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

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ABSTRACT

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.

Keywords: Candida, Hichrom agar, Germ tube test, Morphology, Speciation, Antifungal susceptibility

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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.

Raynaulds 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 chlamydospores 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 Chlamydospores

**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, chlamydospores 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 | Candida Species           |
|----------------|---------------------|---------------------------|
| Positive       | 47 (73%)            | *C. albicans*             |
| Negative       | 17 (27%)            | Non-albicans Candida      |
| Total          | 64 (100%)           |                           |

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

| Species            | Number of *Candida* isolates |
|--------------------|-----------------------------|
| *C. albicans*      | 47 (73%)                    |
| *C. krusei*        | 7 (11%)                     |
| *C. tropicalis*    | 4 (6%)                      |
| *C. parapsilosis*  | 3 (5%)                      |
| *C. glabrata*      | 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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