RESEARCH ARTICLE ISSN: 2467-9151 OPEN ACCESS

Identification of *Candida* species using CHROM agar

Sangeeta Khadka[1]*, Pravesh Regmi[1], Samita Giri[1], Pradeep Kumar Shah[2], Shyam Kumar Mishra[3]

[1]Department of Microbiology, St. Xavier's College, Kathmandu, Nepal
[2]Department of Microbiology, Tri-Chandra College, Kathmandu, Nepal
[3]Department of Microbiology, Institute of Medicine, Kathmandu, Nepal

ABSTRACT

Background: *Candida* is the part of normal microbial flora of human body that potentially causes disease in the immune compromised host. Though *C. albicans* is regarded as most virulent species, non- *albicans Candida* are increasingly being reported in clinical samples which warrant their accurate identification that helps in directing the effective use of antifungal agents.

Methods: The study was done in clinical isolates of *Candida* species obtained at clinical laboratory, Nobel Hospital, Kathmandu from the period of May 2014 to April 2015. Phenotypic characterization was based on the observation of growth characteristic on Sabouraud's Dextrose Agar, Grams' staining, germ tube formation and characteristics appearance in CHROMagar.

Results: A total of 51 isolates were identified as *Candida* species. *C. albicans* (64.7%) predominated non *albicans Candida* (35.3%). The majority of non *albicans Candida* were *C. tropicalis*. Germ tube formation test was positive for all *C. albicans* which produced light green color in CHROM agar.

Conclusion: CHROM agar can be routinely used to identify different Candida species from clinical samples.

Keywords: Yeast, Phenotypic, Chromogenic

BACKGROUND

Medically important yeasts are abundantly present in the environment and some of these species constitute as normal flora in human beings (1). *Candida* species are commonly associated as one of the major agents causing infections in immuno-compromised patients (2). *Candida albicans* is the most virulent among the *Candida* species and is responsible for different forms of Candidiasis including infections in superficial sites and cutaneously (2).

***Correspondence:** **Sangeeta Khadka**
Department of Microbiology, St. Xavier's College, Kathmandu, Nepal
E-mail: sanguinekhadka@gmail.com

Over the last few decades, there has been an increase in the incidence of candidiasis caused by other *Candida* species (non-*albicans Candida*) such as *Candida dubliniensis*¸ *Candida glabarta*¸ *Candida krusei*¸ *Candida tropicalis*¸ and *Candida. Parapsilosis* (3). Though molecular techniques in yeast detection are highly sensitive and specific¸ their implementation in routine diagnostic is limited due to complex nature of test and affordability (4). Routine characterizations of clinical isolates in resource-limited settings rely on conventionally used phenotypic methods. We aim to use a chromogenic media to characterize the *Candida* species obtained from clinical samples.

METHODOLOGY

A hospital based descriptive cross-sectional study was conducted on the isolates of *Candida* species obtained in Clinical Laboratory¸ Nobel Hospital¸ Sinamangal¸ Kathmandu, Nepal. Clinical isolates of *Candida* recovered during May 2014 – April 2015 were included in the study.

For laboratory identification, samples obtained were first grown in blood culture media to rule out any contamination. Preliminary identification was done by observing growth characteristics in Sabouraud's Dextrose Agar (SDA) slant, Grams' staining and germ tube test. For germ tube test, a small inoculum of the test yeast cells from a pure culture was suspended in 0.5 ml human serum. The suspensions were incubated at 37 °C for three hours after which a drop of the incubated serum was placed on a microscope slide and covered with a cover-slip. Normal saline and a preparation of *C. albicans* ATCC 90028 was used as negative control and positive control respectively. Identification in CHROMagar plate was done by following manufacturers (Himedia, India) instruction whereby smooth colonies that appeared light green were interpreted as *C. albicans*. Similarly, blue to metallic blue colored raised colonies were interpreted as *C. tropicalis*, likewise pink smooth colonies were considered as *C. glabrata*, while purple fuzzy colonies were suggestive of *C. krusei* as shown in Figure 1.

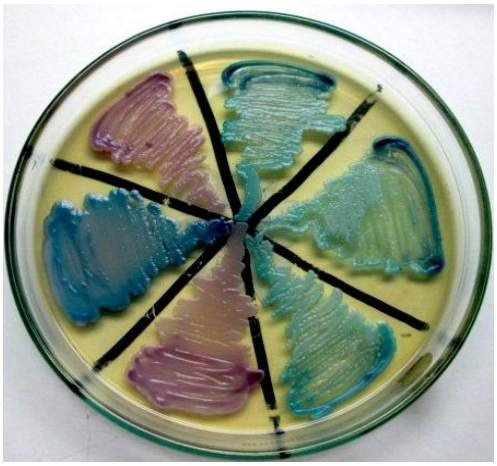

Figure 1: Observation of Candida species in CHROM agar (A/B/C: C. albicans; D/F: C. krusei; E: C. tropicalis)

