Evaluation of Hicrome Candida Differential Agar for Species Identification of *Candida* Isolates from Various Clinical Samples

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ABSTRACT

Introduction: *Candida albicans* remains the most common species causing human infections but recent epidemiological data reveal shift from *C. albicans* to non *albicans Candida* species. The conventional methods of identification are time consuming and difficult to perform. The present study was done to evaluate the performance of conventional identification method (phenotypic and biochemical) and commercially available chromogenic *Candida* speciation media (Hicrome Candida differential agar) for the identification of medically important *Candida* species in a routine clinical microbiology laboratory.

Material and Methods: A total of 115 *Candida* isolates from various clinical specimens received in the Department of Microbiology were taken up for the study over a period of one year i.e. from January 2014 to December 2014. The *Candida* isolates were speciated by using conventional methods and were compared against chromogenic agar medium (Hicrome Candida differential agar).The conventional methods used for speciation of yeast isolates were germ tube test, colony morphology on corn meal agar, sugar fermentation and sugar assimilation test

Results: The isolation of non *albicans Candida* (59.1%) predominated over *Candida albicans* (40.9%). Non *albicans Candida* species isolated were *C. tropicalis* (40%) followed by *C. guilliermondii* (10.43%), *C. krusei* (4.34%), *C. glabrata* (2.60%), *C. kefyr* and *C. parapsilosis* (0.87%) each.

Conclusion: The accurate species identification of *Candida* is important for the treatment, as not all species respond to the same treatment and also because of the problem of anti-fungal resistance. Hicrome Candida differential agar is a convenient and rapid method of identification of *Candida* species even in resource limited poor settings.

Keyword: *Candida albicans*, Non *albicans Candida* species, Hicrome Candida differential agar and Conventional method.

INTRODUCTION

Candida is an asexual, dimorphic fungus which is present in the human body and his surroundings. Candidiasis has emerged itself as an alarming opportunistic disease due to increase in the number of immunocompromised, aged, receiving prolonged antibacterial and aggressive cancer chemotherapy or undergoing invasive surgical procedures and organ transplantation patients.[1] Among *Candida* species, *Candida albicans* is generally considered as the major pathogen. An increase in the prevalence of non-*albicans Candida* species has been noted during the last decades.[2,3]

It has become important to identify yeast isolated from various clinical specimens to the species level.[4] Species identification of *Candida* isolates is conventionally done by germ tube test, inoculation on corn- meal agar, sugar assimilation and fermentation tests. Newer methods which have been developed for yeast identification include CHROM agar, API systems, Vitek 2 ID system and molecular methods.[5-7] Study of colony morphology on cornmeal agar, sugar fermentation and assimilation tests are time consuming and labour intensive.[8,9] Several brands of chromogenic media have been developed to produce rapid yeast identification. These media contain chromogenic substrates that react with enzymes secreted by microorganisms producing colonies with various pigmentation. These enzymes are species specific, allowing organisms to be identified to the species level by their colour and colony characteristics.[10] Hicrome Candida differential agar (Himedia, Mumbai, India) is one such chromogenic medium which is introduced by Himedia laboratory to differentiate *Candida* species namely *C. albicans, C. krusei, C. tropicalis,* and *C. glabrata* based on colony color.[11]

Non *albicans Candida* like *C. tropicalis, C. krusei, C. glabarata* and *C. parapsilosis* are less susceptible to azoles, particularly fluconazole.[12] Therefore, correct identification of *Candida* species is of great importance, as it presents prognostic and therapeutical significance, allowing an early and appropriate antifungal therapy. Thus, the present study was undertaken for species identification of *Candida* isolates in various clinical specimens and to evaluate utility of Hi-Crome Candida Differential Agar to differentiate *Candida* species isolated from various clinical samples on the basis of coloration and colony morphology.

MATERIAL AND METHODS

The present study was laboratory based carried on clinical isolates of *Candida* species. The study was carried out in the Department of Microbiology, MGM's Medical College and Research Centre, Aurangabad, Maharashtra after approval by institutional Ethical Committee. A total of 115 *Candida* species were isolated from various clinical specimens received in the Department of Microbiology, Tertiary Care Centre were taken up for the study over a period of one year i.e. from January 2014 to December 2014.

