

Utility of HiCrome Candida Differential Agar to Speciate Clinical Candida Isolates

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ABSTRACT

As the epidemiological distribution of Candida species changed in recent years and also to decide proper antifungal agents, the rapid and reliable identification of Candida species is essential. The conventional methods are labour intensive and take longer time to identify the Candida species and to judge proper antifungal agent. The automated systems proved to be considerably expensive. Many workers in their studies have been shown that chromogenic agars can identify Candida clearly to species level based upon the characteristic coloured colonies. The present study is carried out to prove the utility of HiCrome Candida differential agar in identification of Candida species. A total of 127 Candida strains, including 77 *C. albicans*, 23 *C. tropicalis*, 18 *C. parapsilosis* and 9 *C. krusei* identified by standard techniques were subcultured on HiCrome Candida differential agar and then incubated at 37^o c for 48 hours to study their characteristics. The HiCrome Candida differential agar showed the sensitivity and specificity of 97.40 % and 100 % for *C. albicans*, 100 % each for *C. tropicalis* and *C. krusei* while in case of *C. parapsilosis* it was 88.88%, 100 % respectively. The HiCrome Candida Differential Agar though easy and reliable method, it should be used with caution when colony colour differs from the standard colour as per the manufacturer's manual.

Key words: Utility, HiCrome Candida Differential Agar, Candida species

INTRODUCTION

Genus Candida comprises about 200 species, of which close to 20 have been associated with pathology in humans and animals.^[1] Though *Candida albicans* is the predominant isolate, rise in frequency of isolation of non-*albicans* Candida species is observed.^[2] As the Candida species epidemiological distribution changed in recent years and expression of putative virulence factors, antifungal susceptibility pattern, risk of developing deep organ involvement, severity of clinical manifestations differs depending on the infecting Candida species, the rapid and reliable identification of these Candida species is essential.^[3,4,5] The procedures like culturing on cornmeal agar, carbohydrate

fermentation and assimilation are labour intensive and take longer time to identify the Candida to species level and to judge proper antifungal agent.^[6] Although automated systems are available to accurately identify the isolates to species level and derive their antifungal susceptibility pattern. These automated systems proved to be considerably expensive and are limited to few sophisticated laboratories.^[2] Many workers in their studies have been shown that chromogenic agars for Candida can identify clearly *C. albicans*, *C. tropicalis*, *C. krusei* and *C. parapsilosis* based upon the characteristic coloured colonies.^[7,8,9] Although the manufacturer also claim that this media shows better performance with

good accuracy in identifying Candida species, there is need to establish its ability in selective isolation and presumptive identification of Candida species, before replacing conventional methods.^[2] Considering these facts, the present study is carried out to prove the utility of HiCrome Candida differential agar in identification of *C. albicans* and non-*albicans* Candida from clinical specimens as compared to identification by conventional methods.

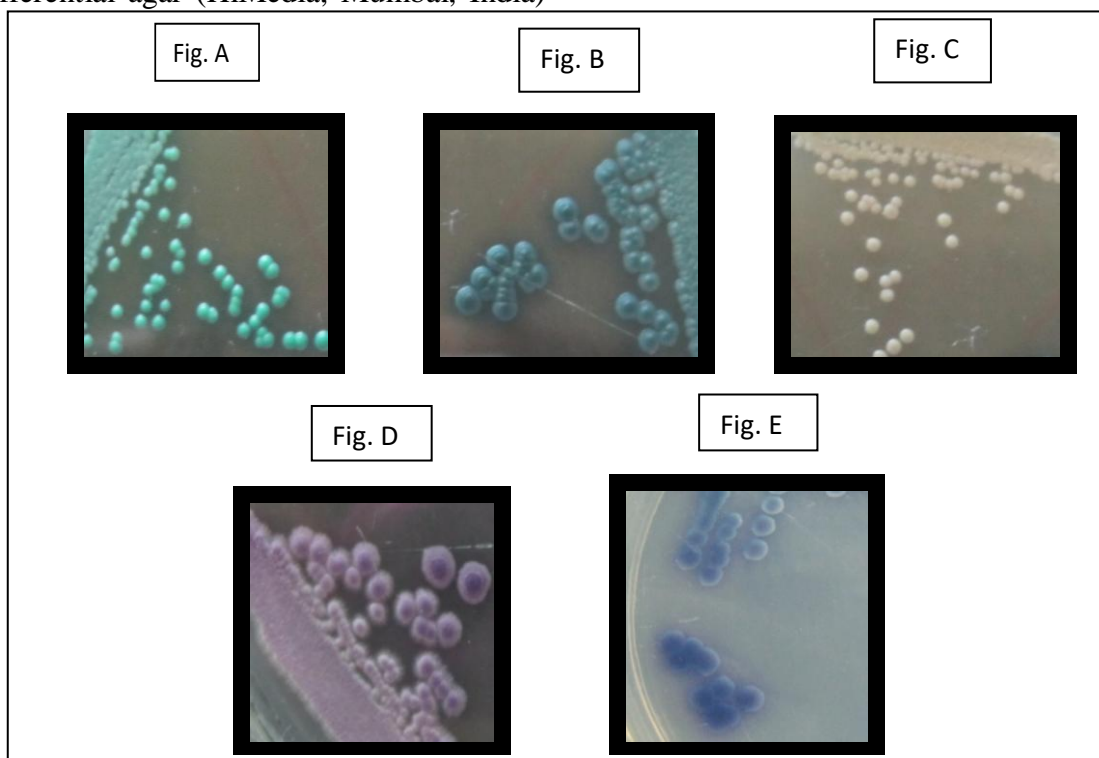
MATERIALS AND METHODS

The present laboratory based prospective study was conducted at microbiology department of Mahatma Gandhi Institute of Medical Sciences, Sevagram, Maharashtra, India over a period of 1 year from June 2011 to May 2012. A total of 127 Candida strains, including 77 *C. albicans*, 23 *C. tropicalis*, 18 *C. Parapsilosis* and 9 *C. krusei* from various clinical specimens identified by standard diagnostic techniques (germ tube test, chlamydospore formation on corn meal agar, sugar fermentation and sugar assimilation tests and growth at 45°C) were included in this study. HiCrome Candida differential agar (HiMedia, Mumbai, India)

