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Original Research Article

Identification and Speciation of Candida Isolates Using CHROM Agar- A Hospital Based Study

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ABSTRACT

Background: Candida species especially non albicans Candida are increasingly being isolated from clinical specimens. Rapid identification of yeast isolates to the species level is essential in order to optimize the antifungal treatment. The conventional methods of identification are time consuming and difficult to perform. The study was done to evaluate the performance of conventional identification methods and commercially available chromogenic Candida speciation media (Hi CHROM agar) for the identification of medically important yeast and yeast-like organisms in a routine clinical microbiology laboratory.
Methods: A total of 98 Candida species were isolated from various clinical specimens including, blood, nail scrapings high vaginal swabs, sputum, skin scrapings and BAL. Identification and speciation of *Candida* was done using Hi CHROM agar and conventional method simultaneously.
Results: *C.* albicans (44.89%) was most common species, followed by C. krusei (26.53%), *C.* tropicalis (24.48%), and *C.* glabrata (4.08%). Isolation rate of non Candida albicans species was higher (55.11%) compared to Candida albicans (44.89%).
Conclusion: The performance of Hi CHROM agar almost paralleled that of conventional methods. Use of this medium is rapid, technically simple and cost effective as compared to time consuming technically demanding expensive conventional method.

Keywords: Candida species, Hi CHROM candida agar, rapid identification.

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 and its appendages to deep-seated or disseminated candidiasis. Estimates suggest that Candida species have been the third most common nosocomial pathogens associated with blood stream infections. [1]

Among the species, although C. albicans has been associated mostly with infections, there has been an increase in the prevalence of infections due to non-albicans Candida in the recent past. [2-5] Of the many pathogenic non-albicans species known, C. tropicalis, C. parapsilosis, C. kefyr, C. krusei, and C. guilliermondii are mostly associated with human infections. Candida species have grown from successful commensals to pathogens in various body sites with the help of many virulence determinants. It is necessary to identify Candida to species level as many non albicans Candida have decreased susceptibility to antifungal agents

Detection of growth patterns on cornmeal agar takes 24-72 hours and sugar

assimilation tests may take 72 hours to two weeks. These procedures are labour intensive and take longer to determine the diagnosis and judge the proper antifungal agent.

In order to facilitate rapid identification, several chromogenic substrate containing culture media have been developed. These special media yield microbial colonies with varying colors secondary to chromogenic substrates that react with enzymes secreted by microorganisms. [6-9]

In the present study, we speciated *Candida* isolates using germ tube test, chlamydospore formation test and also evaluated the performance of commercially available chromogenic Candida speciation media (Hi CHROM agar).

Also among the newer tests, Hi CHROM agar is rapid and cost effective as compared to other expensive systems like API systems, Vitek 2 ID system and molecular methods.

MATERIALS AND METHODS

The present prospective study was carried out between November 2014 and October 2015 in the Department of Microbiology, Government Medical College, Srinagar. The inclusion criteria were from patients having symptoms of candidiasis where other causes were ruled out. A total of 98 consecutive Candida isolates from various clinical specimens obtained from patients attending the Outpatient department of a tertiary care hospital in Srinagar, were included in the study.

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 Sabouraud's 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 48hrs at 25°C. The arrangement of hyphae, pseudohyphae, blastospores, and chlamydospores were noted after incubation of 72 hours. [10-12]

Simultaneously CHROM agar Candida (HiMedia, Mumbai, India) was used to differentiate several Candida spp. by growth of different coloured colonies in it. [6-8] 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 to 48 hours colony morphology and colour of the colony was interpreted as per manufacturer's specifications by two personnel to avoid subjective variation. Light green colonies were identified as C. albicans isolates, whereas C. dubliniensis, Candida tropicalis, Candida krusei were considered in case of dark green, blue to purple and purple colonies respectively. Candida parapsilosis and Candida glabrata were identified by cream to white colonies on CHROM agar agar.

Statistical analysis: Sensitivity and specificity of HiChrom candida agar was calculated as below: Sensitivity [true positive) X 100/ (true positive + false negative)] Specificity [(true negative) X 100/ (true negative + false positive).

RESULTS

A total of 98 isolate of Candida species were recovered from various clinical samples like blood, nail scrapings, High Vaginal swabs, skin scrapings, sputum, pus, and BAL (Figure 1).

