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A STUDY ON IDENTIFICATION AND SPECIATION OF MEDICALLY IMPORTANT CANDIDA SPECIES ISOLATED FROM VARIOUS CLINICAL SAMPLES BY USING HICROME CANDIDA.

Microbiology

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ABSTRACT

Candida albicans remains the most frequently isolated species, but an increase in the prevalence of Non-albicans candida is a matter of concern for various laboratories, as NAC show less susceptibility to antifungal agents particularly azoles[1-3]. Routinely used conventional methods are cumbersome and time consuming. Hence 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. A total of 51 non repetitive clinical isolates of Candida were obtained from various clinical samples. *Candida albicans* was the major species accounting for 23 (45.10%) of the total isolates. *Non albicans Candida* constituted 17 (33.33%) of *C. tropicalis*, 9 (17.64%) of *C. krusei* and 2 (3.92%) of *C. parasilosis*. 49 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.

KEYWORDS

Non-albicans Candida, HiCrome Candida differential agar, conventional method, candida speciation.

INTRODUCTION

Candida species particularly Candida albicans remains the most common cause of superficial and deep fungal infections worldwide[1,2]. But an increase in the prevalence of Non albicans Candida (NAC) during the last few decades is a matter of concern for various laboratories, as NAC show less susceptibility to commonly used antifungal agents particularly azoles ie fluconazole[3-5]. Thus species identification of Candida isolates have become very necessary for providing effective antifungal therapy, which will in turn prevent the emergence of drug resistance and also nosocomial infection[6].

A large variety of techniques from conventional to molecular methods are available for yeast identification[4,5]. Routinely used conventional methods like gram staining of smear, germ tube test, colony morphology on Sabouraud's dextrose agar, urease test, growth on corn meal agar, sugar fermentation and assimilation test, growth at 45^{0}C are labour intensive, cumbersome and time consuming ie may take 3-4 days[7].

Now a days, newer methods like several chromogenic media, BiGGYagar system, API system, Vitek 2ID system and molecular methods, have been developed for rapid identification of Candida species[8-10]. HiCrome Candida agar is one of the selective and differential chromogenic media manufactured by Himedia, Mumbia, India. This 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[11]. 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 ie within 48hrs, can be easily interpreted and is also cost effective as compared to other newer methods like API system, Vitek 2ID system and molecular methods which are expensive[12-15].

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, which inturn 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.

MATERIALAND METHOD

Study design and setting – A prospective study was carried out in the department of Microbiology, NIMS Medical College and Hospital, Jaipur (Rajasthan) from January 2017 to May 2017. A total of 50 non repetitive clinical isolates of *Candida* obtained from various clinical samples, which were received from different Wards, ICUs and OPDs of the hospital and were submitted to microbiology laboratory with all aseptic precautions were included in the study.

Specimen processing [7]

1. Direct examination of sample- Direct microscopic examination by 10% KOH mount and Gram staining reveals presence of oval budding yeast cells with / without pseudohyphae[7].

2. Culture – All the clinical samples were inoculated on to Blood agar and SDA , both were incubated at 37C for 48-72 hrs. All the samples which showed growth were identified by colony characteristics and by gram staining. Once the conformation of colonies was done, they were further speciated[7].

3. Speciation of candida isolates (Fig 1-8)

(a) Standard conventional method - Candida isolates were further speciated by standard method which includes germ tube test, growth pattern on Corn Meal agar , sugar fermentation and assimilation test[7].

(b) Growth on chromogenic agar – Isolated Candida species were also inoculated on to the chromogenic media (HiCrome Candida differential agar (M1297A); Himedia, Mumbia, India.) Inoculated plate was incubated at 37^{0}C for 24 hours. These plates were further incubated for 48 hrs to get better well developed colored colonies. The color of the colony was interpretated as per color code provided on chromogenic media (Table 1) by two observer to avoid subjective variation[11]. All the isolates with doubtful morphology were considered unidentified and excluded from the study.

RESULT

In the present study, a total of 51 (4.5%) Candida species were isolated from 1113 clinical specimens. In our study conventional method was considered gold standard method for speciation. Out of 51 Candida isolates, most common isolated species was Non-albicans Candida ie 28 (55%) followed by Candida albicans ie 23 (45%) (Fig 4). Out of 28 Non-albicans Candida, C.tropicalis was most frequently isolated (33%) followed by C.krusei (18%), C.parasilosis (4%) (Fig 9).

