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Isolation and speciation of *Candida* from various clinical samples using chrome agar in a tertiary care Hospital in Coimbatore

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Abstract

Most common causes of fungal infections worldwide are *Candida* species. It is the fourth leading cause of health care associated infections. *Candida* species have emerged as a major cause of human disease, especially among the immunocompromised and those hospitalized with serious co-morbid conditions. To isolate and identify the various *Candida* species from clinical samples by using chromogenic media. Various samples received in laboratory from patients of all age group and both sexes with suspected *Candida* infection were included in this study and the positive isolates were identified by using chromogenic media. 40 candida isolates were studied. 21-40 years was the most common age group affected and female predominance was seen. *C. tropicalis* was most commonly found followed by *C. albicans* species. It can be concluded from our results that the species level identification of the *Candida* isolates using chrome agar medium would enable the laboratories to rapidly identify and speciate the clinically important *Candida* species. This can greatly influence the treatment options for the clinician and may have an impact on the patient care that can potentially reduce the patient's morbidity and mortality.

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Introduction

The opportunistic yeasts belonging to the genus *Candida* have been associated with a wide range of human infections and significant mortality and morbidity. The clinical manifestations range from infections of the superficial skin to deep seated or disseminated candidiasis. Most common cause of fungal infections worldwide is *Candida* species. It is the leading cause of health care associated infections and the most common cause of central line-associated bloodstream infections (Sajjan *et al.*, 2014).

Candida species are the members of the normal flora of skin, mucous membrane, and gastrointestinal tract. They are endogenous opportunists which cause secondary infection in individuals with some underlying immunocompromised conditions. As a result general risk factors for *Candida* infections are associated with compromised immune system like diabetes mellitus, febrile neutropenia, patients on chemotherapy, and patients on broad spectrum antibiotics, patients on steroid, post transplantation, malignancy, HIV infected people, pregnancy and extremes of age (infancy, old age).

The genus is composed of a heterogeneous group of organisms and more than 17 different *Candida* species are known to be the etiological agents of human infection. In the past decade majority of the infections were caused by *Candida albicans*. However, recently there has been an upsurge in infections caused by non-albicans species of *Candida*. It is necessary to identify *Candida* at species level as many non-albicans *Candida* have decreased susceptibility to antifungal agents.

Candida albicans and non-albicans species are closely related but differ from each other with respect to epidemiology, virulence characteristics, and antifungal susceptibility. All *Candida* species cause disease ranging from superficial infections such as oral thrush to invasive disease, yet they show differences in disease severity and susceptibility to different antifungal agents.

Candida species identification is therefore important for successful management. Speciation helps to understand the epidemiology of *Candida*, particularly the source and mode of transmission. This in turn facilitates the development of effective measures to prevent and control transmission of resistant pathogens (Pfaller, 1996).

Identification of yeast pathogens by traditional methods requires several days and specific mycological media. These methods are thus labor intensive and time consuming. Several brands of chromogenic media are available for rapid identification of *Candida* species.

Chrome agar is a new differential culture medium that allows the isolation and presumptive identification of yeast species of clinical importance rapidly. Hence the aim of this study was to rapidly isolate and identify the distribution of *Candida* species from various clinical samples at a tertiary care hospital (Jaggi *et al.*, 2014).

In this study, we evaluated the performance of a commercially available chromogenic *Candida* speciation media for the identification of medically important yeasts and yeast like organisms in a routine clinical microbiology laboratory.

Materials and methods

The present prospective study was carried out between July 2024 to September 2024 in the Department of Microbiology, Government Coimbatore Medical College Coimbatore. The inclusion criteria were from patients having symptoms of candidiasis where causes were ruled out.

A total of 40 *Candida* isolates from various clinical specimens (urine, high vaginal swabs, pus,

wound swabs, sputum, blood, BAL and skin scraping) were taken up for the study over a period of 3 months from out patients and in patients admitted into various wards and intensive care units. A detailed clinical history was taken with regards to the age of the patient, sex, underlying disease / conditions, immune deficiencies, HIV status, diabetes mellitus, pregnancy, malnutrition, any on-going treatment, burns, cancer and any other co- morbid conditions.

Specimens

Various clinical samples including blood, nail scrapings, High vaginal swabs, skin scrapings, sputum pus and BAL from the patients suspected of having fungal infection were cultured to isolate the infecting fungi.

Specimen processing

All the samples were inoculated on Sabourauds Dextrose Agar [SDA] slants supplemented with chloramphenicol and incubated at 37°C for 24-48 hrs. Any growth found on SDA slope was processed for identification of species. From the isolated colony, Gram staining and germ tube test were performed. Germ tube was done to classify the isolates as albicans and non-albicans. From pure isolated colony heavy inoculum of yeast was streaked across Corn meal agar plate and cover slip was placed over it and incubated for 48 hrs at 25°C. The arrangement of hyphae, pseudohyphae, blastophores and chlamydospores were noted after incubation of 72 hours.