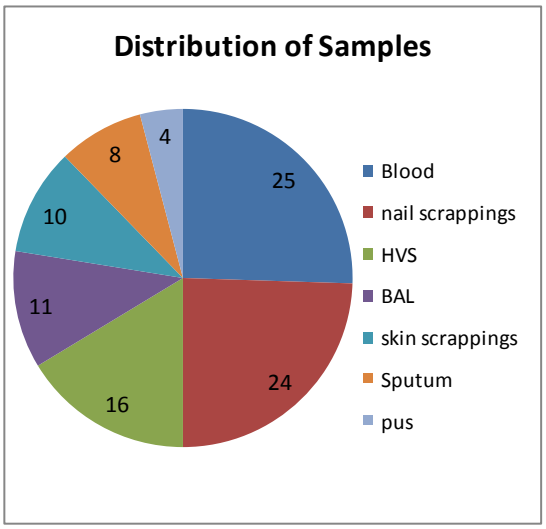


Figure.1 Distribution of various clinical samples

C. albicans (44.89%) was most common species, followed by C. krusei (26.53%), C. tropicalis (24.48%), and C. glabrata (4.08%). Isolation rate of non Candida albicans spp. was higher (55.11%) compared to Candida albicans (44.89%) (Table 1).

Table.1 Distribution of Candida species isolated

Species	No. of isolates
Candida albicans	44(44.89)
Candida krusei	26(26.53)
Candida tropicalis	24 (24.48)
Candida glabrata	4 (4.08)
Total	**98**

Distribution of the different species of candida among various clinical specimens is shown in Table 2.

Table 2 Distribution of the different species of candida among various clinical specimens

Clinical specimens	C. albicans (%)	C. krusei (%)	C. tropicalis (%)	C. glabrata (%)	Total (%)
Blood	5(20)	8(32)	12(48)	0	25(24.48)
Nail scrapings	6 (25)	10(41.66)	5 (20.83)	3(12.5)	24(24.48)
HVS	14 (87.5)	1 (6.2)	1 (6.2)	0	16(16.32)
BAL	9(81.8)	1 (9.09)	1 (9.09)	0	11(11.22)
Skin scrapings	2(20)	5(50)	2 (20)	1(10)	10(10.20)
Sputum	6 (75)	1 (12.5)	1 (12.5)	0	8 (8.16)
Pus	2 (50)	0	2 (25)	0	4(4.08)
Total	**44**	**26**	**24**	**4**	**98**

Figure 2 Appearance of candida species on Hi CHROM Agar and Corn Meal agar

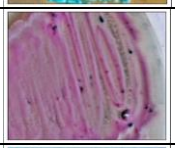
Species	Appearance on Hi CHROM Agar		Appearance on Corn Meal Agar	
Candida albicans	Light green		Pseudohyphae, clusters of blastoconidia along the points of septation, and spherical chlamydospores	
Candida krusei	Pale pink with white edges		Elongated blastoconidia and pseudohyphae	
Candida tropicalis	Blue		Oval blastospores singly orin small groups all along,long pesudohyphae	
Candida glabrata	Pale cream		Small round to oval blastoconidia	

Table 3 Sensitivity and specificity of CHROM agar in identifying Candida species

Candida spp.	No. of Candida spp. identified by conventional method	No. of Candida spp. identified using Hi CHROM agar	Sensitivity of Hi CHROM agar	Specificity of Hi CHROM agar
C.albicans	44	46	100%	96%
C krusei	26	24	92.3%	100%
C.tropicalis	24	22	92.3%	100%
C.glabarata	04	02	66.66%	100%

In this present study, both SDA and Hi CHROM agar were employed as primary plating medium for various samples. There were no major discrepancies between SDA and Hi CHROM agar in growth rate or colony size for primary isolation of

Candida. Both media supported growth of all 98 Candida isolates. After 18-24 h of incubation, colonies of most Candida isolates on Hi CHROM agar started developing characteristic colours, which became more prominent after 48 h. However, false positive and false negative results were also seen. Different colony colours displayed by Candida isolates on Hi CHROM agar and the appearance on Corn Meal Agar are given in Figure 2.

The sensitivity and specificity of candida species on Hi CHROM agar is shown in Table 3.

