ejpmr, 2020,7(10), 466-469

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

EVALUATION OF HICROME DIFFERENTIAL AGAR FOR SPECIES IDENTIFICATION OF CANDIDA ISOLATES FROM VARIOUS SPECIMENS

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Article Received on 24/07/2020 **Article Revised on 14/08/2020** **Article Accepted on 04/09/2020**

ABSTRACT

Candida species especially *Non Albicans Candida* (NAC) are increasingly being isolated from clinical specimens. The conventional methods of identification are time consuming and difficult to perform. The study was done to evaluate the performance of conventional identification method (phenotypic and biochemical) and commercially available chromogenic Candida speciation media (CHROM agar) for the identification of medically important yeast and yeast-like organisms in a routine clinical microbiology laboratory. A total of 50 yeast strains were included during the six months study period. The conventional methods used for speciation of yeast isolates were Germ tube test, chlamydospore formation test on corn meal agar, sugar fermentation test and sugar assimilation test and were compared against chromogenic agar medium (HiCrome agar). *Candida albicans* was the major species accounting for 26 (52.0%) of the total isolates. *Non albicans Candida* constituted 15 (30.0%) of *C. tropicalis*, 7 (14.0%) of *C. krusei* and 2 (4.0%) of *C. parasilosis*. HiCrome agar is a convenient and rapid method of identification of *Candida* species even in resource of poor settings.

KEYWORDS: *Candida* species, HiCrome agar.

INTRODUCTION

Candida spp. are the members of the normal flora of the skin, mucous membranes, and gastrointestinal tract. They are endogenous opportunists which cause secondary infection in individuals with some underlying immunocompromised conditions. Candidiasis is a common fungal disease found in humans affecting mucosa, skin, nails and internal organs of the body. A large variety of techniques from conventional to molecular methods.[1] are available for yeast identification. Routinely used conventional methods like gram staining of smear, germ tube test, colony morphology on Sabouraud's dextrose agar (SDA), urease test, growth on corn meal agar, sugar fermentation and assimilation test, growth at 45*C are labour intensive, cumbersome and time consuming i.e. may take 3-4 days.[2] Now a day, newer methods like several chromogenic media, API system, Vitek 2 ID system and molecular methods, have been developed for rapid identification of *Candida* species.[3] HiCrome *Candida* agar is one of the selective and differential chromogenic media manufactured by Himedia, Mumbai, India. This media contain chromogenic substrates that react with enzymes secreted by microorganisms producing colonies with various pigmentations. These enzymes are species□specific, allowing organisms to be identified to the species level by their colour and colony characteristics. They are used for rapid isolation and identification of *Candida* species directly from clinical samples and also where multiple yeast species are present in the sample. On these media results are obtained in less time i.e. within 48hrs, it can be easily interpreted and also cost effective as compared to other newer methods like API system, Vitek 2 ID system and molecular[4-5] methods which are expensive. As many tests from conventional to molecular methods are available, clinical microbiological laboratories are facing an important challenge regarding selection of method that is cost effective, accurate, easily interpreted and also rapid for identification of *Candida spp*, which in turn is of great importance for clinicians of our hospital to select appropriate prophylactic and therapeutic antifungal drug. Keeping the above point in view, the present study aimed at species identification of *Candida* isolated from various clinical specimens and also to evaluate the usefulness of HiCrome Candida differential agar as compared to routine conventional method for speciation of *Candida* spp.

MATERIAL AND METHODS

The present study was carried out between May 2019 to October 2019 in the Department of Microbiology, Al-Falah School of Medical Sciences & Research Centre Faridabad. A total of 50 consecutive *Candida* isolates from various clinical specimens like urine, blood, sputum, pus, catheter tip, ear swab from patients with Candidiasis and stool sample from patients with antibiotic associated diarrhoea were included in the study. These specimens were processed for the isolation of *Candida spp.* using standard Mycology methods.[6] Gram staining was performed from direct specimen and the specimens were inoculated on Sabouraud's dextrose agar slants, incubated at 37°C for 24 hrs. Germ tube test was done and the positives identified were either *C. albicans* or *C. dubliniensis. C. albicans* were further identified by growth at 45°C and Chlamydospore formation on corn meal agar.[7] All the 50 isolates were subjected to Sugar fermentation test and Sugar assimilation test for final confirmation of species. Simultaneously the *Candida spp.* were inoculated on CHROM agar and incubated at 37°C for 24 hrs and the species were identified by type and colour of the colonies on CHROM agar media as per manufacturer's instructions (Table 1).

Table 1: Colour of various *Candida* Spp. on HiCrome Agar.

