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Laboratory Identification Of Candida Species From Various Clinical Specimens By Convention Method And Hichrom Agar

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Abstract:
Background: *Candidiasis is one of the most common fungal disease of human caused by several species of Candida. Candida spp especially non albicans candida are increasingly being isolated from clinical samples. The epidemiology and susceptibility to antifungals may vary with different species of Candida, accurate and rapid etiological identification of Candida spp is important to provide adequate therapy. The present study was conducted to study the distribution of Candida albicans and non albicans Candida from various clinical samples and to speciate Candida using Hi Chrom Candida differential agar.*
Material & Methods: *A total of 57 Candida species isolated from various clinical samples in our institute from Januauary 2021 to December 2021 were included in the study. The preliminary diagnosis was made by wet mount, Gram stain, Culture on Sabouraud's dextrose agar and negative urease test. Hichrom Candida differential agar were used for speciation of Candida.*
Results: *Total 57 isolates of Candida species were found of which > 60 years age group was the most common age group and female predominance was seen in the present study .Maximum number of candida species isolated from Sputum sample(42.10%) followed by Urine(22.80%). Prevalence of Candida albicans was 24.56%.and Non-albicans candida were 75.43% in our study. Among Non albicans Candida , C.tropicalis 35.08% were the major isolates followed by C.glabrata 21.05%,C. parapsilosis 14.03%% , and C. krusei 5.2%% .*
Conclusion: *Characterization up to species level is important for early treatment decisions and effective management as some NAC species are intrinsically resistant to antifungal agents. Hichrom Candida Differential agar is useful for the primary isolation and differentiation of medically important Candida species.*
Keywords: *Speciation , Candida albicans , Non Albicans Candida ,Hi chrome Candida Differential agar,*

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I. Introduction

Candida spp is the only opportunistic fungi that exist both as a commensal and pathogen[1].Candida spp are the most common opportunistic pathogens that cause severe infections in the immunosuppressive host[2].Candida spp infections have various clinical manifestations from superficial mucocutaneous disorders to an invasive infection affecting multiple organs[3].The increasing incidence of HIV infections, use of steroids and broad spectrum antibiotics, organ transplantations ,advanced life supports and prosthetic devices, all have led to the increasing incidence of Candida spp[4]. Candidaspp are commonly found in the hospital environment in air and various surfaces. They could also be detected on the hands of healthcare personnel that could lead to nosocomial spread among patients[5].

Infections caused by the genus Candida are the main cause of nosocomial fungal infections especially in tertiary care hospitals[6]. There are currently more than 150 species of Candida and approximatlely 20 are known to cause infections in humans [7]. *Candida albicans* is the most frequently reported species causing human infection, but other species are also reported : *C glabrata, C parapsilosis ,Candida tropicalis* and *C krusei,* among others. Although *Candida albicans* still remains responsible for the most yeast infections, Non-albicans species (NAC) appear to be increasing in prevalence[8].As NAC spp significantly vary in their prevalence as per country and health care setups within the country, species identification also plays an important role in formulation of local therapeutic guidelines[9].Hence this study has been undertaken to study the distribution of

Candidaalbicans and Nonalbicans Candida spp from various clinical samples and to speciate Candida using Hi Chrom Candida differential agar.

II. Material And Methods:

This was a prospective study conducted in the Department of Clinical Microbiology of Shridevi Institute of Medical Sciences and Research Hospital, Tumkur, Karnataka from January 2021 to December 2021. All the clinical samples such as Sputum, Urine, High Vaginal swab, Pus submitted to the Microbiology laboratory suspected of fungal infection during the study period were included.Approval of ethical committee was obtained from Institutional ethical committee.

Inclusion Criteria: Patients of all age group and both sex with clinically suspected fungal infection including inpatients and outpatients

Exclusion Criteria:1.Patients who had taken antifungal drug during the last week.