Isolation and identification of *Candida* species

All the clinical samples were screened for budding yeast like cells by direct microscopy of wet mount, gram stain, 10% KOH preparation and Gram stain of a colony on routine

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culture media. All the *Candida* isolates were subcultured on Sabouraud's Dextrose Agar with Chloramphenicol and incubated at 25^{0}C and 37^{0}C. The *Candida* isolates were further speciated by standard protocol that include germ tube test, chlamydospore formation on corn meal agar, sugar fermentation and sugar assimilation test.[13-16] Simultaneously the Candida species were inoculated on HiCrome Candida differential agar and incubated at 37^{0}c for 24 to 48 hours and the species were identified by type and colour of the colonies on Hicrome Candida differential agar media as per manufacturer's instruction.[11,13]

Colony morphology of *Candida* species on HiCrome Candida differential agar

i. *Candida albicans*: light green coloured smooth colonies
ii. *Candida tropicalis*: blue to metallic blue coloured raised colonies
iii. *Candida glabrata*: cream to white smooth colonies
iv. *Candida krusei*: purple fuzzy colonies

We used ATCC strains of *Candida albicans ATCC 10231*, *Candida glabrata ATCC 15126, Candida krusei ATCC 14243* and *Candida tropicalis ATCC 750* as control.

RESULT

A total of 115 *Candida* species were isolated from various clinical specimens processed during the study period. *Candida albicans* was the commonest species isolated 47 (40.9%) followed by *C. tropicalis* 46 (40%), *C. guilliermondii* 12 (10.43%), *C. krusei* 5 (4.34%), *C. glabrata* 3 (2.60%), *C. kefyr* 1 (0.87%) and *C. parapsilosis* 1 (0.87%). Isolation rate of *non albicans Candida* species was higher 68 (59.1%) as compared to *Candida albicans* 47 (40.9%) [Table No. 1]. Among the non *albicans Candida*, *Candida tropicalis* 46 (40%) was the commonest followed by *C. guilliermondii* 12 (10.43%), *C. krusei* 5 (4.34%), *C. glabrata* 3 (2.60%), *C. kefyr* 1 (0.87%) and *C. parapsilosis* 1 (0.87%). These 115 *Candida* isolates were also subjected to identification using Hicrome Candida differential agar. The results of conventional method and Hicrome Candida differential agar for various species are given in [Table No. 3]. There was an agreement in identification by Hicrome Candida differential agar and conventional method for four species. (i.e. *Candida albicans, Candida tropicalis, Candida krusei* and *Candida glabrata*). Three *Candida* isolates (*Candida guilliermondii, Candida parapsilosis* and *Candida kefyr*) were not identified by Hicrome candida differential agar. These three *Candida* species were identified by conventional methods like sugar fermentation and sugar assimilation tests. The colony morphology of *Candida albicans*, *Candida tropicalis, Candida krusei*, *Candida glabrata* and *Candida guilliermondii* on Hicrome Candida differential agar are shown in photograph number 1, 2, 3,4 and 5 respectively.

DISCUSSION

Rapid identification of yeast species are very difficult in resource-limited poor laboratories due to lack of trainings, proper reagents and equipments. The conventional methods like inoculation on corn-meal agar, biochemical assimilation and fermentation tests are not used in these laboratories due to lack of resources, expertise and time required for these

***Candida* isolates**	**Number of isolates**	**Percentage**
Candida albicans	47	40.87%
Non *albicans Candida*	68	59.13%
Total	115	100%

Table-1: Distribution of *Candida albicans* and Non *albicans Candida* isolates

***Candida* species**	**Conventional method**	**Hicrome Candida differential agar**
Candida albicans	47	47
Candida tropicalis	46	46
Candida guilliermondii	12	-
Candida krusei	5	5
Candida glabrata	3	3
Candida kefyr	1	-
Candida parapsilosis	1	-
'p' value – 0.864, Result – No significant difference		

Table-2: Identification of *Candida* species by Conventional method and Hicrome Candida differential agar

