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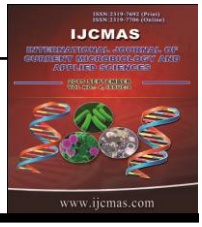

Original Research Article

Isolation of *Candida* and its Speciation in Various Samples in a Tertiary Care Hospital in North Karnataka, India

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Keywords

Candidiasis, *Candida albicans*, antifungal agents, Fluconazole, Ketoconazole

A B S T R A C T

The Candida species are the 4th most common organisms causing blood stream infection, and constitute 8% of all nosocomial infections. 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. The most important species which are considered pathogenic to humans are *C. albicans*, *C. tropicalis*, *C. kruseii*, *C. glabrata*, *C. lusitaniae* and *C.viswanathii*. All the suspected cases of candidiasis like oral thrush, vaginitis, skin and nail infections, diarrhoea, urinary tract infection, respiratory tract infection, diabetic and postoperative wound infections, endocarditis, meningitis, septicemia were included in this study. Samples collected include blood, urine, stool, sputum, oral swabs, vaginal swabs, wound swabs, pus, CSF, skin and nail samples and other body fluids. The preliminary diagnosis was made by wet mount, Gram's stain, culture on SDA and negative urease test. Direct examination of all samples was performed doing a wet mount and Gram's stain. For culture Sabouraud's dextrose agar (SDA) with Chloramphenicol and Gentamicin was used. Colonies were identified by the colony characters and by Gram's stain. Once the colonies were confirmed speciation was done by germ tube test, corn meal agar inoculation, sugar fermentation, sugar assimilation and CHROMagar Candida inoculation. Antifungal susceptibility testing was done by disc diffusion method. The antifungal agents used for disc diffusion method were Amphotericin B (10µg), Fluconazole (10µg), Nystatin (10µg) and Ketoconazole (10µg). In the present study, total of 312 various specimens were collected, out of which 136 (43.5%) Candida isolates were obtained. *Candida albicans* (69) was the most isolated species followed by *C. tropicalis* (39), *C. krusei* (19), *C. glabrata* (8) and *C. guillermondii* (1). All species of Candida isolates were susceptible to amphotericin B and nystatin. The next effective antifungal drug was ketoconazole with 84.6% (115) Candida isolates susceptible to it. Fluconazole was least effective with only 55.8% (76) isolates susceptible to it. Identification of Candida isolated from various clinical specimens and speciation has become increasingly important as the changing epidemiology of Candida infections calls for monitoring of species distribution and susceptibility of Candida in order to successfully manage such cases.

Introduction

Candida is an yeast like fungus causing commonest fungal infections. It is a normal inhabitant in the skin, mucous membrane of oral cavity, gastrointestinal tract, respiratory tract and genitourinary tract and may invade other parts of the body particularly in immunocompromised individuals. Many changes in the internal and external environment induce this harmless saprophyte to become a true pathogen. These predisposing factors include ageing, pregnancy, AIDS, diabetes, steroidal therapy and Candida infection can also occur secondary to bacterial infections. Candidiasis is usually endogenous in origin. The spectrum of disease caused by candida is extensive. The range of manifestations extends from simple mucosal colonization to multiple organ invasion or invasive candidiasis in the neonate and elderly and can also cause nosocomial infections.

There has been an increase in treatment failure of candidiasis, because of drug resistance. Change in drug susceptibility of different species of *Candida* and the introduction of newer antifungal agents has made the in vitro susceptibility testing of antifungal agents more important which helps in rational use of the same.

The *Candida* species are the 4th most common organisms causing blood stream infection, and constitute 8% of all nosocomial infections. 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. The most important species which are considered pathogenic to humans are *C. albicans*, *C. tropicalis*, *C. kruseii*, *C. glabrata*, *C. lusitaniae* and *C.viswanathii* (Shivaprakash *et al.*, 2007).

This study has been undertaken to identify the most common *Candida* species in various clinical specimens and to study the distribution of *Candida albicans* and non-albicans *Candida* species in clinical specimens and to determine the anti-fungal susceptibility pattern of *Candida* species.

Materials and Methods

Present study was carried out in the department of Microbiology, in a tertiary care hospital in north Karnataka for a period of one year from April 2013 to April 2014. All the clinical samples submitted to the microbiology laboratory suspected of fungal infection during the study period were included. All the suspected cases of candidiasis like oral thrush, vaginitis, skin and nail infections, diarrhoea, urinary tract infection, respiratory tract infection, diabetic and postoperative wound infections, endocarditis, meningitis, septicemia were included in this study. Samples collected include blood, urine, stool, sputum, oral swabs, vaginal swabs, wound swabs, pus, CSF, skin and nail samples and other body fluids. The preliminary diagnosis was made by wet mount, Gram's stain, culture on SDA and negative urease test. Isolates diagnosed to be fungus other than Candida species were excluded from the study. Direct examination of all samples was performed doing a wet mount and Gram's stain. For culture Sabouraud's dextrose agar (SDA) with chloramphenicol and gentamicin was used. Colonies were identified by the colony characters and by Gram's stain. Once the colonies were confirmed speciation was done by germ tube test, corn meal agar inoculation, sugar fermentation, sugar assimilation and CHROMagar Candida inoculation. Antifungal susceptibility testing was done by disc diffusion method as recommended by CLSI M-44A guidelines on Mueller Hilton agar (CLSI, 2009). The

antifungal agents used for disc diffusion method were amphotericin b (10μg), fluconazole (10μg), nystatin (10μg) and ketoconazole (10μg).

