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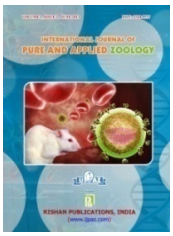

ISOLATION AND RAPID IDENTIFICATION OF CANDIDA SPECIES FROM THE ORAL CAVITY

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ABSTRACT

CHROMagar Candida is a new differential culture medium that allows the isolation and presumptive identification of species of yeast of clinical importance. During the past two decades, there has been a significant increase in the prevalence of fungal infections caused by *Candida* species. The aim of the study was to isolate and identify *Candida* species from the oral cavity with CHROMagar Candida. This study was carried out in 30 patients with infections aged 21 to 90 years. Swabs samples were taken from oral cavity and were cultured directly on Sabouraud dextrose agar medium. In this study shows that CHROMagar Candida can easily identify four species of Candida on the basis of colonial color and morphology, and accurately differentiate between them i.e. *Candida albicans, Candida glabrata, Candida tropicalis and Candida krusei.* Results showed the prevalence of *C.albicans* (n = 21, 70%), *C. glabrata* (n = 5, 16.6%), *C. krusei* (n = 2, 6.7%), and *C. tropicalis* (n =2, 6.7%). In this investigation, CHROMagar produced light green colonies and were considered as *Candida albicans*, Pink with a darker mauve center colonies were identified as *Candida glabrata,* pink with pale borders colonies were identified as *Candida krusei,* and dark blue, purple diffusion colonies were identified as *Candida tropicalis.* CHROMagar is extremely useful in making a rapid presumptive identification of common yeast species. This capability plus the ability to detect mixed cultures of *Candida* species promises to improve and streamline the work flow in the mycology and clinical microbiology laboratory.

Keywords: CHROMagar candida, oral cavity, *Candida albicans, Candida glabrata.*

INTRODUCTION

Candida species are considered as one of the most important causes of human infections. Candidacies are a common infection caused by yeast-like fungus (Chander, 2002). The oral cavity is inhabited by more than 700 microbial species and many intrinsic and extrinsic factors affect the composition, metabolic activity and pathogenicity of the highly diversified oral microflora (Samaranayake *et al.,* 2002; *Aas et al.,* 2005 and Zahir and Himratul-Aznita, 2013). *Candida* is not harmful in healthy hosts, but may cause opportunistic infections in immune-compromised hosts, such as patients suffering from AIDS, leukemia and diabetes (Batool *et al.,* 2011; Sayyada *et al.,* 2010 and Khaled *et al.,* 2006). It is generally believed that candidiasis arises from endogenous commensal strains inhabiting the oral cavity, gastrointestinal tract and genitourinary system (Cannon *et al.,* 1995 and Bharathi and Usharani, 2011).

Several brands of chromogenic media are available for rapid identification of yeast (Cooke *et al.,* 2002). These special media yield

microbial colonies with varying pigmentation secondary substrates that react with enzymes secreted by microorganisms. These media are specific, allowing the organisms to be identified to the species level by their color and colonial characteristics.

The manufacturer of CHROMagar Candida currently advertises its product as able to detect and differentiate many species, *C. albicans* by growth as light to medium green colonies, *C.tropicalis* by growth as steel blue colonies accompanied by purple pigmentation diffused into surrounding agar, and *C. krusei* by growth as large, fuzzy, rose colored colonies with white edges, after incubation for 48 hours at 37 °C, as also reported in several studies (Topley and Wilson, 2005). Use of chromogenic media in clinical microbiology laboratories for the isolation and presumptive identification of important Candida species is easy to perform, requires less time and is cost effective too (Pfaller *et al.,* 1996 andWillinger *et al*., 2001). In this study our goal was to evaluate the usefulness of CHROMagar Candida for detection and identification of major Candida species with accuracy to reduce the time of identification, and its characterization from poly fungal specimens.

MATERIAL METHODS

A total of 30 Candida species isolated from randomly selected 70 clinical specimens from the oral cavity swabs samples. The study was conducted from June 2011 to December 2012 for a period of six months, in the Gangasaras Diagnostic centre, Pattukkottai.