	***Candida* species**	**Colony colour on HicromeCandida differential agar**	**No. of *Candida*isolates. (n=115)**
1	*Candida albicans*	Light green	47
2	*Candida tropicalis*	Blue	46
3	*Candida guilliermondii*	Light pink to pink	12
4	*Candida krusei*	Purple fuzzy	5
5	*Candida glabrata*	White to cream	3
6	*Candida kefyr*	Light pink	1
7	*Candida parapsilosis*	Light pink	1

Table-3: Colony colour of *Candida* isolates on Hicrome Candida differential agar

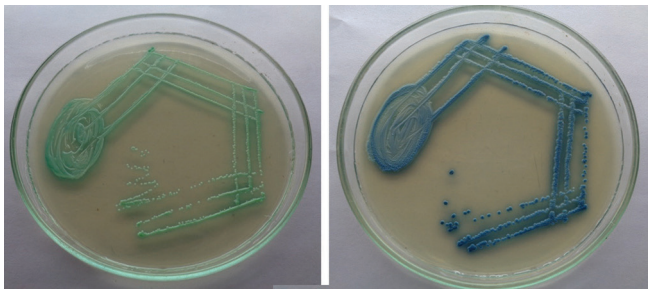

Figure-1: Light green coloured colonies of Candida albicans on Hicrome Candida differential agar, **Figure-2:** Blue coloured colonies of Candida tropicalis on Hicrome Candida differential agar

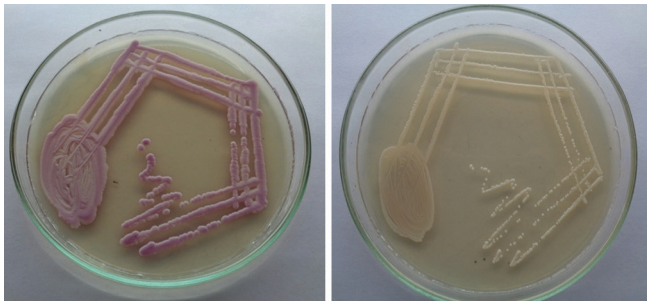

Figure-3: Purple fuzzy colonies of Candida krusei on Hicrome Candida differential agar, **Figure-4:** Cream coloured colonies of Candida glabrata on Hicrome Candida differential agar

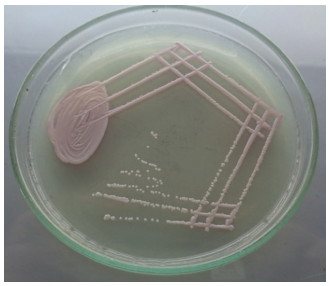

Figure-5: Light pink coloured colonies of Candida guilliermondii on Hicrome Candida differential agar

tests which increase the cost of mycology cultures. Therefore, these laboratories do not go beyond the germ tube test and limit their diagnosis to *C. albicans* or non *albicans Candida*.[17] As a result of which, selection of appropriate agent for antifungal therapy becomes almost impossible. Without standard diagnostic tools, a safe and effective drug treatment, prevention of resistance to antimicrobial therapy and monitoring of resistance are not possible. So, in these settings there is always a need of a medium which helps in the isolation and identification of the agent at the species level.[4] It is necessary to evaluate simple, cost effective and rapid method like chromogenic medium to identify *Candida* to species level. Chromogenic agar is a newer and more rapid method to speciate *Candida* species. It contains enzymatic substrates that are linked to chromogenic compounds. When a specific enzyme cleaves the substrate, the chromogenic substances produce colour. The action of different enzymes produced by yeast species results in colour variation which is useful for presumptive identification of some yeast.[18]

In the present study, conventional method and Hicrome Candida differential agar were used for identification of *Candida* isolates from various clinical samples and utility of Hicrome Candida differential agar was noted. *Candida albicans* produces light green colonies on Hicrome Candida differential agar. In our study, all the isolates of *Candida albicans* produced light green colonies on Hicrome Candida differential agar. This is in agreement with Sivakumar *et al* (2009),[19] Kumar *et al* (2013),[20] Sukumaran *et al* (2012),[21] who reported that all the isolates of *Candida albicans* produced light green colonies on Hichrome agar (Himedia, Mumbai, India). Nadeem *et al* (2010),[4] also reported that *Candida albicans* produced green coloured colonies however media used was CHROMagar Candida, (France).

