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Original Article

Detection of *Candida* Species by Hichrom Agar and Their Antimycotic Sensitivity in Hadoti Region

Divya Dadhich[1], Naveen Saxena[2], Anita E Chand[3], Ghanshyam Soni[2], Suchitra Morya[4]

[1]Assistant Professor, Department of Microbiology, Government Medical College, Kota, Rajasthan, India, [2]Professor, Department of Microbiology, Government Medical College, Kota, Rajasthan, India, [3]Senior Professor and Head, Department of Microbiology, Government Medical College, Kota, Rajasthan, India, [4]Senior Demonstrator, Department of Microbiology, Government Medical College, Kota, Rajasthan, India

Abstract

Introduction: Hichrom agar is a differential culture medium which facilitates the isolation and identification of some clinically important species of *Candida*.

Materials and Methods: A total of 100 *Candida* species were isolated from various mucocutaneous clinical specimens including oral thrush, vaginitis, balanitis, and angular cheilitis. Speciation of *Candida* was done using Hichrom agar and conventional methods simultaneously. Antifungal susceptibility testing was done by the disc diffusion method to amphotericin B, fluconazole, nystatin, itraconazole, ketoconazole, and clotrimazole.

Results: *Candida albicans* (54%) was the predominant species isolated. Non-albicans *Candida* spp. isolated was *Candida tropicalis* (22%), *Candida glabrata* (12%), *Candida krusei* (06 %), *Candida parapsilosis* (04%), and *Candida kefyr* (02%). Antifungal susceptibility testing was done using antimycotic sensitivity testing by disc diffusion method. Overall antifungal drug resistance for *Candida* in the present study was 26% for fluconazole, 24% for itraconazole, 29% for clotrimazole, 18% for ketoconazole, and 10% for nystatin. No resistance was observed for amphotericin B.

Conclusion: The advantage of using Hichrom agar is that it helps in the isolation and identification of *Candida* to species level. The performance of Hichrom agar paralleled that of conventional methods. Use of this medium is rapid, technically simple, and cost-effective compared to time-consuming technically demanding expensive conventional method. Hichrom agar serves as a primary isolation and differentiation medium for clinical specimens that could allow mycology laboratories to rapidly identify *Candida* spp., enabling clinicians to choose appropriate antifungal agents, thus decreasing patient's morbidity and mortality.

Key words: Antifungal susceptibility testing, *Candida*, Hichrom agar, Fluconazole

INTRODUCTION

Candida is the most common fungal infection found in the humans affecting mucosa, skin, nails, and internal organs. *Candida* species colonize the mucosal surfaces of all humans soon after birth, and the risk of endogenous infection is ever-present.[1] *Candida* species are a component of the normal flora of human beings and commonly found on the skin, gastrointestinal tract, and female genital tract, particularly higher in the vagina during pregnancy.[1] Carriage rate of *Candida* species tends to increase with age. *Candida* species are the fifth most common cause of blood stream infections and fourth common cause of nosocomial infections.[1,2] *Candida* species produces various cutaneous, mucocutaneous, and systemic manifestations depending on the immune status of the host and underlying predisposing factors. In developed countries, *Candida albicans* accounts for 40-60% of yeasts isolates, whereas Indian reports show an increased predominance of non-*C. albicans* (NAC) isolates.[3,4] Increase in the prevalence of non-albicans species such as *Candida glabrata* and *Candida krusei*[5] has been noted during the past decade because of the extensive use of antimycotic drugs particularly azoles for prolonged periods. *C. glabrata* is associated with severe complications than other species.[5]

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Corresponding Author: Dr. Anita E Chand, House No. 2-k-9 Rangbari Yojna, Kota - 324 005, Rajasthan, India. Phone: +91-09829577983. E-mail: dr.williammasih@yahoo.com

Several brands of chromogenic media have been developed to produce rapid yeast identification. *C. albicans* produces an enzyme b-N-acetyl-galactosaminidase[6] and incorporation of chromogenic or fluorogenic hexosaminidase substrates into the growth medium helps in identification of *C. albicans* isolates directly on primary isolation.[7] Hichrom *Candida* differential agar is a selective and differential medium, which facilitates rapid isolation of yeasts from mixed cultures and allows differentiation of *Candida* species namely *C. albicans, C. krusei, Candida Tropicalis,* and *C. glabrata* on the basis of coloration and colony morphology.