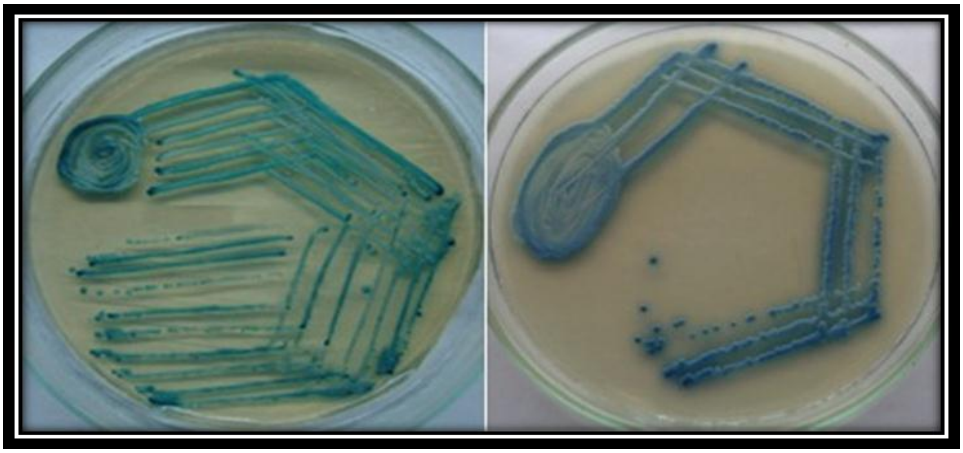
Candida Species	Colour on HiCrome Agar
Candida albicans	Light Green
Candida dubliniensis	Dark Green
Candida glabrata	Cream to White
Candida krusei	Pink, Fuzzy
Candida. parasilosis	White to Cream
Candida tropicalis	Blue to Purple

RESULTS

A total of 50 *Candida* spp. were isolated from various clinical specimens .Table 2 gives the distribution and sources of *Candida* spp. identified by the gold standard conventional methods. *Candida albicans* was the major species accounting for 26 (52.0%) of the total isolates. *Non albicans Candida* constituted 15 (30.0%) of *C. tropicalis*, 7 (14.0%) of *C. krusei* and 2 (4.0%) of *C. parasilosis*. These 50 strains were also identified by using HiCrome *Candida* differential agar. 48 *Candida* species which were correctly identified by HiCrome *Candida* agar except 2 species of *C. parasilosis* (identified by conventional method) which were identified as *C. glabrata* by HiCrome *Candida* agar (Fig 10). 100% sensitivity and specificity was observed for *Candida albicans, Candida tropicalis, Candida krusei*. For *Candida parasilosis* 0% sensitivity and 0% specificity were observed (Table 2).

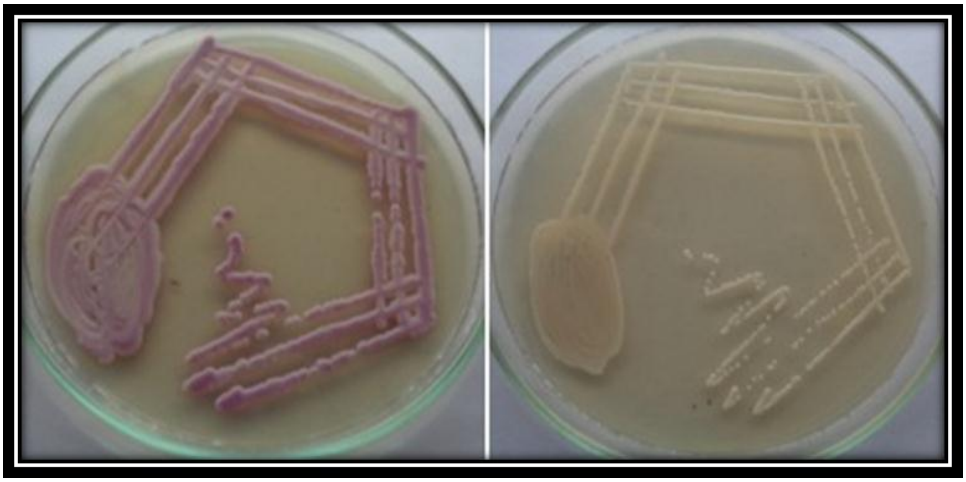
Table 2: Sensitivity & Specificity of HiCrome agar for identification of various *Candida spp.* (n=50).

