

Original Research Article

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Isolation and Speciation of *Candida* from Various Clinical Samples in a Tertiary Care Hospital

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ABSTRACT

Keywords

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Infections due to *Candida* species and other fungi have increased dramatically in recent years. *Candida* isolates from various clinical samples were collected and inoculated on SDA. Speciation was done by Germ tube test, Hichrom agar, and morphology on corn meal with Tween 80. 64 were culture positive. Of this, 47(73%) *C. albicans* and 17 (27%) non albicans *Candida* were isolated. Hichrom agar can be used for rapid, presumptive identification of *Candida* species.

Introduction

Infections due to candida species and other fungi have increased dramatically in recent years. (Fraser *et al.*, 1992) *Candida* is a yeast like fungi belongs to the sub class of Ascomycota. The genus is composed of a heterogeneous group of organisms, and nearly 20 of these species are considered to be significant pathogens of which 7 of them are well known pathogens to cause opportunistic infections.

Although *C. albicans* remains the most common cause of human candidiasis, now for the past four decades non-albicans candida species like *C. glabrata*, *C. krusei*, *C.*

parapsilosis, *C. tropicalis* and *C. rugosa*, are emerging as important opportunistic pathogen which have shown increased resistance to antifungal agents (Abi-said *et al.*, 1997). These candida species differ in their expression of putative virulence factors and antifungal Susceptibility (Baillie and Douglas, 1998). This necessitates the rapid species level identification of *Candida* as an essential task for the clinical mycology laboratory since it has a direct bearing on treatment decisions

Materials and Methods

About 64 *Candida* isolates from various clinical samples (Urine, Blood, High vaginal swab, Pus, Sputum) are taken up for the study

for a period of 3 months. A detailed clinical history was taken with regards to the age, sex, underlying disease/ conditions, Immunodeficiencies like Diabetes mellitus, Human Immunodeficiencies virus infection etc....., As per the standard operating Procedures all the clinical specimens were inoculated on blood agar and Mac Conkey agar Except blood samples which were inoculated in biphasic brain heart infusion broth. The culture plates are incubated aerobically at 37°C for 24 to 48 hours. The visual growth is Stained and the one which revealed gram positive budding yeast cells with or without pseudohyphae are confirmed as Candida. All the isolated Candida colonies are inoculated immediately on Sabourauds Dextrose Agar (SDA) and incubated at 37°C for 24- 48 hrs.

Raynauld's Braude phenomenon was observed by inoculating the colonies in 0.5 ml of human serum and incubating at 37 ° C for 1- 2 hrs. (Forbes *et al.*, 2007)

Inoculation on Hichrome agar media and incubated at 37 ° C for 24 – 48 hrs. The characteristic colour and morphology of the colonies were noted as per the manufacturer's instruction. (Hichromagar; Hi Media, Mumbai, India) (Baradkar *et al.*, 2010)

Formation of chlamydo spores was identified by Dalmau plate culture method in Corn meal agar with 1% tween-80 incubated for 2- 3 days at room temperature. (Ann P. Koehler *et al.*, 1999) Observed for the presence of true hyphae or pseudohyphae, blastoconidia, arthroconidia and Chlamydo spores

Results and Discussion

About 64 isolates were culture positive for Candida species on SDA plate. 27 urine samples, 17 high vaginal swabs, 13 pus samples, 4 sputum and 3 blood samples had

shown a growth of candida species (Table 1). Of this 23 (36%) were males and 41 (64%) were females (Table 2). Among the 64 isolates, Germ tube test was positive in 47 (73%) isolates and were identified as *C. albicans* (Table 3).

Out of the 64 isolates, 47 (73%) isolates were produced apple green colonies (*C. albicans*), 7(11%) pink colonies (*C. krusei*), 4(6%) white to pale pink colonies (*C. tropicalis*), 3 (5%) blue colonies (*C. parapsilosis*), and 3 (5%) purple colonies (*C. glabrata*) on Hichrom agar (Table 4).

On Corn meal agar with Tween-80, chlamydo spores were demonstrated in all 47 (73%) *C. albicans* isolates.

Fungal infection especially by *Candida* species, are becoming increasingly common. So their optimum identification and isolation will help the clinicians to know the pathogen and their susceptibility pattern. This will guide them to institute proper drugs thereby avoiding any treatment failures. Further the high level resistance to azoles among *non albicans Candida* isolates emphasizes the need for species directed treatment.

About 64 isolates were culture positive for Candida species on SDA plate. 27 urine samples, 17 high vaginal swabs, 13 pus samples, 4 sputum and 3 blood samples has shown a growth of candida species. Of this 23 (36%) were males and 41 (64%) were females. Among the 64 isolates, Germ tube test was positive in 47 (73%) isolates and were identified as *C. albicans*.

Growth was observed in 23 (36%) males and 41(64%) females indicating that women are at increased risk to develop UTI than men. (Koneman *et al.*, 2006) Of the 64 *Candida* isolates, 47 (73%) were *Candida albicans* and 17 (27%) were *non- albicans Candida*.

Table.1 Culture positivity of *Candida* species from various clinical samples

S. No	Nature of sample	No of samples shown growth on SDA	Percentage (%)
1.	Urine	27	54
2.	High vaginal swab	17	17
3.	Pus	13	15
4.	Sputum	4	8
5.	Blood	3	6
	Total	64	100

Table.2 Gender distribution of *Candida* species from various clinical samples

Gender	No of patients	Percentage (%)
Male	23	36%
Female	41	64%
Total	64	100%

Table.3 Distribution of *Candida albicans* and *Non albicans* isolates (n=64) based on Germ Tube Test

Germ Tube Test	No. (%) of isolates	<i>Candida</i> Species
Positive	47 (73%)	<i>C. albicans</i>
Negative	17 (27%)	Non- <i>albicans</i> <i>Candida</i>
Total	64 (100%)	

Table.4 Species distribution of the *Candida* isolates (n=64) by HiChrom agar

Species	Number of <i>Candida</i> isolates
<i>C. albicans</i>	47 (73%)
<i>C. krusei</i>	7 (11%)
<i>C. tropicalis</i>	4 (6%)
<i>C. parapsilosis</i>	3 (5%)
<i>C.glabrata</i>	3 (5%)

Among the 17 non-*albicans* *Candida* species, *C. krusei* 7 (11%) *C. tropicalis* 4 (6%), *C. parapsilosis* 3 (5%), and *C. glabrata* 3 (5%) were isolated. In our study *C. krusei* 7 (11%) was the most common non *albicans* species isolated. Whereas (Latiff *et al.*, 2004) reported that *C. parapsilosis* was the most common non *albicans* species accounting for 21% and 8% respectively. Whereas (Shivprakash *et al.*, 2007) (36%) and (Enwuru *et al.*, 2008) (18%) documented *C. tropicalis* was the most common

non *albicans* species. In this present study *C. dubliensis* was not isolated.

As PCR is expensive and not available at all places, it was observed in the present study that species identification of *Candida* can be done by using Hichrome agar (Mokaddas *et al.*, 2007) and the isolates were further confirmed by sugar assimilation and other biochemical tests. This indicates that Hichrome agar can be used at field level for rapid presumptive

identification. This medium also carries the potential of improving identification of *Candida* from mixed cultures

This study implies that Hichrom agar can be used for rapid, presumptive identification of *Candida species*, which improves the identification of mixed candidial infection. Since intrinsic resistance was observed in *non albicans Candida species*, speciation is in need of the day. Knowledge of the susceptibility pattern will prevent the resistance of available few antifungal agents.

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