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Research Paper

Prevalence of Candida Species in the Clinical Samples in a Tertiary Care Hospital

Medical Science

KEYWORDS : Candida albicans, Non-albicans Candida, HiCrome Candida Agar, Candidiasis

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ABSTRACT

Candidiasis is the commonest fungal disease found in humans. Candida albicans is the representative species. But in recent times, Non albicans Candida is gaining medical importance as it is causing persistence of infection, thus increasing the morbidity and mortality. Therefore, this study was undertaken to speciate the Candida isolates from the inpatients in various Departments attached to the Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai ,using HiCrome Candida agar and conventional methods.

Summary : Non albicans Candida accounted for 86% as compared to Candida albicans which was isolated in only 14%,out of 50 Candida isolates. C.tropicalis had a higher prevalence rate of 30% followed by C.glabrata and C.krusei with a prevalence rate 22%.

Introduction

Candida is a yeast like fungus, found as a part of the normal microbiota, is responsible for the various clinical manifestations from mucocutaneous overgrowth to bloodstream infections. Though the more common species *C.albicans,*were responsible for about 70-80% of all *Candida* infections, dramatic alterations in the prevalence of different *Candida* species and emergence of newer species have occurred during the past few decades especially in immunocompromised patients and/or those hospitalized with serious underlying diseases [1, 2,3].Therefore, this study was undertaken to identify the prevalence of the various *Candida* species in the samples collected from infection sites.

Objectives

To isolate the fungus *Candida* from the various clinical samples.

To identify and speciate *Candida* isolates using conventional tests and HiCrome *Candida* agar.

Methodology

Study design

This cross-sectional study was undertaken among 50 non-repetitive consecutive *Candida* isolates from the inpatients admitted for a period of three months in various Departments attached to the Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai, after obtaining approval from the Institutional Ethics Committee.

Statistical analysis

Association between prevalence and clinical variables was analysed using SPSS and Epi Info statistical software. All statistical tests were two tail tests. Results were given in properties with 95% Confidence intervals(CI).

Sample Processing

Samples like Bronchoalveolar lavage(BAL), sputum, urine, tongue scraping, blood, gastric lavage, aural swab and other body fluids were collected under aseptic precautions in a sterile wide mouthed container, transported and processed as per standard operating procedure of the Microbiology laboratory.

Identification of *Candida* species

Gram's stain

All the inoculated culture plates were inspected for tiny, dry or dull creamy white colonies at 24hours and 48hours. On microscopic examination by Gram's stain, *Candida* showed Gram positive oval budding yeast cells with or without pseudohyphae, under oil immersion objective lens of light microscope.[1] Other tests to identify the *Candida* yeast were as follows;

Germ tube test

A small inoculum of yeast cells obtained from an isolated colony is suspended in 0.5 ml of human serum and incubated at 35-37°C for no longer than 3 hours. After incubation, a drop of suspension was observed microscopically for the presence of germ tubes under low power microscope.If positive within 2 hours, it was considered as *C.albicans* or C.*dubliniensis*[1, 4].

HiCrome *Candida* agar

Isolates were subcultured twice on Sabouraud dextrose agar prior to inoculation on HiCrome *Candida* agar chromogenic media from Himedia. A single yeast colony was streaked on to the plates and incubated at 37°C in the dark. The results were read after 48 hours. Identification of the most commonly isolated *Candida* species was done based on the colour of colonies obtained as indicated by Himedia as follows: *Candida albicans* - Light green; *Candida tropicalis* -Steel blue with pink halo; *Candida krusei* – Pink;*Candida parapsilosis* -Cream coloured; *Candida glabrata* -Purple; *Candida dubliniensis*- Dark green; *Candida guilliermondii* - Pale pink to purple.[5]

Sugar Fermentation Test

A loopful of 24-48 hours culture from a sugar free media was suspended in a sterile distilled water.0.2 ml of this suspension was added to 2 % sugar fermentation media with bromothymol blue indicator. Inoculated sugar tubes with Dextrose, Lactose, Sucrose and Maltose sugars were incubated at 30°C for 48 to 72hours.The ability to ferment a sugar was noted by the presence of acid and gas trapped in Durham's tube.[6]

Sugar Assimilation Test

A yeast suspension was made from a 24-48 hours culture, grown in a sugar free media, into 2 ml of yeast Nitrogen base by adding a heavy inoculum. The suspension was added to 18ml of molten agar cooled to 45°C and mixed well and the entire volume was poured into a 90mm sterile Petri dish. The plate was allowed to set at a room temperature until the agar surface hardens. With the help of sterile forceps, discs (Himedia) containing sugars like dextrose, sucrose, lactose, raffinose, trehalose were placed on the surface of the inoculated agar and incubated at 30°C for 24-48hours and observed for the growth of yeast around the sugar discs, indicating assimilation of that particular carbohydrate. Each *Candida* species utilizes specific carbohydrate substrate and the characteristic carbohydrate profiles were used to identify the species[7].

