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ORIGINAL ARTICLE

ISOLATION AND SPECIATION OF CANDIDA FROM CLINICAL SAMPLES IN A TERTIARY CARE HOSPITAL AT KURNOOL, ANDHRAPRADESH, INDIA

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ABSTRACT: Candida is one of the most frequently encountered opportunistic fungi that cause infection in humans. The pathogenesis of Candida is complex and probably varies with each infection. This study was conducted to understand the prevalence of Candida from various clinical specimens of patients and to show the emergence of Non albicans Candida in clinical samples. This study also focused on the antifungal susceptibility which guides the clinicians to treat the infection effectively. **METHODS:** Clinical samples were collected from outpatients and inpatients of Government General Hospital, Kurnool over a period of one year from March2008 to June2009. Isolation, culture, speciation of Candida was done by using standard methods. Antifungal susceptibility testing was done by disc diffusion technique against amphotericin B, nystatin, fluconazole and clotrimazole. **RESULTS:** Candida manifests in various sites depending on the predisposing factors and immune status of the person. In this study we found the association of Candida with various predisposing factors (Pregnancy, Oral contraceptive pills's, Immune suppression, Diabetes). This study observed the dominance of non-albicans Candida (51%) in the clinical samples over Candida albicans (49%). The maximum antifungal susceptibility was observed against amphotericin B in both the albicans and non-albicans Candia, but non-albicans Candida showed maximum resistance to azoles. **CONCLUSION:** Candida albicans was the most predominant species (49%) isolated in various clinical samples. There was an increase in the prevalence of non albicans Candida in this study. Among the nonalbicans Candida (51%) Candid tropicalis was the commonest species isolated. Candida albicans showed maximum susceptibility to amphotericin B and maximum resistance to azoles was seen in nonalbicans Candida.

KEYWORDS: Candida, isolation, speciation, antifungal susceptibility.

INTRODUCTION: Fungi are commonly recognized as medically significant organisms causing potentially life threatening diseases. With the control of most bacterial infections, fungal infections have assumed great importance. The yeast like fungi typically colonize mucocutaneous surface which can be portals of entry into deeper tissues when the host defenses are compromised.[1] The clinical manifestations of candidiasis include mucocutaneous candidiasis, cutaneous candidiasis and systemic candidiasis. Many types of yeast have the ability to cause disease in the suitable environment. Some of the risk factors for yeast infections are AIDS, Burn Patients, Pregnancy, Steroids, Antibiotic therapy, Immunosuppressant, Cancer treatment, Heart Surgery, Genetic deficiency, Endocrine deficiency (Diabetes), Use of catheters and unsterile needles.[2]

Rapid identification of Candida isolates to the Species level in the clinical laboratory has become more important because of the incidence of candidiasis. It has been on rise in proportion to an increasing number of patients at risk for infection with Candida albicans and recently, with innately azole resistant non-albicans Candida species.[3] Difference in expression of putative virulence

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factors and in antifungal susceptibility among different Candida species has raised the need for species level identification.[4]

MATERIALS AND METHODS: 400 clinical samples from patients attending outpatient and inpatient departments of Government General Hospital, Kurnool were processed in the microbiology department over a period of one year. 100 samples from healthy persons who had no signs and symptoms suggestive of candidiasis were included as controls. Out of 400 samples in the study group 100 yielded Candida species. Out of 200 controls samples 10 yielded Candida species.

SPECIMEN COLLECTION AND PROCESSING: Various clinical samples were collected from the study group which includes oral lesions, throat swab, urine sample, high vaginal swabs, scraping from skin & nail bed. Samples were inoculated onto sabouraud's dextrose agar media with antibiotics and incubated at 25^0C for 2days. Cream colored, smooth and pasty colonies were selected; gram staining and further biochemical tests were performed. Gram staining was performed from the culture isolates and observed the blastoconidia, hyphae and pseudohyphae seen as gram positive. Germ tube test was also done. An isolated colony from the sabourand dextrose agar was inoculated on a plate of cornmeal agar to identify the blastoconidia, arthoconidia pseudohyphae, hyphae or chlamydoconidia. An isolated colony was inoculated on Hichrome candida differential agar for the growth of colonies with characteristic colors for different species. Carbohydrate fermentation test were done by using Glucose, Lactose, Sucrose and Maltose. Carbohydrate Assimilation Test: Yeast Nitrogen base was used for testing assimilation using carbohydrate discs Glucose, Maltose, Sucrose, Lactose Galactose, Trehalose and raffinose. Antifungal susceptibility was tested for amphotericin B, nystatin, fluconazole and clotrimazole. For antifungal susceptibility testing of azoles yeast nitrogen base with glucose and asparagine is used, for amphotericin B and nystatin, yeast nitrogen base with glucose and without asparagine was used.

RESULTS: In the present study a total of 500 clinical samples which included 400 study group and 100 control group were processed. There were 160 males and 240 females. Age varied between 10 to 60 years.

Sample group	Number of cases studied	Number of Isolates	Prevalence
Study group	400	100	25%
Control group	100	7	7%

Table 1: Prevalence of Candidiasis
P value- 0.000087 (< 0.05), Statistically significant

Study group yielded 100 Candida species and 7 were isolated from control group. Candida albicans was found to be the predominant species among the various clinical samples. Out of 100 isolates 49 were Candida albicans (49%) 51 (51%) were non albicans Candida (Table: 2). Candida. albicans (49%) was the predominant species isolated, followed by Candida tropicalis (20%), wheras isolates of other species varied between 3-9% (Table:3).

