

Candida Speciation Using Chrom Agar

VIJAYA D., HARSHA T.R., NAGARATNAMMA T.

ABSTRACT

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

Materials and Methods: A total of 102 *Candida* species were isolated from various clinical specimens (100) including stool, sputum, nasal, oral, ear swabs, diabetic foot, skin and nail scrapings. Speciation of *Candida* was done using CHROM agar and conventional method simultaneously.

Observations: Non albicans candida (NAC) predominated (54.1%) over *Candida albicans* (45.9%). Non albicans candida spp isolated were *C.tropicalis* (35.29%), *C.krusei* (10.78%), *C.parapsilosis* (7.84%), and *C. dubliniensis* (0.9%). Antifungal susceptibility testing was done using antimycotic sensitivity testing by disc diffusion method. Isolates were 100% sensitive to

ketoconazole, clotrimazole, nystatin and amphotericin B. 87.5% of *C.krusei*, 36% *C.tropicalis*, 6.25% *C. albicans* were resistant to itraconazole. 25% *C.krusei* and 8% *C.tropicalis* were resistant to fluconazole. *C.dubliniensis* was resistant to itraconazole only.

Conclusion:The advantage of using CHROM agar is that it facilitates the isolation and identification of *Candida* to species level. The performance of CHROM agar exactly 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.

CHROM 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 patients morbidity and mortality.

Key Words: Candida, CHROM agar, Antifungal susceptibility

INTRODUCTION

Candida albicans historically has been predominant cause of Candidiasis. In 1980s *C.albicans* accounted for greater than 80% of all candidial isolates recovered from nosocomial yeast infections [1]. More recently non albicans candida (NAC) species has been recovered with increasing frequency.

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 [1]. 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 CHROM agar over conventional method for speciation of *Candida* isolates and their susceptibility to antifungal agents by disc diffusion method.

MATERIALS AND METHODS

A total of 102 *Candida spp* isolated from randomly selected 100 clinical specimens which included stool, sputum samples, nasal, oral and ear swabs, diabetic foot, skin and nail scrapings formed the study group. The study was conducted from Jan 2008 to Dec 2008, in the department of Microbiology, Bowring and L.C. Hospital, Bangalore.

CHROM agar was prepared as per the instruction manual (Hi media India). *Candida spp* isolated were inoculated simultaneously to CHROM agar plates and SDA tubes. These were incubated

at 37°C for 48 hours. Species were identified on CHROM agar by morphology and colour of the colony. Growth on SDA were speciated by standard methods using germ tube, corn meal agar, slide culture, sugar fermentation and assimilation test. Ten faecal samples and two oral swabs from AIDS patients were directly inoculated onto SDA and CHROM agar.

A variety of species specific colony were seen.

Appearances of *Candida spp* on CHROM agar were as follows:

- *C.albicans* – blue green
- *C.tropicalis* – dark blue gray centre with pink halo
- *C. krusei* – pink large rough spreading colonies with pale edge.
- *C.parapsilosis* – pale cream coloured colonies.
- *C.dubliniensis* – dark green colonies.

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

RESULTS

[Table/Fig-1] shows *Candida spp* isolated in various clinical samples. All isolates of candida grew on CHROM agar after 48 hr of incubation at 37°C.

C.dubliniensis isolated from faecal sample of AIDS patient grew only on CHROM agar. *C.dubliniensis* was further identified by germ tube, chlamydospore formation and no growth at 45°C.

A mixed growth of *C.tropicalis* +*C.parapsilosis*, *C.albicans* + *C.tropicalis* were isolated in two sputum samples.

Isolates were 100% sensitive to amphotericin B, clotrimazole, nystatin and ketaconazole 87.5% of *C.krusei*, 36% *C.tropicalis*, 6.5% *C.albicans* were resistant to itraconazole. 25% of *C.krusei*, 28% *C.tropicalis* showed resistant to fluconazole. *C.dubliniensis* was resistant to itraconazole only.

DISCUSSION

In the present study NAC (55.8%) was isolated at a higher rate than *C.albicans* as reported by other workers. *Candida spp* isolated by various workers is shown in [Table/Fig-2] [2-9]. In our 1997 study *C.albicans* predominated [10]. Among the stool samples received 90% were from antibiotic associated diarrhoea. *C.dubliniensis* isolated on CHROM agar did not grow on SDA. Isolation of this species has been reported by Raut [11]. Using CHROM agar species like *C.dubliniensis* can be isolated as in the present study. Mixed growth of *Candida spp* found in faecal specimens also has been reported by Frank [6].