2. Isolates diagnosed to be fungus other than Candida species.

Gram's Stain and Wet mount was performed from direct samples. It was inoculated on Blood agar and Sabourad's dextrose agar, incubated at 37^0C for 24 - 72 hours.Creamy, pasty and yeasty colonies on Sabouraud's dextrose agar and Gram positive budding yeast like cells with pseudohyphae on microscopic examination and negative urea hydrolysis test were processed further as clinically suspected Candida species. Germ tube test was performed[10].

The Isolated *Candida spp*. were inoculated on Hi Chrome Candida differential agar (Hi-media ,India) and incubated at 37^0C for 24 - 48 hours and the species were identified by type and colour of the colonies on Hi Chrome Candida differential agar media as per manufacturers instruction.

Staistical analysis: Data was entered in Microsoft Excel. Qualitative data was described using proportion and percentages.

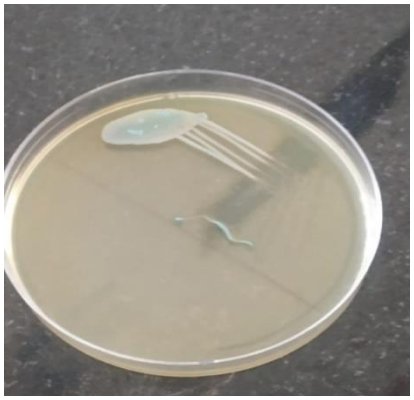

Fig 1: Light blue colored colonies of *Candida tropicalis* on HiChrom agar.

Fig 2: White coloured colonies of *Candida glabrata* on HiChrom agar

III. Results

Out of 185 clinically suspected fungal infection, a total 57 Candida spp was isolated from various clinical samples in our institute over a period of one year . Gender wise distribution showed that 32(53.01%) candida isolates were from females and 25(43.85%) were from males**(Table 1)**.Of the 57 isolates 23(40.35%) were obtained from the age group of > 60yrs and least number 2 (3.50%) isolates was seen in age <10rs**(Table 2)**.Majority of the isolates were from sputum 24(42.10%) followed by Urine 13(22.80%) and Blood 10(17.54%)**(Table 3)**.*Candida albicans* comprised of 14(24.56%) whereas the Non Albicans Candida spp comprised of 43(75.43%)**(Table 4)**.Maximum isolate of *Candida albicans* were from sputum 6(42.85%) followed by blood(28.57%). Where as Majority of Non Albicans Candida spp were from Sputum 18(41.86%) followed by Urine 11(25.58%)**(Table 5)**. Among NAC spp *Candida tropicalis* 22(35.08%) was the most predominant species and least was *Candida krusie* 03(05.26%) **(Table 6).**

Table 1: Gender wise distribution of isolated candida species from various clinical samples

Sl.No	Gender	Total Count	Percentage
1	Male	25	43.85%
2	Female	32	53.01%
	Total	57	100%

Table 2: Age wise distribution of Candida spp isolated from various clinical samples

Sl.no	Age group (yrs)	No. of Samples	Percentage
1	<10	2	3.50%
2	11-20	5	8.77%
3	21-40	11	19.29%
4	41-60	15	28.07%
5	>60	23	40.35%
	Total	57	100%

Table 3: Sample wise distribution of isolated Candida spp from various clinical samples

Sl.No	Sample Type	No. of Samples	Percentage
1	Blood	10	17.54%
2	Ear Swab	1	1.75%
3	Body Fluids	3	5.26%
4	Sputum	24	42.10%
5	Vaginal Swab	6	10.52%
6	Urine	13	22.80%
	Total	57	100%

Table 4: Prevalence of Candida spp and Nonalbicans from various clinical samples.

Sl.no	Candida spp	Number	Percentage
1	*Candida albicans*	14	24.56
2	*Non albican Candida*	43	75.43

Table 5: Distribution of *Candida albicans* and *Non albicans Candida(NAC)* from various clinical samples.