was prepared following manufacturer's instructions. 42.72 grams HiCrome Candida differential agar was suspended in 1000 ml distilled water. It was heated to boiling gently to dissolve the medium completely. Then it was allowed to cool to 50°C and poured into sterile Petri plates. The Candida isolates were subcultured on HiCrome Candida differential agar and then incubated at 37°C for 48 hours. The Candida species were identified by characteristic colony colour as per manufacturer's instructions: *C. albicans*- Light green smooth colonies, *C. dubliniensis*- Dark green colonies, *C. glabrata*- Pink colonies, *C. krusei*- large purple rough spreading colonies, *C. parapsilosis*-cream colored colonies, *C. tropicalis*- Blue colonies. The sensitivity and specificity of HiCrome Candida differential agar was calculated with following formulas:

$$\text{Sensitivity} = \frac{(\text{true positive})}{(\text{true positive} + \text{false negative})} \times 100$$

$$\text{Specificity} = \frac{(\text{true negative})}{(\text{true negative} + \text{false positive})} \times 100$$



Figures: A- *C. albicans*, B- *C. dubliniensis*, C- *C. parapsilosis*, D- *C. krusei*, E- *C. tropicalis*

RESULTS

Table 1: Candida species identification on HiCrome Candida Differential Agar (HCCDA) in comparison to standard techniques.

Candida Species	Standard techniques	HCCDA
Candida albicans	77	75
Candida tropicalis	23	23
Candida parapsilosis	18	16
Candida krusei	09	09

HCCDA: HiCrome Candida Differential Agar

All 23 *C. tropicalis* and 9 *C. krusei* isolates were perfectly identified on HCCDA in comparison to standard techniques. However, among all 77 *C. albicans* and 18 *C. parapsilosis* identified by standard techniques, 2 of each were mismatched on HCCDA.

Table 2: Colony color of Candida species on HCCDA

Species	No.	Light green	Dark green	Cream	Pink	Blue	Purple	Sensitivity (%)	Specificity (%)
<i>C. albicans</i>	77	75	2	-	-	-	-	97.40	100
<i>C. parapsilosis</i>	18	-	-	16	2	-	-	88.88	100
<i>C. tropicalis</i>	23	-	-	-	-	23	-	100	100
<i>C. krusei</i>	9	-	-	-	-	-	9	100	100

123 (96.85%) of the *Candida* isolates produced characteristic pigmented colonies as per the manufacturer's manual, when matched with the confirmed isolates studied. Two confirmed *C. albicans* isolates produced dark green colonies on HiCrome Candida differential agar, indicating it to be *C. dubliniensis* while two confirmed *C. parapsilosis* isolates produced pink colonies indicating it to be *C. glabrata*. The sensitivity and specificity values are shown in Table 2.

DISCUSSION

Many workers^[7,9,10,11,12,13,14,15] in their studies reported that the use of chromogenic medium is an easy and reliable method for the presumptive identification of most commonly isolated *Candida* species, especially *C. albicans*, *C. parapsilosis*, *C. tropicalis* and *C. krusei* with sufficient sensitivity and specificity. The sensitivity and specificity of 97.40 % and 100 % respectively for *C. albicans* with HiCrome Candida differential agar found in our study can be correlated with the sensitivity of 98.8% and specificity of 100% for *C. albicans* reported by Willinger et al^[10] in their study using CHROM agar *Candida*. Our findings can also be comparable with the sensitivity and specificity of 99.4% and 100% respectively for *C. albicans* reported by Yucesoy M et al^[11] using CHROM agar *Candida*. The present study findings of 100 % sensitivity and specificity each for *C.*

tropicalis and *C. krusei* were in concordance with that of Deaf et al^[12] and Sagar K et al^[13] who used the same chromogenic agar. Baradkar et al,^[9] Yucesoy M et al^[11] and L. Sumitra Devi et al^[14] also reported the 100 % sensitivity and specificity for *C. tropicalis* and *C. krusei*, however the chromogenic medias used were Hichrom *Candida* agar, CHROM agar *Candida* and CHROM agar respectively. In case of *C. parapsilosis*, the sensitivity of 88.88% and specificity of 100 % found in this study was lower than that of Pravin Charles MV et al^[15] (sensitivity 100% and specificity 100%) with HiCrome agar. In comparison to our findings, Baradkar et al^[9] reported low sensitivity of 80% and specificity of 98.03% for *C. parapsilosis*.

CONCLUSION

HiCrome *Candida* differential agar though easy and reliable method for identification of *Candida* species, it should be used with caution when colony colour differs from the standard colour as per the manufacturer's manual. To avoid any ambiguity, we recommend HiCrome *Candida* differential agar to be improved further.

Conflicts of Interest

There are no conflicts of interest.

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