These 51 strains were also identified using Hicrome Candida differential agar. 49 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. 0% sensitivity and100% specificity for Candida parasilosis (Table 2).

DISCUSSION

In the present study, out of 51 Candida isolates, 23(45.1%) were identified as C. albicans and 28 (54.9%) were NAC species. Our study showed that Non albicans Candida were isolated at higher rate (54.9%) than C. albicans (45.1%), which was in agreement with the studies conducted by Saroj Golia et al[17] and N.Pahwa et al[19] who also showed the Non albicans Candida incidence (64% and 58% respectively) to be higher than that of C. albicans . This change in pattern has been partly attributed to increased immune suppression resulting in higher number of susceptibility in immunocompromised patients and also due to the prophylactic use of antifungal agents in critically ill patients. Hospitalization (especially in ICU), placement of central venous catheters and the other indwelling devices, previous antimicrobial therapies have played significant role in this changing pattern of Candidiasis[22,23].

In our study, the most frequently isolated species was C. albicans in 45.1% of the infections, followed by C. tropicalis 33.3%, C. krusei 17.6% and C. glabrata 3.92% respectively. Hence C. tropicalis (33.3%) was the second most common species reported in the present study. This finding was comparable with other workers like L.Sumitra Devi et al.[18] (22.9%), Divya Dadhich et al.[20] (26.4%), and Khan and Bobade O, et al.[21] (21%) [table3].

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 studies conducted by D. Dadhich et al[20] and Nayak et al[24].,who also showed cent percent sensitivity and specificity to these Candida species when compared with conventional method.

In our study low sensitivity was reported for C. parasilosis which correlates well with the study conducted by tha of Baradkar et al[11].

These values are in contrast to the study conducted by various other scientists[24,25]. As both the species of C.parasilosis showed characteristic morphology on corn meal agar. Hence a combination of HiCrome and Corn meal agar can identify this Candida species with in 48 hours of culture.

CONCLUSION

On completion of this study it is concluded that *Non- albicans* Candida 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 advocated. The advantages of HiCrome Candida agar is that it is easy to prepare i.e. boiling and dispensing in petri plates. It is technically simple and cost effective compared to technically demanding and time consuming conventional methods. It also facilitates identification of two or more different species present in a single clinical sample as is seen in our study. 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.

Table 1: Colour of different Candida species on Hicrome agar

Species	Color of the colony
C. albicans	Light-green
C. dubliniensis	Dark-green
C. glabrata	Cream to white
C. krusei	Pink, fuzzy
C. parapsilosis	White to Cream
C. tropicalis	Blue to purple

Table 2: Sensitivity and specificity of HiChrom agar for identification of various Candida spp.

Candida spp	No.of Candida spp. identified by conventional method	No.of Candida spp. identified using HiCrome agar	Sensitivity of HiCrome agar	Specificity of HiCrome agar
C. albicans	23	23	100%	100%
C. tropicalis	17	17	100%	100%
C. krusei	9	9	100%	100%
C. parasilosis	2	0	0	100%

Table- 3 Candida species isolated in various studies

S. No.	Author	Year, Place	C. albicans	C. tropicalis	C. Krusei
1	**Badiee P[16]**	Iran, 2011	48%	1.7%	16.1%
2	Saroj Golia[17]	Karnataka, 2013	35.71%	26.78%	4.46%
3	L. Sumitra Devi[18]	Haryana, 2014	51.6%	25%	16.6%
4	N Pahwa[19]	Indore, 2014	42.2%	22.4%	3.4%
5	Divya Dadhich[20]	Kota, 2016	54%	22%	6%
6	Present Study	Jaipur, 2017	45.1%	33.3%	17.6%

Fig 1 –Microscopic appearance of various Candida species on Corn meal agar

(a) C. albicans - elongated pseudohyphal cells with terminal chlamydospores.
(b) C. tropicalis – blastoconidia formed along the abundant long branching pseudohyphal cells.
(c) C. krusei - chains of blastoconidia extend from the junction of pseudohypal cells.
(d) C. parasilosis - blastoconidia occur singly, in clusters and in short chains along the pseudohyphae.

