Percentage
Cervical swab	52	34.6
Skin scrapings	24	16
Oral swabs	23	15.3
Nail scrapings	14	9.3
Sputum	12	8.0
Urine	9	6.0
Blood	5	3.3
Pus	5	3.3
Ear swabs	4	2.6
Stool	2	1.3

C. albicans was the major species isolated accounting for 92 (61.3%) of the total isolates. Non albicans *Candida* constituted 27(18%) of *C. tropicalis* followed by *C. glabrata* 16(10.6%), *C. guilliermondii* 10(6.6%) and *C. krusei* 5 (3.3%)-(Table-2).

Table 2
Different species of Candida isolated

Species	No. of Isolates	Percentage
<i>C. albicans</i>	92	61.3
<i>C. tropicalis</i>	27	18
<i>C. glabrata</i>	16	10.6
<i>C. Guilliermondii</i>	10	6.6
<i>C. krusei</i>	5	3.3

In the study, pregnancy is the major predisposing factor constituting 42 (28%) followed by HIV seropositives 29(19.3%), diabetes 21(14%), secondary to infections 21(14%), prolonged contact with water 14(9.3%) and prolonged use of drugs 8(5.3%). Others 15(10%) in predisposing factors include the presence of malignancies, catheterisation, stay in ICU, parenteral nutrition, prematurity and major surgery-(Table-3).

Table 3
Distribution of different species of Candida among various predisposing factors

Predisposing factors	C.albicans	C.tropicalis	C.glabrata	C.guilliermondii	C.krusei	Total
Pregnancy	26	9	4	2	1	42
HIV seropositive	14	6	4	3	2	29
Diabetes	13	4	2	1	1	21
Secondary to other infections	13	3	1	3	1	21
Prolonged contact with water	10	3	1	-	-	14
Prolonged use of steroid/antibiotics	6	1	1	-	-	8
Others	10	1	3	1	-	15
	92	27	16	10	5	

Among 150 Candida isolates, 83(55.3%) were sensitive to Fluconazole, 26(17.3%) were resistant and 41(27.3%) were intermediate sensitive. For Ketoconazole 102(68%) were sensitive, 17(11.3%) were resistant and 31(20.6%) were intermediate sensitive. For Nystatin 121(80.6%) were sensitive, 8(5.3%) were resistant and 21(14%) were intermediate sensitive. The sensitivity pattern for Amphotericin-B 139(92.6%) were sensitive, 3(2%) were resistant and 8(5.3%) were intermediate sensitive-(Table-4).

Table 4
Drug susceptibility pattern

Species	Fluconazole			Ketoconazole			Nystatin			Amphotericin B		
	S	IS	R	S	IS	R	S	IS	R	S	IS	R
C.albicans	54	23	15	65	17	10	76	11	5	85	4	3
C.tropicalis	14	8	5	19	4	4	20	4	3	23	4	0
C.glabrata	7	5	4	7	6	3	12	4	0	16	0	0
C.guilliermondii	6	3	1	7	3	0	8	2	0	10	0	0
C.krusei	2	2	1	4	1	0	5	0	0	5	0	0
Total	83	41	26	102	31	17	121	21	8	139	8	3

DISCUSSION

Candida is yeast like fungus and is a normal commensal of skin and mucous membrane. Candida with change in external and internal environment causes candidiasis. The disease may be mild to severe, superficial to deep, acute to chronic. As Candida is also isolated normally from nonsterile sites, the presence in clinical specimens must be interpreted with caution.¹⁰The highest number of cases occurred in the age group 20-40years which accounted for 53.33%. The highest incidence was found to be vulvovaginitis. The predominant species isolated in the present study is *Candida albicans* constituting 92 (61.33%). The non-*albicans* Candida constituted 58(39.66%).The major non-*albicans* species isolated were *C.tropicalis*, *C.glabrata* *C.guilliermondii* followed by *C.krusei*.The pregnancy (28%)and HIV(19.3%) infection were the major predisposing factors. Prevalence of *C.albicans* found to be more than non-*albicans* candida. *C.albicans* is the major species isolated. The high hormone level leads to a proportional increase in the glycogen content of the vagina that favour colonization by *Candida* in pregnancy¹¹. In HIV the decreased cell mediated immunity leads to opportunistic infections. *Candida* is found to be the major fungus causing opportunistic infections in AIDS, mainly oral and esophageal candidiasis¹². In the present study diabetes accounted for 14% the cases. The diabetes is a individual risk factor causing increased colonization & the infection¹³. The present study shows that 14 (11.67%) cases have a history of prolonged contact with water. The prolonged use of corticosteroids accounted for 8 (6.67%) cases. The candida infection Secondary to other bacterial infections found to be 21(14%).Most cases isolated were from the respiratory system, secondary to chronic bronchopulmonary disease. The presence of

primary infection accompanied by malnutrition and debilitation favour candidiasis.Others comprised 15(10%) in predisposing factors which include presence of malignancies, catheterisation, stay in ICU, parenteral nutrition and prematurity. In the present study among 150 *Candida* isolates, 26(17.3%) were resistant to Fluconazole, 17(11.3%) were resistant to Ketoconazole For Nystatin 8(5.3%) & Amphotericin-B 3(2%) were resistant.The resistance to fluconazole was higher when compared to other drugs in the study. This might be due to widespread and indiscriminate use of fluconazole for extended periods. It is also noted that resistance was found in all the species. Disc diffusion method is simple, less cumbersome & economical for testing antifungal susceptibility of candida.⁴

CONCLUSION

Candidiasis is one of the frequently encountered fungal disease. It affects all age groups mainly 20-40yrs. The incidence of disease is found to be increased due to predisposing factors like pregnancy, HIV infection, diabetes etc.The most common form is vulvovaginal candidiasis followed by oral & other mucocutaneous candidiasis. The candida infection should be looked in patients with HIV,diabetes,other bacterial infection & patients on drugs like steroids.*Candida albicans* was the main species isolated but increase in nonalbicans *Candida* species noted. The development of resistance to commonly used antifungal drugs indicates need for rational use, preferably after in- vitro antifungal susceptibility testing. Disc diffusion method can be employed to find antifungal susceptibility of candida .

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