Candida tropicalis produces blue to metallic blue colonies on Hicrome Candida differential agar. In our study, all the isolates of *Candida tropicalis* produced blue colonies on Hicrome Candida differential agar. This is in agreement with Sukumaran *et al* (2012),[21] Manikandan *et al* (2013),[22] who reported that all the isolates of *C. tropicalis* produced blue colonies on Hicrome agar (HiMedia). This is also in agreement with Devi *et al* (2014),[23] who found that all the isolates of *Candida tropicalis* produced metallic blue coloured colonies.

Candida krusei produces purple fuzzy colonies on Hicrome Candida differential agar. In our study, all the isolates of *Candida krusei* produced purple fuzzy colony on Hicrome Candida differential agar. Our study is in accordance with Deaf *et al* (2014),[17] who reported that all the isolates of *Candida krusei* produced purple fuzzy colonies on Hicrome Candida agar. Dharwad *et al* (2011),[24] also reported that *Candida krusei* produced pale pink to purple rough colonies. This is also in agreement with Omar *et al* (2010),[25] who reported that all the isolates of *Candida krusei* were correctly identified however the media used was CHROMagar Candida (CHROMagar Microbiology, Paris, France).

Candida glabrata produces white to cream coloured colonies on Hicrome Candida differential agar. In our study, all the isolates of *Candida glabrata* produced white to cream coloured colonies on Hicrome Candida differential agar. This is in agreement with Deaf et al (2014),[17] who found that *Candida glabrata* produced white to cream colonies on Hicrome Candida agar (Himedia) This is also in agreement with Devi *et al* (2014),[23] Yashavanth *et al* (2013),[26] who found that *Candida glabrata* produced white colonies however the media used was CHROM agar.

In our study, three other species (*C. guilliermondii, C. kefyr* and *C. parapsilosis*) produced light pink to pink coloured colonies on Hicrome Candida differential agar leading to difficulties in identification. These species were identified by conventional method like sugar fermentation and sugar assimilation tests. This is in accordance with Sivakumar *et al* (2009)[19] who reported that *Candida guilliermondii*, *Candida parapsilosis, Candida kefyr* produced varying shades of pinkish purple-coloured colonies however the chromogenic media used was CHROMagar Candida (CHROMagar Company, Paris, France).

In our study, all the isolates *Candida* species namely *Candida albicans, Candida tropicalis, Candida krusei and Candida glabrata* were identified by conventional method. *Candida* isolates were inoculated on Hicrome Candida differential agar. We observed that performance of Hicrome Candida differential agar for identification of these four species was exactly paralleled that of conventional method. Similar findings were observed in various studies however they used Chrome agar for identification of *Candida* species. Devi *et al* (2014),[23] Vijaya *et al* (2011),[27] Amar *et al* (2012),[28] Sajjan *et al* (2014),[29] also reported that results of CHROM agar for identification of *Candida* species were consistent with the results of the conventional methods.

The colony colour produced by *Candida albicans, Candida tropicalis, Candida krusei*, and *Candida glabrata* were typical, this medium can be recommended for identification of these species in resource limited settings as it will not require any expertise. Use of Hicrome Candida differential agar may replace conventional methods like germ tube test, colony morphology on cornmeal agar, sugar fermentation and sugar assimilation tests there by reducing the time and expenses required for identification of common isolates of *Candida* species.

CONCLUSION

Conventional methods like germ tube test, morphology on corn meal agar, sugar fermentation and sugar assimilation tests used for the identification of *Candida* species up to species level were very time consuming and cumbersome. The present study highlights the fact that Hicrome Candida dif-

ferential agar is useful in identification of four major isolates of *Candida* species like *Candida albicans, Candida tropicalis, Candida krusei* and *Candida glabrata* and this will also help clinician to make early decision regarding appropriate antifungal therapy thereby decreasing patient morbidity and mortality. Routine use of this media carries the potential for cost saving in the clinical microbiology laboratory and can save the time and expense of performing fermentation and assimilation tests required for identification of *Candida* species. However conventional techniques need to be performed for correct identification of *Candida* species like *Candida guilliermondii, Candida parapsilosis* and *Candida kefyr.*

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