Preparation of CHROMagar Candida: CHROMagar Candida (Himedia) was prepared according to the manufacturer's instructions. CHROMagar Candida is composed of (per litre): peptone (10 g), glucose (20 g), agar (15 g), chloramphenicol (0.5 g) and "chromogenic mix" (2 g). Twelve grams of CHROMagar Candida powder which was added to 250 ml of sterile distilled water in a sterile Erlenmeyer flask. The suspension was completely dissolved by boiling (<100°C) and mixing. The medium dose not require sterilization by autoclave, therefore after cooling in a water bath to 45°C the agar was poured into sterile petri dishes (Odds and Bernaerts, 1994). After allowing cooling, the plates were stored at 4°C prior to use.

Samples were obtained by swabbing oral cavity area of buccal mucosa and tongue with a sterile cotton swab, then were plated onto Sabouraud's dextrose agar (SDA) (Himedia) and incubated at 37°C for 48 hours. Growth on SDA colonies were inoculated into germ tube test. Yeast colonies growing on each SDA tube were resuspended and 10 µL of suspension solution was used to inoculate plates with CHROMagar Candida agar medium. Inoculated plates were incubated at 37°C and read for up to 7 days. Plates were observed for fungal growth using morphology and colour to determine the presence of yeasts. As per the manufacturer, *Candida albicans, C.tropicalis, C.krusei* and *Candida glabrata* were identified by the production of green, dark blue, pink and Pink with a darker mauve center coloured colonies, respectively (www.chromagar.com).

RESULTS

A total of randomly selected 70 oral cavity swab samples were collected from the infected patients attending the various private Hospitals in Pattukkottai, Tamil Nadu. Out of 70 samples, Candida species were isolated from 30 oral swab samples (43.0%) (Table 1). This study was carried out in 30 patients with infections aged 21 to 90 years. All of the yeast isolates tested grew on CHROMagar Candida medium. After 24 hours of incubation at 37 °C, the majority of yeasts had grown well, forming colonies of 1 to 5 mm in diameter; however, growth and colony color development were inconsistent after 24 hours of incubation, and color readings were therefore made only after 48 hours of incubation, as specified in the manufacturer's instructions.

The result of this study shows that CHROMagar Candida can easily identified four important species of Candida on the basis of colonial color and morphology, and accurately differentiate between them i.e. *Candida albicans, Candida glabrata Candida tropicalis* and *Candida krusei.* The majority of *Candida* species amongst the Candida isolates were *Candida albicans* (70%), followed by *C. glabrata* (16.6%), *C. krusei* (6.7%) and *C. tropicalis* (6.7%).

In this investigation, all the isolates of *Candida albicans* formed light green to green colonies on CHROMagar (Figure 1). *Candida*

glabrata isolates formed Pink with a darker mauve center coloured colonies on CHROMagar Candida (Figure 4). After 48 hours, *Candida krusei* colonies were easily distinguishable from those of other yeasts that formed smooth, brownish pink to brownish purple colonies on CHROMagar Candida (Figures 2 and 3). *Candida tropicalis* isolates all developed a distinctive dark blue gray central color after 48 hours of incubation (Figure 4). *Candida* species were isolated from 21 male patients and only from 9 female patients. The highest rate of isolation of Candida was between the age of 50 and 90 (Table 2).