A total of 110 patients had associated predisposing factors out of 400 in the present study. Diabetes mellitus was present in 20 patients out of them, Candida species were isolated in 12 patients

(60%). 25 females had the history of using oral contraceptives for duration of 4months to1year.13 (52%) of them yielded Candida species.12 (24%) Candida isolates were obtained from 50 pregnant women included in the study.40% of association was seen in patients with immune suppression and HIV (Table: 4).

Candida albicans isolates were susceptible to amphotericin B and nystatin whereas susceptibility to fluconazole and clotrimazole was 85.71%, 81.63% respectively. Among non-albicans Candida, susceptibility to amphotericin B and fluconazole varied from 66.66% to 100%, clotrimazole between 33.33% to 75% and Nystatin varied from 87.5% to 100% (Table:5).

DISCUSSION: 100 samples which were positive for culture of Candida species out of 400 samples were processed and further speciated. In the present study, prevalence of candidiasis was 25% which includes 19.62% in Urine, 25.23% in sputum correlating with study by L.Srinivasan[5] 34.73% in vaginal swabs which is comparable with studies like 33.1% by Saporiti et al[6,] 32.4% by abbot J et al,[7] 35% from oral and throat samples which is slightly more than 21.5% obtained in a study by Arnaldo L et al.[8]

In our study most common species isolated was Candida albicans (49%) followed by Candida tropicalis (20%) Candida glabrata (9%), Candida kefyr (8%) Candida krusei (8%), Candida parapsilosis (3%) and Candida dubliniensis 3% similar observations were documented by L. Srinivasan et.al. In the study by Abbas Ali Jafari et.al 61.47%, 14.93%, 8.87%, 6.70%, 3.03%, 2.38%, 2.16% of Candida albicans, Candida tropicalis, Candida parapsilosis, Candida glabrata, Candida Krusei, Candida guilliermondi, Candida kefyr were isolated which is comparable with our study.

In the present study, CHROM agar Candida identified all Candida albicans, Candida tropicalis, Candida glabrata, Candida parapsilosis, Candida dubliniensis and Candida krusei correctly which correlates with study by Willinger B et al,[9] Yucesoy M et al[10] Momani OM et al[11] and Gultekin et al.[12]

In our study antifungal susceptibility showed 100% susceptibility for amphotericin B by Candida albicans, 70% by Candida tropicalis, 66.66% by Candida parapsilosis 88.88% Candida glabrata, 75%, Candida kefyr & Candida krusei and 100% by Candida dubliniensis.

CONCLUSION: From the study group of 400, Candida was isolated from 100 samples out of which 49% was Candida albicans and 51% non-albicans Candida. Our study shows that Candida albicans was the most predominant species isolated. There is an increase in the prevalence of non-albicans Candida. Prevalence of Candida was found to be higher in patients associated with predisposing factors. Candida albicans showed maximum susceptibility to amphotericin B followed by nystatin and fluconazole. Resistance to fluconazole was more pronounced in non-albicans Candida than Candid albicans.

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Species	Numbers	Percentage
Candida albicans	49	49
Candida tropicalis	20	20
Candida glabrata	09	09
Candida kefyr	08	08
Candida krusei	08	08
Candida parasilosis	03	03
Candida dubliniensis	03	03
Total	**100**	

Table 2: Species distribution of Candida in candidiasis

Species	Oral& throat samples	Urine Samples	Vaginal Swabs	Sputum	Stool
Candida albicans	2 (28.57%)	18(58.06%)	16(48.48%)	12(44.44%)	1(50%)
Candida glabrata	_	2 (6.45%)	4 (12.12%)	3 (11.11%)	0
Candida krusei	_	4 (12.90%)	2 (6.06%)	2 (7.40%)	0
Candida dubliniensis	2 (28.57%)	0	1 (3.03%)	0	0
Candida parapsilosis	1 (14.28%)	0	2 (6.06%)	0	0
Candida tropicalis	1 (14.28%)	4 (12.90%)	4 (12.12%)	10 (37.03%)	1 (50%)
Candida kefyr	1 (14.28%)	3 (9.67%)	4 (12.12%)	0	0
Total	**7**	**31**	**33**	**27**	**2**

Table 3: Species distribution of Candida isolates in various samples

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Predisposing Factor	Number of Samples	Positive Samples	Percentage
Diabetes Mellitus	20	12	60%
OCP(oral Contraceptive)	25	13	52%
Pregnancy	50	12	24%
Immune Suppression	10	4	40%
HIV	5	2	40%
Total	**110**	**45**	

Table 4: Predisposing Factors

	Amphotericin B		Fluconazole		Clotrimazole		Nystatin	
	S	R	S	R	S	R	S	R
Candida albicans n=49	49 (100%)	—	42 (85.71%)	7 (14.28%)	0 (81.63%)	9 (18.36%)	49 (100%)	—
Candida tropicalis n = 20	14 (70%)	6 (30%)	16 (80%)	4 (20%)	15 (75%)	5 (25%)	9 (95%)	1 (5%)
Candida glabrata n = 9	8 (88.88%)	1 (11.11%)	7 (77.77%)	2 (22.22%)	5 (55.55%)	4 (44.44%)	9 (100%)	—
Candida kefyr n = 8	6 (75%)	2 (25%)	8 (100%)	—	6 (75%)	2 (25%)	8 (100%)	—
Candida krusei n= 8	6 (75%)	2 (25%)	—	8 (100%)	4 (50%)	4 (50%)	7 (87.5%)	1 (12.5%)
Candida parapsilosis n n= 3	2 (66.66%)	1 (33.33%)	3 (100%)	—	2 (66.66%)	1 (33.33%)	3 (100%)	—
Candida dubliniensis n = 3	3 (100%)	—	2 (66.66%)	1 (33.33%)	1 (33.33%)	2 (66.66%)	3 (100%)	—

Table 5: Antifungal susceptibility of Candida species

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