RESULTS

A total of 51 *Candida* isolates were obtained. On further speciation¸ the most predominant species was *Candida albicans* 64.7% (n=33) ¸ followed by *Candida tropicalis* 11.7% (n=6)¸ *Candida krusei* 7.8% (n=4)¸ *Candida parapsilosis* 7.8% (n=4) and *Candida glabrata* 7.8% (n=4) as shown in Figure 2. The presence of germ tubes was recognized as a yeast cell having about half the width and 3 to 4 times the length of the mother cell and not has the constriction at the neck of the parent cell. More than 50 % of the cells producing such a germ tubes per field were interpreted as germ tube producer *Candida* species. Morphologically, yeast colonies in SDA slant were white to cream colored, smooth, and glabrous. The Grams' stain of the yeast showed Gram-positive, round-oval irregular cells which did not pose typical characteristics that could be used to differentiate them in species level. In the case of CHROM agar, the above-mentioned color profile of different colonies was used to speciate the *Candida*.

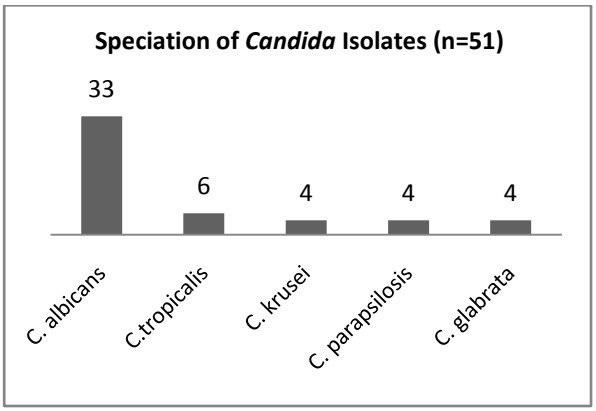

Figure 2: Different types of Candida species identified using CHROMagar

Table 1 depicts the findings comparing observation in CHROMagar and germ tube test. The observation for *C. albicans* in germ tube test and CHROM agar were consistent where the earlier could presumably be used to confirm the species. However, all other species of Candida had negative germ tube test and exhibited similar morphological characteristics in SDA agar and Grams' staining. These species though had a different phenotypic appearance in CHROMagar.

Table 1: Phenotypic characteristics of Candida species

Species	**CHROM agar**	**Germ Tube Test**	
		Positive	Negative
***C. albicans* (N=33)**	Light green	33	-
***C. tropicalis* (N=6)**	Blue purple	-	6
***C. krusei* (N=4)**	Purple fuzzy	-	4
***C. parapsilosis* (N=4)**	Pale	-	4
***C. glabrata* (N=4)**	Pink smooth	-	4

DISCUSSION

This study has made an attempt to investigate the prevalence of different species of *Candida* isolated from various clinical samples submitted at the Laboratory Unit of Nobel Hospital¸ Kathmandu. The study was done to evaluate the performance of conventional identification phenotypic methods and CHROMagar method to identify medically important yeast in a routine clinical microbiology laboratory. *Candida albicans* and non-*albicans Candida* are increasingly being isolated from clinical specimens (5, 6). *C. albicans* is commonly regarded as an endogenous pathogen (7), however, the spread of this organism between patients and from hospital staffs is well recognized (8). In terms of laboratory-based identification, conventionally *Candida* species are identified by their growth characteristics in SDA culture media and expression of germ tube (9) which lacks in species level identification. The potential clinical importance of species-level identification has been recognized as *Candida* species differ in the expression of virulence factors and antifungal susceptibility (10).

CHROMagar *Candida* is a selective and differential medium which is widely used for the rapid identification and differentiation of *Candida* species from the clinical specimen. Its superiority to SDA lies on its ability to selectively inhibit bacterial growth (11). It facilitates the detection and identification of *Candida* species from mixed culture and provides result in 24-48 hours and has an advantage of being technically simple, rapid and cost-effective as compared to the conventional methods (12). In our study, we identified different *Candida* species based on color exhibited. Horvath et al in their study described that CHROMagar *Candida* can readily be used in isolation of yeast from clinical specimen and reported that use of this medium allows rapid identification of clinically important *Candida* species (13). Similarly¸ Melissa and co-workers proposed that CHROMagar can only be used as principle medium to isolate yeasts from clinical samples (14). A newly described yeast¸ *C. dubliniensis* has very similar characteristics as of *Candida albicans* being the producer of both germ tube and chlamydospore (15) which increases the likelihood of misidentification of the causative agent. However¸ CHROMagar *Candida* has been described to identify these two species, where after 48 hours of incubation at 37ºC, *C. albicans* grows as light blue-green colonies as opposed to dark green colonies of *C. dubliniensis* (16).