Sl.No	Sample Type	No. of Samples	*Candida albicans*	Percentage of *C.albicans*	*Non albicans Candida*	Percentage of NAC
1	Blood	10	04	28.57%	06	13.09%
2	Ear Swab	01	0	0%	01	2.32%
3	Body Fluids	03	01	7.14%	02	4.65%
5	Sputum	24	06	42.85%	18	41.86%
6	Vaginal Swab	06	01	7.14%	05	11.62%
7	Urine	13	02	14.28%	11	25.58%
	Total	57	14	100%	43	100%

Table 6: Distribution of Candida spp on Hi Chrom Candida differential agar

Sr no	Candida species	No of isolates	Percentage
1	*Candida albicans*	14	24.56%
2	*Candida tropicalis*	20	35.08
3	*Candida glabrata*	12	21.05
4	*Candida parapsilosis*	08	14.03
5	*Candida krusie*	03	05.26%

IV. Discussion

Candida species are the most common cause of fungal infections world wide. They are the third most dominant cause of health care associated infections[11] .The increased incidence of systemic mycoses caused by Candida species in hospitalized patients is an important cause of morbidity and mortality world wide, especially in critically ill patients[12] .High mortality rate (30-50%) along with raising resistance to antifungal agents among NAC species imposes serious ,medical, and economic problems to the society[13].

In the present study, Candida spp isolates were found to be highest in females 56.14% with afemale to male ratio of 1.3:1 which is similar to the study by Dharwad S et al [14]with female preponderance. The reason for this disparity may be due to the higher number of females selected for the study.The isolation rate of Candida spp was highest in the age group more than 60yrs (40.35%).Similar findings were observed by Shwetha DC et al[15] and Liu et al[16] in their studies. Predominance of Candida species in elderly group might be due to the presence of significant co morbid conditions .The highest number of candida isolates were obtained from sputum (42.40%) followed by urine (22.80%) and blood (17.54%) in our study which is similar to the study conducted by Chongtham U et al[17] where maximum isolates were obtained sputum (43%) followed by urine (34%). Roopa C et al[18] in their study observed that highest number of isolates were from High vaginal swabs(38%) followed by sputum(32%). Whereas study by Shukla et al[19] observed that maximum candida spp isolates were seen in blood (75%).Gopi A and Murthy NS[20] also observed that predominant isolates were from Sputum(41.6%) and Urine(20.4%).However studies by ShaikN et al[21] and Joseph K et al [22]recovered maximum number of isolates from urine(60% an 46.9% respectively) followed by respiratory samples (17.3% and 20.4% respectively.

HiChrom agar is a commercially available selective and differential media for isolation and identification of Candida spp.This medium contains chromogenic substrates that react with species specific enzymes secreted by yeast cells , resulting in development contrasting colored colonies.Chromogenic medium facilitates presumptive identification of yeast isolates upto the species level within 24hrs of incubation[1].In this study only 24.56 % isolates were *Candida albicans* and rest of the isolates were NAC spp which was similar to the studies by Deepthi KN et al Shukla et al and Chongtham U et al .[23,19,17]Incidence of nonalbicans ranging from 54-74% have been reported in numerous studies[24,25]. In contrast studies conducted by Khannna BV et al and Liu et al reported that Candida albicans was the predominant isolates[26,16].

In the present study among NAC spp , *Candida tropicalis* 20 (35.08%) was the most predominant species followed by *Candida glabrata* 12 (21.05%)which is similar to the studies conducted by Bhatacharjee et al and Chakrabarthy et al[27,28]. Studies conducted by Western countries , *Candida glabrata* and *Candida parapsilosis* formed the predominant NAC species[29].Limitation of this study was inability to do antifungal susceptibility tests.

V. Conclusion

There has been a significant increase in infections caused by NAC spp. The trend in the resistace acquired by the NAC spp leads to the importance of identification of Candida to the species level. Hi Chrom Candida differential agar is a simple ,rapid ,inexpensive valuation method for identification of Candida spp even in resource poor settings.

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