Simultaneously CHROM agar *Candida* was used to differentiate several *Candida* spp.by growths of different coloured colonies in it. This is based on the direct detection of specific enzymatic activities by adding certain fluorochromes to the media. A subculture was made from primary isolation media in CHROM agar *Candida* media, and it was incubated at 37°C for 24 hours. After 24-48 hours colony morphology and colour of the colony was interpreted as per manufacturers' specifications.

Growth on chrome agar

Isolated species were inoculated on HiCrome *Candida* differential agar to improve species identification based on coloured colony morphology. These agar plates were incubated at 37°C for 48 hours. The species were identified by characteristic colony colour and morphology as per HiMedia technical data MI1297A. Appropriate controls were used. A variety of species specific colonies were seen. Appearance of *Candida* species on chrome agar were as follows.

1. *C. albicans* - Light green coloured smooth colonies
2. *C. tropicalis*- Blue to metallic blue coloured raised colonies
3. *C. glabrata* – Cream to white smooth colonies
4. *C. krusei* – Purple fuzzy colonies

Results

In our study, candidiasis was found to be more common among 21-40 years (62%) followed by 41-60 years (15%). The rate of isolation of the candida species was more in females (62%) than in males (38%) (Table 1).

Table 1. Age and sex wise distribution of patients with Candidiasis

Age of patients	Males	Females
0-20 years	2	1
21-40 years	8	17
41-60 years	2	4
60 years	3	3
Total	15	25

Table 2. Distribution of *Candida* species in various clinical isolates

Samples	*C. albicans*	*C. tropicalis*	*C. glabrata*	*C. Krusei*	Total
Urine	4	8	3	0	15
High vaginal swab	3	2	2	0	7
Pus	3	2	2	0	7
Wound swabs	3	2	2	1	8
BAL	0	0	0	2	2
Skin scraping	1	0	0	0	1
Total	14	14	9	3	40

The distribution of *Candida* species in various clinical samples were as follows urine 38% , high

vaginal swab 18% Pus 18%, wound swab 21%, BAL 5% and skin scraping 1% (Table 2).

Table 3. Distribution of predisposing factors in patients with Candidiasis

Predisposing factors	No. of patients
Diabetes mellitus	14
History of antibiotics/steroids intake	8
In dwelling catheters	4
Sepsis	4
Prolonged hospitalization	4
Pregnancy/ IUCD/ Oral contraceptive pills	3
HIV positive	3

Table 4. Distribution of *Candida albicans* and Non-albicans *Candida* isolates

Isolates		No. of isolates	Percentage
Non-albicans *Candida* (49)	*Candida tropicalis*	14	35%
	Candida glabrata	9	23%
	Candida krusei	3	7%
Candida albicans (40)		14	35%
Total		40	100%

Table 5. Colony colour of *Candida* isolates on Hicrome *Candida* differential agar

Candida species	Colony colour on Hicrome *Candida* differential agar	No. of *Candida* isolates(n=40)
C. albicans	Light green	14
C. tropicalis	Blue	14
C. glabrata	White to cream	9
C. krusei	Purple fuzzy	3

Analysis of the risk/predisposing factors in patients from whom the *Candida* species were isolated showed, 35% with underlying diabetes mellitus, 20% on multiple antibiotics and 10% with history of catheter usage (Table 3).

Among *Candida* species isolated 14 (35%) were found to be candida non-albicans and 26(65%) were *Candida albicans* (Table 4). The most common species among the candida isolates were *C. tropicalis* (35%) followed by *C. glabrata* (23%) and *C. krusei* (7%).

Table 6. *Candida* isolates on CHROME agar and corn meal agar

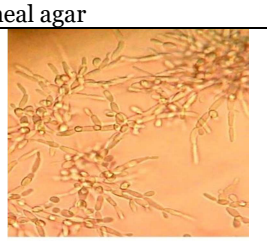
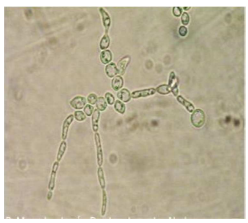
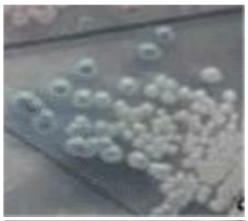
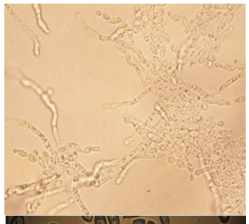
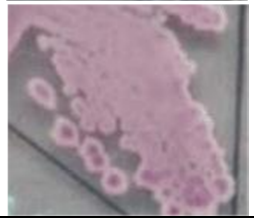
Species	Appearance on H CHROM agar		Appearance on corn meal agar	
Candida albicans	Light Green		Pseudohyphae, clusters of blastoconidia along the points of septation, and spherical chlamydospores	
Candida krusel	Pale pink with white edges		Elongated blastoconidia and pseudohyphae	
Candida tropicalis	Blue		Oval blastospores singly orin small groups all along,long pesudohyphae	
Candida glabrata	Pale cream		Small round to oval blastoconidia	

Table 5 and Fig. 1 together emphasize the utility of HiCrome *Candida* differential agar in identifying *Candida* species through distinct colony color and morphology. On HiCrome agar, *Candida albicans* formed light green colonies, *Candida tropicalis* appeared as blue colonies, *Candida glabrata* produced white to cream colonies, and *Candida krusei* exhibited purple fuzzy colonies. These visually distinct features facilitate easy and rapid differentiation of species.