It is necessary to identify *Candida* to species level as many NAC have decreased susceptibility to antifungal agents. The present study was undertaken to evaluate the advantages of Hichrom agar over conventional method for speciation of *Candida* isolates and their susceptibility to antifungal agents by disc diffusion method.

MATERIALS AND METHODS

The study was conducted at the Department of Microbiology, Government Medical College and M.B.S Hospital, Kota, from June 2012 to September 2013. A total of 100 strains of *Candida* were isolated from various mucocutaneous clinical samples of patients admitted in MBS Hospital and associated Group of Hospitals - JK Lone Hospital and NMC Hospital, Kota, Rajasthan.

Hichrom agar was prepared as per the instruction manual (Himedia India) (Table 3). *Candida* spp. isolated was inoculated simultaneously to Hichrom agar plates and Sabouraud dextrose agar (SDA) tubes. These were incubated at 37°C for 48 h. Species were identified on Hichrom agar by morphology and color of the colony. Growth on SDA was speciated by standard methods using germ tube, corn meal agar, sugar fermentation, and assimilation test. Appearances of *Candida* spp. on Hichrom agar were as follows:[8]

- *C. albicans* - blue green
- *C. tropicalis* - dark blue-gray center with pink halo
- *C. krusei* - pink large rough spreading colonies with pale edge
- *C. parapsilisis* - pale cream colored colonies
- *C. glabrata* - cream to white smooth colonies.

Antifungal susceptibility was performed by disc diffusion method using antimycotic sensitivity test agar. Discs used were amphotericin B (100 units), fluconazole (10 mcg), clotrimazole (10 mcg), nystatin (100 mcg), itraconazole (10 mcg), ketoconazole (10 mcg), and sensitivity zones were measured as for the instruction manual (Himedia).[8,9] ATCC strain of *C. albicans* was used as control.

RESULTS

Table 1 shows *Candida* spp. isolated in various clinical samples. All isolates of candida grew on Hichrom agar after 48 h of incubation at 37°C.

Overall antifungal drug resistance for *Candida* in the present study was 26% for fluconazole, 24% for itraconazole, 29% for clotrimazole, 18% for ketoconazole, and 10% for nystatin. No resistance was observed for amphotericin B. The results of this study have been presented in Tables 1 and 2.

DISCUSSION

C. albicans (54%) was the most prevalent species of *Candida* reported in the present study (Table 1). This finding was consistent with the findings of other workers who reported that the incidence of *C. albicans* was 61.3% (Biradar *et al.*),[10] 49.3% (Feglo and Narkwa),[11] and 47% (Dominic and Dharwad).[12] However, Kashid *et al.*[13] found that *C. tropicalis* was the most prevalent species accounted for 46.2% followed by *C. albicans* (29.2%). *C. tropicalis* (22%) was the second most common species reported in the present study. This finding was comparable with other workers, Bobade *et al.*[14] (22.9%), Babin *et al.*[15] (26.4%), and Khan and Baqai *et al.*[16] (21%). However, *C. glabrata* was reported as second most common species by Feglo and Narkwa *et al.*[11] (17.9%) and Saldhei *et al.*[17] (11.9%).

Overall antifungal drug resistance for *Candida* in the present study was 26% for fluconazole, 24% for itraconazole, 29% for clotrimazole, 18% for ketoconazole, and 10% for nystatin. No resistance was observed for amphotericin B. In present study, 22.2% of *C. albicans* was found to be fluconazole resistant which is in consonance with Kashid *et al.*[13] and Babin *et al.*[15] However, a higher resistance was observed by Saldhei *et al.*[17] (81.1%). For NAC, the resistance varies from 0% in *Candida kefyr* to 100% in *C. krusei*. For AmB, no resistance was observed in the present study. This was in consonance with Kashid *et al.*[13] Bobade *et al.*[14] reported 7.5% resistance in *C. albicans*.