Figure 1. *Candida albicans.*

Figure 2. *Candida krusei.*

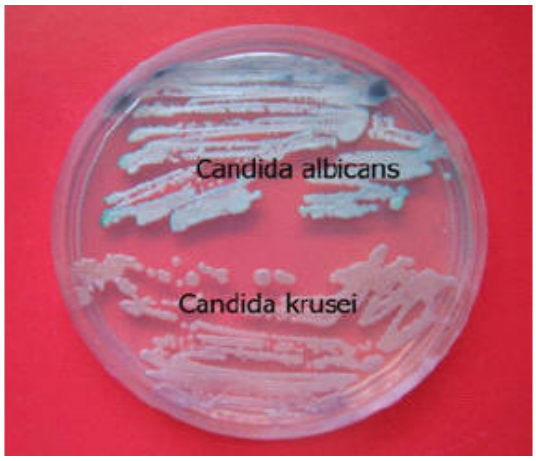

Figure 3. *C. albicans and C.krusei.*

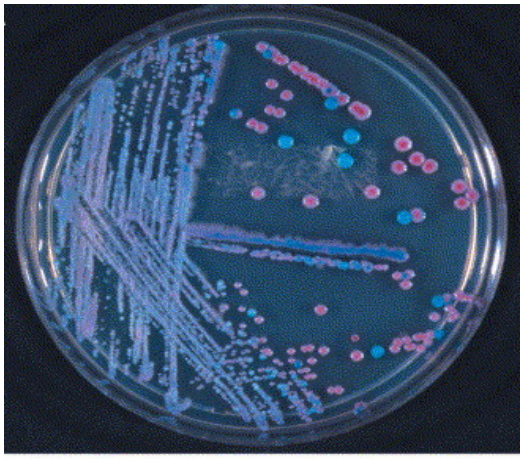

Figure 4. *Candida glabrata* (Pink)

Table 1. Different species of Candida species isolated from oral cavity.

Species	No. of isolates	% of Isolates	Colour on CHROMagar	Germ Tube
C.albicans	21	70%	Green	+
C. glabrata	5	16.6%	Pink with a darker mauve center	-
C.krusei	2	6.7%	Pink	-
C.tropicalis	2	6.7%	Dark blue	-

Table 2. Age distribution among which Candida species isolated.

Age (Years)	No. of isolates	% of isolates
21-40	3	10
41-60	12	40
61-80	11	36.7
Above 80	4	13.3
Total	30	100

DISCUSSION

The total numbers of 30 Candida species were isolated in the oral cavity. *Candida albicans* was found to be the predominant with 70% (21/30). In agreement with findings of others (Back-Brito *et al.,* 2009; Williams and Lewis, 2000), the majority of yeast isolates from oral cavity swabs were *C. albicans* (70%), but it was often recovered in association with other yeasts. This was followed by *C.glabrata* 16.6% (5/30), *C.krusei* 6.7% (2/30) and *C.tropicalis* 6.7% (2/30) (Table 1). Germain *et al.,* (2001) found the distribution of Candida species to be as follows: *C. albicans* 54%, *C.glabrata* 15%, *C.parapsilosis* 12%, *C.tropicalis* 9% and *C.krusei* 3%. Raju and Rajappa (2011) reported a similar pattern of distribution of species. The findings of the present study are more or less similar with the previous study. This could be due to variation in geographical distribution of various Candida species.

CHROMagar candida is one of the most widely used media in the mycology laboratory. Colony characteristics presented in Table 1 for identification of *C. albicans*, *C. glabrata, C. krusei* and *C. tropicalis* using CHROMagar were in agreement with previously published reports (Beighton *et al.*, 1995 and Hospenthal *et al.*, 2002). The sensitivity and specificity of CHROMagar andida media for identifying *C. krusei* and *C. tropicalis* were over 99 %. However, other Candida species C.*glabrata* also produce Pink with a darker mauve center coloured colonies on CHROMagar (Beighton *et al.*, 1995 and San Millan *et al.*, 1996). In the present study, the isolation rates of Candida species is high in ages ranging from 41-80 years old. The similar studies were conducted by Pinho (2002) and Zaremba *et al.* (2006), and found the isolation rates of Candida species to be high in ages ranging from 60-80 years.

CONCLUSION

C. albicans is the most frequently isolated yeast from the oral cavity infection patients. CHROMagar Candida is a useful culture medium for the isolation and direct identification of *Candida* species, especially *Candida albicans, Candida glabrata, Candida krusei* and *Candida tropicalis.* Easy to prepare, with low cost, CHROMagar Candida proves to be a useful medium for the identification of species of yeast that are isolated with greater frequency in clinical material and for the identification of mixed cultures.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this article.

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