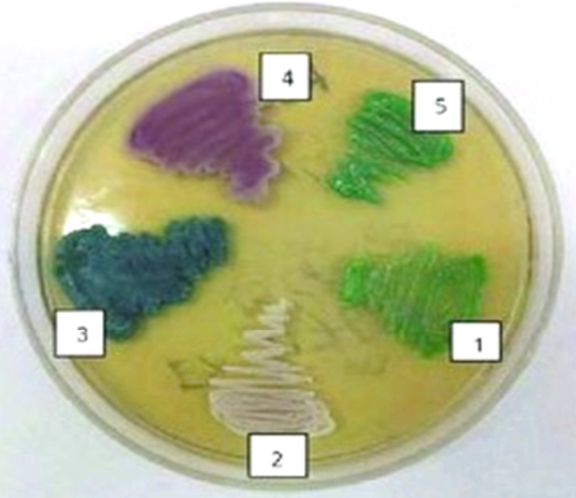


Fig. 1. *Candida* isolates

Fig. 1 provides a visual depiction of these colony morphologies, reinforcing the reliability of chromogenic media in distinguishing between *Candida* species. Table 6 adds further depth by comparing the appearance of these species on HiCrome agar with their morphological features on Corn Meal Agar. For instance, *Candida albicans* showed light green colonies on HiCrome agar and distinct pseudohyphae, clusters of blastoconidia, and spherical chlamydospores on corn meal agar. Similarly, *Candida krusei* exhibited pale pink colonies with white edges on HiCrome agar and elongated blastoconidia with pseudohyphae on corn meal agar. This combination of chromogenic media and corn meal agar provides a comprehensive approach to identifying *Candida* species, ensuring accurate and efficient diagnostics in clinical microbiology.

Discussion

In our study prevalence of *Candida* was predominant in females than in males. We found female (60%) preponderance in our study which was concordant with the study conducted by Sajjan *et al.*, 2014 (65%). But Pfaller recorded the high prevalence of candida in male in their study (Pfaller, 1996).

In our study the distribution of *Candida* species in different samples showed that the highest number of isolates from urine 37% followed by high vaginal swab 18%, Pus 18%, wound swab 20%, BAL 5% and skin scraping 2%.

This is similar to study done by Pfaller (1996) who has highest *Candida* isolates from urine (25%). But Jaggi *et al.*, 2014 isolated *Candida* mostly from blood (33.6%) and respiratory samples (20%) and least from urine 8%. Similarly Saldhana *et al.*, 2011 isolated highest number of *Candida* from high vaginal swabs (38%) followed by blood (16%) and urine (12%).

Considering the predisposing factors in association with *Candida* infection 31% of them had underlying diabetes mellitus, 22% were on multiple antibiotic therapies and 13% had a history of catheter usage in our study. In a study conducted by Sajjan *et al.*, 2014 diabetes was the most common risk factor followed by pregnancy and drug intake. A study done by Arora *et al.*, 2011 found intravenous catheter as most common risk factor followed by prolonged antibiotic usage and immunosuppression.

In our study, *Candida* non-albicans species (64%) were more than *Candida albicans* species (36%). Similarly studies by Saldhana *et al.*, 2011 and Mokadas *et al.*, 2007 showed higher rate of *Candida* non-albicans species 53% and 60.5% respectively. Factors like increased use of antifungal drugs, use of broad spectrum antibiotics, long term use of catheters and increase in the number of immunocompromised

patients contributes to the emergence of non-albicans *Candida* species. Various studies by Madhumati and Rajendran, 2015; Dharwad and Dominic, 2011; Saldhana *et al.*, 2011 showed that *Candida albicans* is the most common species.

Among non-albicans *Candida*, it was found that *C. tropicalis* was the most common isolate in our study. It was concordant with study conducted by Xessi *et al.*, 2007; Mokadas *et al.*, 2007 reported *C. parapsilosis* (30.6%) as the most common isolate. In study by Mujika *et al.*, 2004 *C. krusei* (23%) were more than other Candida non-albicans species.

CHROM agar has the advantage of rapid identification of *Candida* species. It is technically simple, rapid and cost effective when compared to technically demanding, time consuming and expensive conventional method. It is superior to SDA in terms of suppressing the bacterial growth.

Use of CHROM agar medium would allow mycology laboratories to identify clinically important *Candida* species rapidly and potentially decreasing laboratory cost.

Conclusion

Percentage of non–albicans species (55%) has increased in prevalence as compared to *C. albicans* (45%) from clinical samples in recent years. CHROM agar when used to speciate can give excellent results in short time. The use of chrome agar medium would enable the laboratories to rapidly identify and speciate the clinically important *Candida* species.

Therefore isolation and prompt identification of the infecting organism to the species level is essential to optimize early antifungal therapy. Hence CHROM agar can be routinely used instead of Sabourads Dextrose agar.

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