Candida Spp.	No. of *Candida spp.* identified by conventional method	No. of *Candida spp.* identified by HiCrome method	Sensitivity of HiCrome agar	Specificity of HiCrome agar
C. albicans	26	26	100%	100%
C. tropicalis	15	15	100%	100%
C. krusei	07	07	100%	100%
C. parasilosis	02	00	0%	0%

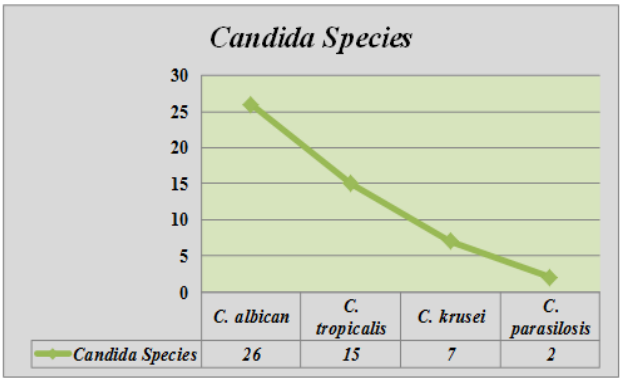


(A) *C. albicans* **(B) *C. tropicalis***

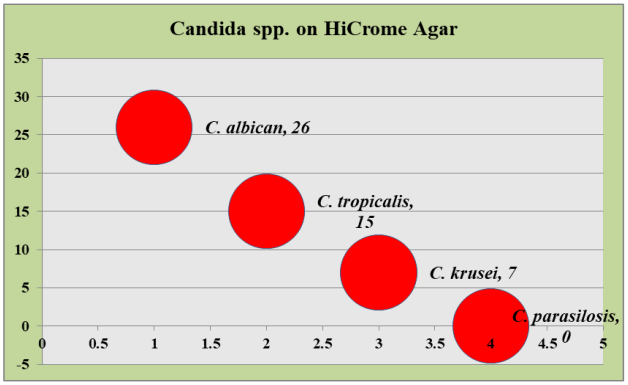
Fig 1: Differentiation of various species of *Candida* on HiCrome agar.



(C) *C. krusei* **(D) *C. parasilosis***



Graph 1: Isolation of different *Candida spp.* by Conventional Method.



Graph 2: Isolation of different *Candida spp.* by HiCrome Agar.

DISCUSSION

The potential clinical importance of species-level identification has been recognized as *Candida* species differ in the expression of virulence factors and antifungal susceptibility.

Non Albicans Candida (NAC) are on the rise due to increasing immunocompromised states. Non Albicans Candida (NAC) are more resistant to fluconazole, 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. In the present study *C. albicans* predominated i.e., 52%. Predominance of *C. albicans* was also seen in the study of Manjunath V, Vidya GS et al in 2012. However higher incidence of non albicans Candida ranging from 50 - 74% have been seen in various studies like Golia S, Reddy KM et al in 2013, Vijaya D, harsha TR et in 2011. Among the non albicans species, *Candida tropicalis* is reported to be the most predominant species as discussed elsewhere. In our study also *C. tropicalis* was the most common non albicans species. 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. 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. In the present study, HiCrome agar showed 100% sensitivity and 100% specificity for *C. albicans*, *C. tropicalis* and *C. krusei*. Our study agrees with that of the study conducted by D. Dadhich et al in 2016, which also showed cent percent sensitivity and specificity to these *Candida* species when compared with conventional method. Our study had its own limitations of small sample size, inability to perform antifungal susceptibility tests. However CHROM agar has proved to be a valuable method for identification of *Candida* species even in resource poor settings.

CONCLUSION

Along with *Candida albicans*, *Non Albicans Candida* (NAC) spp like *C. tropicalis*, *C, krusei* and *C. glabrata* are increasingly being isolated from clinical specimens. On completion of this study it is concluded that *Non-albicans Candida* (NAC) which was earlier considered to be non-pathogenic has emerged as important pathogen. It can no longer be discarded as a lab contaminant. Hence speciation of *Candida* species is of utmost importance in the present clinical scenario. Speciation of *Candida* using conventional methods is quite cumbersome and

time consuming. Therefore species level identification using HiCrome *Candida* agar medium is recommended. The advantages of HiCrome Candida agar is that it is easy to prepare i.e. boiling and dispensing in petri dishes. It also facilitates identify the two or more different species present in a single clinical sample. As a result it can be concluded that the use of HiCrome agar *Candida* is an easy reliable method for presumptive identification of most of the *Candida* species. Furthermore, the species level identification of the *Candida* isolates along with their antifungal susceptibility patterns can greatly influence the treatment options for the clinician and may have an impact on the patient care.

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