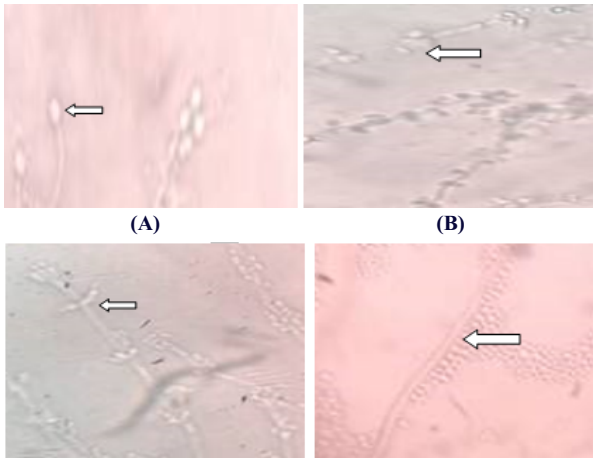


Fig 2 Differentiation of various species of Candida on HiCrome agar

(a) C. albicans (b) C. tropicalis (c) C. krusei(d) C. parasilosis

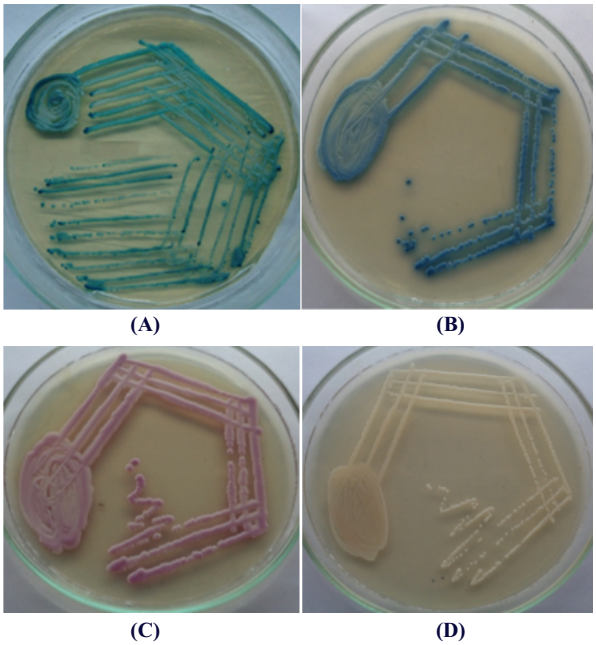


Figure 9 : Isolation of different species of Candida by Conventional methods

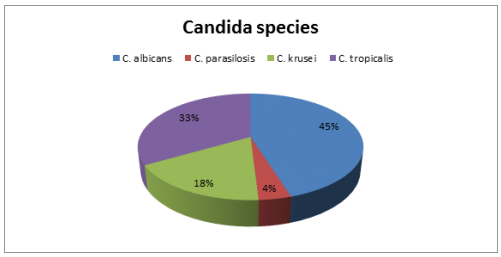
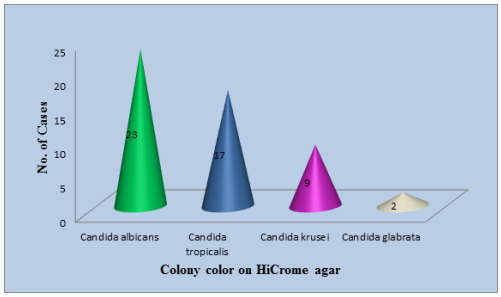