DISCUSSION

Pathogenic yeast from the genus Candida can cause serious infections in humans and are now recognized as a major agent of hospital acquired (nosocomial) infections. [13] The growing problem of mucosal and systemic candidiasis reflects the enormous increase in the number of patients at risk and the increased opportunity that exists for Candida species to invade tissues normally resistant to invasion. [14] Candida species are true opportunistic pathogens that exploit interventional therapeutic advances to gain access to the circulation and deep tissues. Systemic infections due to yeasts and resistance to antifungals are on the rise in Indian hospitals. [15] Increasing resistance to azoles and amphotericin B has been reported both from India and other countries. [16-18] Identification of Candida to species level is definitely warranted as there is increase in the incidence of Candida non albicans infections. [13] The antifungal susceptibility of fluconazole has changed against the Candida species, Candida non albicans are being more resistant to fluconazole. [19] Therefore species level identification has a direct impact on choice of empirical antifungal treatment. Also there may be geographic variation in the species isolated which necessitates that we have data on the distribution of candida species in different geographic regions.

For differentiation between different species of candida conventionally Germ tube test, chlamydospore formation, sugar fermentation and assimilation tests are being used which are laborious and time consuming. In most laboratories, non albicans Candida isolates were reported based on germ tube test only, without species level identification and antifungal susceptibility test. [20]

CHROM AGAR is a rapid method to differentiate between different candida species. It facilitates the detection and identification of candida species from mixed culture and provides result in 24-48 hours. [21-23]

In the present study Candida albicans was the predominant species (44.89%). The following non-albicans species were isolated; C.krusei (26.53%), C. tropicalis (24.48%), and C. glabrata (4.08%). Isolation rate of non Candida albicans species was higher (55.11%) compared to Candida albicans (44.89%). Similar findings have been reported by Shivanand Dharwad and Saldanha Dominic et al. [24] and Tavleen Jaggi et al. [25] where in the rate of isolation was 47% and 44% respectively. Higher incidence of non albicans candida ranging from 54 - 74% has also been seen in various studies. [26-28]

We obtained sensitivity and specificity of Hi CHROM Candida agar for C. albicans as 100% and 96.42% respectively. Baradkar et al., [29] who used Hi Crome media (Himedia Mumbai, India) reported a sensitivity of 96.55% and specificity of 96.42%; Willinger and Manafi [30] reported 98.8 and 100% sensitivity and specificity while Peng et al., 100 and 94.6%. [8]

Yucesoy et al., reported sensitivity and specificity of 100% in case of C. krusei. [6] Our study shows sensitivity of 92.3% and specificity of 100% in detecting Candida krusei.

As regards C. tropicalis, wide ranging differences in sensitivity and specificity have been reported by various authors. Sensitivity ranged from 66.7 to

100%, specificity from 78.8 to 100%.[6,29,30] our study showed a sensitivity of 92.3%% and specificity of 100% for C. tropicalis, which is in between the rates reported by Willinger and Manafi and Yucesoy et al. [6,30]

Rapid identification of C. glabrata has a special importance because C. glabrata is less sensitive than other species to ketoconazole and fluconazole. [31]A sensitivity of 66.66% and specificity of 100% were reported in our study for C. glabrata. Willinger et al. [30] reported 98% sensitivity and 95.7% specificity for C. glabrata on chromagar. Peng et al. [8] reported sensitivity and specificity values of chrome agar for C. glabrata as 90.2 and 95.4% respectively. Yucesoy et al. [9] reported 90.9% sensitivity and 100% specificity of chrome agar for C. glabrata. Pfaller et al. [32] and Willinger et al. [30] concluded that chrome agar allowed identification of C. glabrata.

We found that Hi CHROM agar Candida, easily identifies several species of Candida on the basis of colony color and morphology and accurately differentiates between the three most common species of Candida, i.e. C. albicans, C. tropicalis, and C. krusei, which has also been reported by Murray et al. [7] This medium easily facilitates the detection of more than two species in a single specimen by giving different colored colonies on a plate at the same time.

Chromogenic agar has the advantage of rapid identification of Candida species, being technically simple, rapid and cost effective compared to technically demanding time consuming and laborious conventional method.

CONCLUSION

CHROM agar when used to speciate can give excellent results within short time. Presumptive identification becomes easier especially in case of non-albicans candida. Along with Candida albicans, non albicans Candida spp. like C. tropicalis, C, krusei and C. glabrata are increasingly being isolated from clinical specimens. CHROM agar is a simple, rapid and inexpensive method with good sensitivity and specificity for identification of such species.

Hence CHROM agar can be routinely used instead of Sabourad's Dextrose agar.

DECLARATIONS

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Ethical approval: Was obtained from the institutional Ethical Committee.

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