Results and Discussion

In the present study, total of 312 various specimens were collected, out of which 136 (43.5%) Candida isolates were obtained. The highest number of candida isolates were obtained from high vaginal swabs (38) followed by sputum (32) and oral swabs (20).The distribution of candida isolates in various specimens is displayed in Table 1.

Candida albicans (69) was the most isolated species followed by *C. tropicalis* (39), *C. krusei* (19), *C. glabrata* (8) and *C. guillermondii* (1). The distribution of different *Candida* species is displayed in Table 2.

All species of candida isolates were susceptible to amphotericin B and nystatin. The next effective antifungal drug was ketoconazole with 84.6% (115) Candida isolates susceptible to it. Fluconazole was least effective with only 55.8% (76) isolates susceptible to it. The antifungal susceptibility of all isolates is displayed in Table 3.

Speciation of *Candida* isolates were done by sugar assimilation and sugar fermentation tests but we found that CHROMagar candida is an effective and fast screening agar which can be used for speciation of *Candida*.

The frequency of fungal infections caused by *Candida* species, has amplified over the past few years, especially in immunocompromised patients. There is also an increase in the frequency of non albicans species causing infection commonly *Candida tropicalis, Candida glabrata, Candida krusei*, and *Candida guillermondii*. In our study, incidence of candida isolation was 43.6% which correlates with study done by Mohandas *et al.* (2011).

In the present study 50.7% of the Candida isolates were *Candida albicans* followed by *C. tropicalis* (28.6%), *C. krusei* (13.9%), *C. glabrata* (5.8%) and *C. guillermondii* (0.73%). These findings co relate with study done by Mohandas *et al.* (2011)

Chromogenic agar is a newer and more rapid method to speciate *Candida*, which contains enzymatic substrates that are linked to chromogenic compounds. When specific enzyme cleaves the substrate, the chromogenic substances produce colour. The action of different enzymes produced by yeast species results in color variation which is useful for the presumptive identification of some yeasts (Odds and Bernaerts, 1994).

In our study, speciation of *Candida* isolates were done by sugar assimilation and sugar fermentation tests but we found that CHROMagar *Candida* is an effective and faster screening method which can be used for speciation of *Candida*. In the present study CHROM agar could identify, *Candida albicans, Candida tropicalis* and *Candida glabrata*. Similar findings have been reported by different studies (Odds and Bernaerts, 1994; Golia *et al.,* 2013). The Chromogenic medium facilitates presumptive identification of yeast isolates upto the species level within 24hrs of incubation. Primary inoculation of the clinical specimen on chromogenic medium which are positive for yeast cells can be identified by direct Gram's stain. This can hasten the presumptive species identification of yeast in clinical specimens. This will allow early initiation of appropriate therapy.

Table.1 The distribution of *Candida* isolates in various specimens

Specimen	Number of isolates	Percentage
Vaginal swabs	38	27.94%
Sputum	32	23.52%
Oral swabs	20	14.70%
Urine	15	11.02%
Blood	4	2.94%
Stool	10	7.35%
Pus and wound swabs	11	8.08%
Skin scrapings	2	1.47%
Nail samples	1	0.73%
Body fluids	3	2.20%

Table.2 The distribution of different *Candida* species

Candida species	Number of isolates	Percentage
C.albicans	69	50.7%
C.tropicalis	39	28.6%
C.krusei	19	13.9%
C.glabrata	8	5.8%
C.guillermondi	1	0.73%
Total	136	

Table.3 Antifungal sensitivity pattern of *Candida* isolates

Candida species / **Antifungal agents**	*C. albicans* (n= 69)	*C. tropicalis* (n=39)	*C. krusei* (n=19)	*C. glabrata* (n=8)	*C. guillermondi* (n=1)
Amphotericin B	100%	100%	100%	100%	100%
Nystatin	100%	100%	100%	100%	100%
Ketoconazole	94.2%	82%	73.6%	50%	R
Fluconazole	65.2%	64.1%	31.5%	R	R

The antifungal susceptibility of the *Candida* isolates to Amphotericin B revealed that all the isolates were sensitive to it. Our study correlates with findings of similar studies done on candida (CLSI, 2009; Maria Fátima Sugizaki *et al.*, 1998). The next effective antifungal drug was Ketoconazole with 84.6% (115) Candida isolates susceptible to it. Fluconazole was least effective with only 55.8% (76) isolates susceptible to it. These findings correlate with study done by Ragini Ananth Kashid *et al.* (2011).

In conclusion, identification of *Candida* isolated from various clinical specimens and speciation has become increasingly

important as the changing epidemiology of *Candida* infections calls for monitoring of species distribution and susceptibility of *Candida* in order to successfully manage such cases.

We also found CHROMagar to be an effective screening tool for speciation of *Candida* isolates.

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