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Observation and Results

Table 1: Age&Genderwise distribution of cinical samples (n=50)

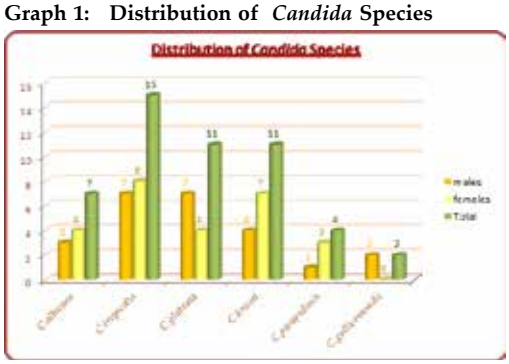
AGE GROUP (YEARS)	NO. OF SAMPLES (%)	MALE (%)	FEMALE (%)
11-20	7(14)	2(4)	5(10)
21-30	5(10)	3(6)	2(4)
31-40	9(18)	4(8)	5(10)
41-50	10(20)	3(6)	7(14)
51-60	9(18)	3(6)	6(12)
61-70	5(10)	5(10)	0(0)
71-80	5(10)	24 (48)	26(52)

Table 2: Distribution of *Candida* species in various samples (n=50)

Species	Urine	Sputum	BAL	Gastric lavage	Tongue scraping	Blood	Aural Swab	No. of Samples	%(Confidence Interval)
C.albicans	**4**	**0**	**1**	**0**	**1**	**0**	**1**	**7**	**14 (6-27%)**
C.tropicalis	**12**	**1**	**2**	**0**	**0**	**0**	**0**	**15**	**30 (18-45%)**
C. glabrata	**8**	**1**	**1**	**1**	**0**	**0**	**0**	**11**	**22 (12-36%)**
C. krusei	**8**	**1**	**1**	**0**	**0**	**1**	**0**	**11**	**22 (12-36%)**
C.parapsilosis	**4**	**0**	**0**	**0**	**0**	**0**	**0**	**4**	**8 (2-19%)**
C.gulliermondii	**2**	**0**	**0**	**0**	**0**	**0**	**0**	**2**	**4 (0-14%)**
Candida albicans **7** (14 %) (CI=6-27%)					Non *albicans Candida* (NAC) **43** (86%) (CI=73-94%)				

(Proportions given with 95% Confidence Interval(CI))

Graph 1: Distribution of *Candida* Species



Discussion

Among 50 *Candida* isolates collected from the inpatients, majority of the patients were females 26(52%) and 10(20%) belonged to the age group of 41-50years **(Table 1)**. This age preponderance might be due to the association with the predisposing factors found in this age group such as Diabetes mellitus or iatrogenic factors such as the medications like steroids, oral contraceptives, omeprazole, tranquilizers etc[8,9,10] Due to the anatomical position of the female urethra, the females had increased susceptibility to urinary tract infections and genital tract infections. In women, Candiduria may be due to extension of *Candida* vaginitis.[9]

Majority of the isolates were from the Medicine Department 21(42%) followed by Nephrology Department 8(16%), Medical and Surgical Intensive Care Units 7 (14%), where critically ill patients had increased usage of intravenous lines, indwelling urinary catheters and other prosthetic devices, which support the growth of *Candida*. In Nephrology Department, many patients had stones in the urinary tract and were treated with stents, which had to be left insitu for atleast two weeks, which could lead to Candiduria. These observations correlate with the study conducted on *Candida* infections of Medical devices.[11]

In the present study, *Candida* was isolated from samples like BAL, sputum, urine, tongue scraping, blood, gastric lavage and aural swab. Majority of the samples showing Candida growth were from urine 38(76%), this might be due to the increased use of indwelling urinary catheters as well as hematogenous dissemination from other infected regions.[9-11]

The distribution of *Candida albicans* and *Non albicans Candida*(NAC) species showed that NAC accounted for 43 (86% - Interval range:73 - 94%) as compared to *Candida albicans* which was isolated in only 7 (14% - Interval range:6-27%) of patients.(Table2) On comparison of the gender distribution, NAC was common in females **(Graph1)** and on statistical analysis found to be statistically insignificant (X^2 =0.09;p=0.76). On comparison of the age distribution, NAC species was common in the age group greater than 40 years and on statistical analysis found to be statistically significant(X^2=2.89; p=0.09).

The results of the study conducted in India and Abroad showed the prevalence of *Candida albicans* as around 36%, [10,12] in some studies and whereas many other studies indicate the prevalence of *Candida albicans* as >40%,predicting a major variation in this study.[2,3,12] The reasons for the increased prevalence of Non *albicans Candida* species, might be that being a tertiary care hospital, most of the patients report late with terminally ill conditions, where high end antibiotics were used to save the life of

the patients. Improper and inappropriate use of antibiotics allows growth of Candida and cause systemic infection in immunocompromised condition, due to their great adaptability to different host niches.

Yet another fact noted was that prevalence of *Candida albicans* was on a decreasing pace rather considering it to be in a steady state. It was considered that the widespread indiscriminate use of antifungal drugs leads to the emergence of newer species and decreasing incidence of *Candida albicans.*

In the present study, HiCrome *Candida* agar was used to speciate the *Candida* isolates and found to be very useful, as it produced specific colour for the common *Candida* species, like *C.albicans, C.tropicalis, C.glabrata, C.krusei, C.parapsilosis* and *C.gulliermondii.* Conventional sugar fermentation and sugar assimilation tests were also performed which correlated with HiCrome *Candida* agar.

C.tropicalis was the most commonly isolated *Non albicans Candida* species[2, 13]. Among the NAC species **(Table 2)**, *C.tropicalis* had a higher prevalence rate of 30% (Confidence Interval range :18-45%) followed by *C.glabrata* and *C.krusei* with a prevalence rate 22% (Confidence Interval range:12-36%).

Conclusion

In concusion, this study clearly indicates that *Non albicans Candida* species is emerging as a serious pathogen than Candida albicans. Thus, the study provides an insight about the alarming increase in the *Non albicans Candida* infections. Hence, it is essential to have a complete knowledge about NAC for better management of the patients. It was also found that HiCHROMagar *Candida* was very useful and reliable in identifying the *Candida* species, like *C.albicans, C.tropicalis, C.glabrata, C.krusei.*

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