Figure 10 Isolation of different Candida species on HiCrome agar



REFERENCES

1) Rippon, J.W. 1988. Medical mycology: the pathogenic fungi and the pathogenic actinomycetes. :1-797 .
2) Cortegiani A, Misseri G, Chowdhary A. What's new on emerging resistant Candida species. Intensive Care Med. 2019 Apr;45(4):512–5.
3) Abi-Said D, Anaissie E, Uzun O, Raad I, Pinzcowski H, Vartivarian S. The epidemiology of hematogenous candidiasis caused by different Candida species. Clin Infect Dis. 1997 Jun;24(6):1122–8.
4) Price MF, LaRocco MT, Gentry LO. Fluconazole susceptibilities of Candida species and distribution of species recovered from blood cultures over a 5-year period. Antimicrobial Agents and Chemotherapy. 1994 Jun 1;38(6):1422–4.
5) Antifungal susceptibility testing of isolates from a randomized, multicenter trial of fluconazole versus amphotericin B as treatment of nonneutrope... - PubMed - NCBI [Internet]. [cited 2019 Dec 22].
6) Dagi HT, Findik D, Senkeles C, Arslan U. Identification and antifungal susceptibility of Candida species isolated from bloodstream infections in Konya, Turkey. Ann Clin Microbiol Antimicrob [Internet]. 2016 May 31 [cited 2019 Dec 22];15. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4888423/
7) Jagdish Chander. Candidiasis. Text Book of Medical Mycology. 3rd edition. 2009: p. 266-90.
8) Utility of the germ tube test for direct identification of Candida albicans from positive blood culture bottles. - PubMed - NCBI [Internet]. [cited 2019 Dec 22].
9) A Comparison of updated Vitek Yeast Biochemical Card and API 20C yeast identification systems. [Internet]. [cited 2019 Dec 22].
10) Evaluation of the VITEK 2 system for rapid identification of yeasts and yeast-like organisms. - PubMed - NCBI [Internet]. [cited 2019 Dec 22].
11) Baradkar VP, Mathur M, Kumar S. Hichrom candida agar for identification of Candida species. Indian J Pathol Microbiol . 2010;53:93-5.
12) CHROMagar Candida, a new differential isolation medium for presumptive identification of clinically important Candida species. [Internet]. [cited 2019 Dec 22]. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC263904/
13) Species distribution and drug susceptibility of candida in clinical isolates from a tertiary care centre at Indore Pahwa N, Kumar R, Nirkhiwale S, Bandi A - Indian J Med Microbiol [Internet]. [cited 2019 Dec 22].
14) High rate of non-albicans candidemia in Brazilian tertiary care hospitals. - PubMed - NCBI [Internet]. [cited 2019 Dec 22]. Available from: https :// www. ncbi. nlm.nih.gov/pubmed/10459478
15) Nosocomial candidiasis: emerging species, reservoirs, and modes of transmission. - PubMed - NCBI [Internet]. [cited 2019 Dec 22].9. Utility of the germ tube test for direct identification of Candida albicans from positive blood culture bottles. - PubMed - NCBI [Internet]. [cited 2019 Dec 22]. Available from: https:// www. ncbi. nlm. nih. gov/pubmed/18685004.
16) Badiee P, Alborzi A Susceptibility of clinical Candida species isolates to antifungal agents by E-test, Southern Iran: A five year study IRAN. J. MICROBIOL. 3 (4) : 183-188.
17) Saroj Golia, K. Mallika Reddy K. Sujatha Karjigi and Vivek Hittinahalli Speciation of Candida using chromogenic and cornmeal agar with determination of fluconazole sensitivity Al Ameen J Med Sci 2013; 6(2) :163-166.
18) L. Sumitra Devi, Megha Maheshwari. Speciation of Candida species isolated from clinical samples by using chrom agar and conventional methods. International Journal of Scientific and Research publications. March 2014, volume 4, issue 3.
19) Pahwa N, Kumar R, Nirkhiwale S, Bandi A. Species distribution and drug susceptibility of Candida in clinical isolates from a tertiary care centre at Indore. Indian J Med Microbiol 2014;32:44-8.
20) Dadhich D, Saxena N, Chand AE, Soni G, Morya S. Detection of Candida Species by Hichrom Agar and Their Antimycotic Sensitivity in Hadoti Region. Int J Sci Stud 2016;4(4):23-2
21) Bobade O, Waghmare M, Chhabrani P, Kaur I. Species distribution and antifungal susceptibility profile of Candida isolated from urine samples. Int J Med Sci Public Health 2013;2:867-70.
22) The changing face of candidemia: emergence of non-Candida albicans species and antifungal resistance. - PubMed - NCBI [Internet]. [cited 2019 Dec 22].
23) High rate of non-albicans candidemia in Brazilian tertiary care hospitals. - PubMed - NCBI [Internet]. [cited 2019 Dec 22].
24) Nayak S, Kavitha B, Sriram G, Saraswathi TR, Sivapathasundharam B, Dorothy AL. Comparative study of Candida by conventional and CHROMagar method in non-denture and denture.
25) Peng CF, Lee KM, Lee SH. Characterization of two chromogenic media of Candida ID2 and CHROMagar Candida for preliminary identification of yeasts. J Biomed Lab Sci 2007;19:63-8.