[Table/Fig-3] shows the antifungal susceptibility as reported by various workers [12-14]. Variability found in antifungal susceptibility testing using disc diffusion methods as shown in [Table/Fig-3], as it is dependent on the disc potency, inoculum size, temperature and duration of incubation.

The advantages of CHROMagar are easy to prepare ie boiling and dispensing in Petri plates, facilitates the rapid isolation and identification of yeast species. CHROM agar facilitates identification between yeast spp from specimens containing mixture of yeast spp as in the present study and do not affect the viability on subsequent subcultures [6]. CHROM 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.

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

Specimen(100)	<i>C.albicans</i>	<i>C.tropicalis</i>	<i>C.krusei</i>	<i>C.parapsilosis</i>	<i>C.dubliniensis</i>
Stool-47	12	24	4	6	1
Sputum-23	18	5	0	2	0
CSOM-10	4	5	1	0	0
Nasal swab-6	3	0	3	0	0
Oral swab-5	5	0	0	0	0
Diabetic foot-5	2	2	1	0	0
Skin&Nail -4	2	0	2	0	0
Total 100 %	46 45.09	36 35.29	11 10.78	8 7.84	1 0.9

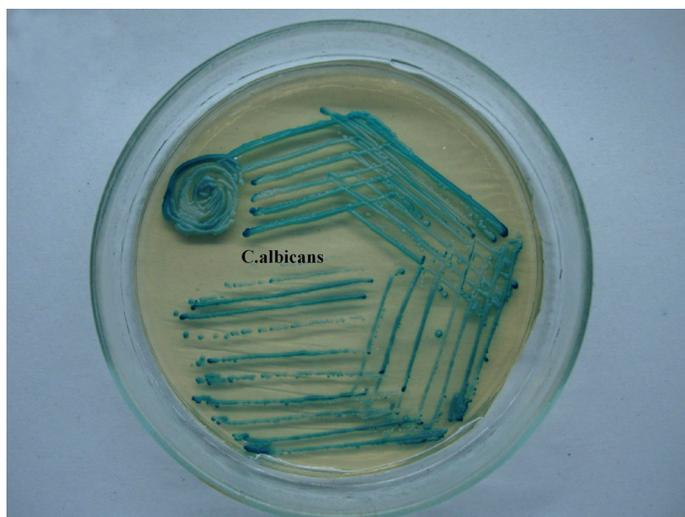
[Table/Fig-1]: *Candida spp* isolated in various clinical samples

	Present study 2009	Shiva Prakash ² 2007	Cooper ³ 2007	Vijaya ⁴ 2000	Grace ⁵ 2005	Lymn ¹ 2003	Frank ⁶ 1994	Prasad ⁷ 1999	Baradkar ⁸ 1999	Pfeller ⁹ 1999
<i>C.albicans</i>	45.9	3.4	25.80	46	43.15	24	39.25	47.6	76.92	52
<i>C.tropicalis</i>	35.29	35.6	38.70	30	9.47	24	7.43	35.4	17.94	11
<i>C.krusei</i>	10.78	–	6.45	10	1.05	10	5.92	4.9	–	2
<i>C.parapsilosis</i>	7.84	28.8	25.8	–	21.05	6	10.19	1.2	–	–
<i>C.dubliniensis</i>	0.9	–	–	–	–	–	–	–	5.12	–

[Table/Fig-2]: *Candida spp* isolated by various workers in percentage

	<i>C.albicans</i>			<i>C.tropicalis</i>			<i>C.krusei</i>			<i>C.parapsilosis</i>		
	Fluconazole	Itraconazole	Amphotericin	Fluconazole	Itraconazole	Amphotericin	Fluconazole	Itraconazole	Amphotericin	Fluconazole	Itraconazole	Amphotericin
Vijaya Present study 2009	100	93.75	100	92	64	100	75	12.5	100	100	100	100
Srinivasan ¹² 2006	30.6	75	100	22.2	77.8	88.9	0	14.3	42.9	0	75	100
Vijaya 2001	80	–	100	60	–	100	0	–	100	–	–	–
Ashok rathan ¹³ 1999	10	11		3	5		100	31		0	3	
Susan verghese ¹⁴ 1998	96.66	96.66	100	97.7	98.84	100	79.43	85.8	100	100	–	–

[Table/Fig-3]: Antifungal susceptibility as reported by various workers.(%)



[Table/Fig-4]: C.albicans on Chromagar



[Table/Fig-6]: C.krusei on Chrom agar



[Table/Fig-5]: C.tropicalis on Chromagar

importantly this capability will also enable clinicians to choose appropriate anti fungal agents, thus decreasing patient's morbidity and mortality [Table/Fig-4, 5, 6].