The conventional methods and the CHROM agar method were compared and were found to give similar results. This was similar to the findings of Nayak *et al.*,[18] who found that CHROM agar showed 100% specificity and 100% sensitivity when compared to SDA and conventional methods. The advantages of Hichrom agar are easy to prepare, i.e., boiling, facilitate the rapid isolation, and identification of yeast species. Hichrom agar facilitates identification between yeast spp. from specimens containing mixture of yeast spp. and do not affect the

Table 1: *Candida* species isolated from different clinical conditions

Clinical condition	*C. albicans* (%)	*C. tropicalis* (%)	*C. glabrata* (%)	*C. krusei* (%)	*C. parapsilosis* (%)	*C. kefyr* (%)	Total (%)
Oral thrush	23 (42.5)	17 (31.4)	6 (11.1)	6 (11.1)	0	2 (3.7)	54 (54)
Vaginitis	26 (68.4)	3 (7.8)	6 (15.7)	0	3 (7.8)	0	38 (38)
Balanitis	4 (80)	0	0	0	1 (20)	0	5 (5)
Angular cheilitis	1 (33.3)	2 (66.7)	0	0	0	0	3 (3)
Total	54 (54)	22 (22)	12 (12)	6 (6)	4 (4)	2 (2)	100

C. albicans: Candida albicans, C. tropicalis: Candida tropicalis, C. glabrata: Candida glabrata, C. krusei: Candida krusei, C. parapsilosis: Candida parapsilosis, C. kefyr: Candida kefyr

Table 2: Antifungal sensitivity profile of *Candida* isolates (in percentage)

Antifungal drugs	Fluconazole (25 µg)			Itraconazole (10 µg)			Clotrimazole (10 µg)			Ketoconazole (10 µg)			Nystatin (100 U/disc)			AmB (100 U)		
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
C. albicans	48.1	29.6	22.2	29.6	44.4	25.9	14.8	55.5	29.6	29.6	44.4	25.9	66.7	29.6	3.7	100	00	00
C. tropicalis	54.5	31.8	13.6	54.5	31.8	13.6	40.9	36.3	22.7	90.9	9.1	00	40.9	36.3	22.7	100	00	00
C. glabrata	50	16.6	33.4	50	16.6	33.4	16.6	33.4	50	50	33.4	16.6	33.4	50	16.6	100	00	00
C. kefyr	100	00	00	100	00	00	100	00	00	100	00	00	100	00	00	100	00	00
C. parapsilosis	50	25	25	50	25	25	25	50	25	50	25	25	25	50	25	100	00	00
C. krusei	00	00	100	00	00	100	66.6	16.6	16.6	66.6	16.6	16.6	100	00	00	100	00	00
Total (in percentage)	48	26	26	38	38	24	26	45	29	50	32	18	58	32	10	100	00	00

S: Sensitive, I: Intermediate sensitive, R: Resistant, *C. albicans: Candida albicans, C. tropicalis: Candida tropicalis, C. glabrata: Candida glabrata, C. krusei: Candida krusei, C. parapsilosis: Candida parapsilosis, C. kefyr: Candida kefyr*

Table 3: Comparison of various studies showing *Candida* species recovered from clinical samples (in percentage)

Candida species	Present study	Bobade *et al.* 2013[14]	Saldhei *et al.* 2012[17]	Vijaya *et al.* 2011[8]	Kashid *et al.* 2011[13]	Feglo and Narkwa 2011[11]	Babin *et al.* 2011[15]	Shivanand *et al.* 2011[21]	Fouzia *et al.* 2010[16]	Birader *et al.* 2009[10]	Usharani *et al.* 2005[20]
C. albicans	54	36.6	79.1	45.9	29.2	49.3	35.5	47	30	61.3	62.5
C. tropicalis	22	22.9	5.9	35.29	46.2	11.9	22.9	30	21	18	15.6
C. glabrata	12	13.7	11.9	-	6.12	17.9	20.6	09	08	10.6	9.3
C. krusei	6	08.7	2.9	10.78	-	4.5	15.7	14	03	3.3	-
C. parapsilosis	4	03.6	-	7.84	10.2	1.5	-	-	10	-	-
C. kefyr	2	11.46	-	-	1.36	-	-	-	-	-	-

viability on subsequent subcultures.[19] Hichrom agar has the advantage of rapid identification of *Candida* species, technically simple, rapid, and cost-effective compared to technically demanding time consuming and expensive conventional method.

CONCLUSION

Although the results on Hichrom agar exactly paralleled that of the conventional method, it is superior to SDA in terms of suppressing the bacterial growth. Use of Hichrom agar medium would allow mycology laboratories to identify rapidly, clinically important *Candida* spp. while potentially decreasing laboratory cost. 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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