ACKNOWLEDGEMENT

This study was supported by Medical Education and Research Trust, Bangalore. Authors are grateful to the trustee of MERT Bangalore.

REFERENCES

[1] Lynn L. H., Duane R. H., Elirton K. M. Dooley D. Direct isolation of candida spp from blood cultures on the chromogenic medium CHROM agar candida. *J Clin Microbiol.* 2003;41:6:2629-2632

- [2] Shivaprakasha S,Radhakrishan K,Karim PMS.Candida spp other Candida albicans: A major cause of fungemia in a tertiary care centre. *Indian J Med Microbiol* 2007;25:4:405-407.
- [3] Capoor MR, Rawat D,Nair D,Deb M, Aggarwal P.Evaluation of glucose-methylene blue Mueller Hinton agar for E test minimum inhibitory concentration determination in candida spp. *Indian J Med Microbiol* 2007;25:4:432-433
- [4] Vijaya D. Malini.Characterisation & antifungal susceptibility of candida spices. A Preliminary study. *J Acad Clin Microbiol.* 2000;2(2):55-57
- [5] Grace L, Tan, Ellena M Peterson. CHROM agar candida medium for direct susceptibility testing of yeast from blood culture . *J Clin Microbiol* 2005;43:4:1727-1731
- [6] Frank C.ODDS, Ria Bernaets.CHROMO agar candida a new differential medium for presumptive identification of clinically important candida species. *J Clin Microbiol* 1994;12:1923-1929.
- [7] Prasad KN, Agarwal J, Dixit AK, Tiwani DP, Dhole TN, Ayyageni A. Role of yeasts as nosocomial pathogens & their susceptibility to fluconazole & amphotericin B. *Indian J Med Res* 1999;110:11-17
- [8] Baradkar VP, Karyakarte RP. Isolation & characterization of candida species in acquired immunodeficiency syndrome. *Indian J Med Microbiol* 1999;17:1:42-44.
- [9] Pfler MA,Messer SA,,Hollis RJ et al. Trends in species distribution and susceptibility of fluconazole among blood stream isolates of Candida species in the United States. *Diag Microbiol Infect Dis* 1999;33:4:311-7
- [10] Vijaya D,Nagarathamma T.Opportunistic pathogenic fungi associated with chronic bronchopulmonary diseases. *Indian J Microbiol* 1997; 37:159-160.
- [11] Raut SH,Varaiya A.Differentiation of candida dubiliniensis on CHRO Magar and Pals agar. *Indian J Med Microbiol* 2009;27:1:55-58.
- [12] SrinivasanL,Kenneth J. Antibiotic susceptibility of candida isolates in a tertiary care hospital in southern India. *Indian J Med Microbiol* 2006;24:1:80.
- [13] Ashok rathan.Antifungal susceptibility testing. *Indian J Med Microbiol* 1999;17:3:125-128.
- [14] Susan verghese ,Sapna MC,Padmaja P,Sudha P. Disc diffusion sensitivity testing of candida species. *Ann Natl Acad Med Sci* 1998; 34:2:135-142.

AUTHOR(S):

1. Dr. Vijaya D.
2. Dr. Harsha T.R.
3. Dr. Nagarathamma T.

PARTICULARS OF CONTRIBUTORS:

1. Corresponding Author
2. Department of Microbiology, AIMS, B.G.Nagara, Bellur 571448.
3. Bowring & L.C.Hospital Bangalore, India.

NAME, ADDRESS, TELEPHONE, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Dr. D.Vijaya
130, 11nd main road Email-vijayadanand@rediffmail.com
Sheshadripuram, Bangalore 560020, Karnataka.
Phone: 080-23446667; 9482009120

DECLARATION ON COMPETING INTERESTS:

No competing Interests.

Date of Submission: Jan 07, 2011
Date of Peer Review: Mar 05, 2011
Date of Acceptance: Jul 20, 2011
Date of Publishing: Aug 08, 2011