C. albicans was the commonest species (64.7%) identified in our study. An increased proportion of *C. albicans* as compared to non-albicans *Candida* is reported in different instances (1, 17). In contrast, higher prevalence of non-*albicans Candida* (63.3% versus 36.7%) has also been reported (5, 10). Over the past decade¸ there has been a significant increase in the number of reports of systemic and mucosal yeast infections with both *albicans* and non-*albicans Candida* species worldwide (18). Among the non-*albicans* species, *C. tropicalis* was predominating species in our study which correlates with findings of similar studies done in Nepal (19) and elsewhere (20). Among 35.3% of non-*albicans Candida*¸ *C. tropicalis* (11.7%) was the most common species isolated in this study. *Candida* species have been found to be one of the most common pathogens causing up to 15% of nosocomial bloodstream infection and mortality rate ranging from 5-71% (21). Nevertheless in our study¸ no *Candida* species was isolated from the blood specimen.

The present study was a laboratory-based study focused on characterization of *Candida* species using both conventional tools and chromogenic agar based media. A further correlation of the isolated *Candida* species with the clinical features and the predisposing factors of each patient are warranted.

CONCLUSION

CHROMagar being relatively user friendly can routinely be used to identify different *Candida* species from clinical samples. Selective nature of the media and direct speciation based on color production makes it superior in terms of commonly used Sabouraud's Dextrose Agar.

COMPETING INTERESTS

The authors declare that they have no competing interest.

AUTHORS' CONTRIBUTION

SK designed study, performed laboratory works and drafted manuscript. PR and SG significantly contributed in manuscript preparation. PKS and SKM supervised in designing protocol and conducting laboratory works.

REFERENCES

1. Marinho SA, Teixeira AB, Santos OS, Cazanova RF, Ferreira CAS, Cherubini K, et al. Identification of Candida spp. by phenotypic tests and PCR. Brazilian Journal of Microbiology. 2010;41(2):286-94.

2. Pfaller MA, Pappas PG, Wingard JR. Invasive Fungal Pathogens: Current Epidemiological Trends. Clinical Infectious Diseases. 2006;43(Supplement 1):S3-S14.

3. Wingard JR. Importance of Candida Species Other than C. albicans as Pathogens in Oncology Patients. Clinical Infectious Diseases. 1995;20(1):115-25.

4. Sahiner F EK, Ozyurt M, Ardıç N, Hoşbul T, Haznedaroğlu T. Phenotypic and genotypic identification of Candida strains isolated as nosocomial pathogens. Mikrobiyol Bul. 2011;45(3):478-88.

5. Deorukhkar SC, Saini S, Mathew S. Non-albicans Candida Infection: An Emerging Threat. Interdisciplinary Perspectives on Infectious Diseases. 2014;2014:7.

6. Achkar JM, Fries BC. Candida Infections of the Genitourinary Tract. Clinical Microbiology Reviews. 2010;23(2):253-73.

7. Pfaller MA. Nosocomial Candidiasis: Emerging Species, Reservoirs, and Modes of Transmission. Clinical Infectious Diseases. 1996;22:889-94.

8. Marco F, Lockhart SR, Pfaller MA, Pujol C, Rangel-Frausto MS, Wiblin T, et al. Elucidating the Origins of Nosocomial Infections with Candida albicans by DNA Fingerprinting with the Complex Probe Ca3. Journal of Clinical Microbiology. 1999;37(9):2817-28.

9. Alam MZ, Alam Q, Jiman-Fatani A, Kamal MA, Abuzenadah AM, Chaudhary AG, et al. Candida identification: a journey from conventional to molecular methods in medical mycology. World Journal of Microbiology and Biotechnology. 2014;30(5):1437-51.

10. Mohandas V, Ballal M. Distribution of Candida Species in Different Clinical Samples and Their Virulence: Biofilm Formation, Proteinase and Phospholipase Production: A Study on Hospitalized Patients in Southern India. Journal of Global Infectious Diseases. 2011;3(1):4-8.

11. Silva JO FS, Lavrador MA, Candido RC. Performance of selective and differential media in the primary isolation of yeasts from different biological samples. Mycopathologia. 2004;151(1):29-36.

12. Vijaya D. HTR, Nagaratnamma T. Candida Speciation Using Chrom Agar. Journal of Clinical and Diagnostic Research 2011;5(4):755-7.

13. Horvath LL, Hospenthal DR, Murray CK, Dooley DP. Direct Isolation of Candida spp. from Blood Cultures on the Chromogenic Medium CHROMagar Candida. Journal of Clinical Microbiology. 2003;41(6):2629-32.

14. Murray MP, Zinchuk R, Larone DH. CHROMagar Candida as the Sole Primary Medium for Isolation of Yeasts and as a Source Medium for the Rapid-Assimilation-of-Trehalose Test. Journal of Clinical Microbiology. 2005;43(3):1210-2.

15. Pincus DH, Coleman DC, Pruitt WR, Padhye AA, Salkin IF, Geimer M, et al. Rapid Identification of Candida dubliniensis with Commercial Yeast Identification Systems. Journal of Clinical Microbiology. 1999;37(11):3533-9.

16. Sullivan D, Coleman D. Candida dubliniensis: Characteristics and Identification. Journal of Clinical Microbiology. 1998;36(2):329-34.

17. Yang CW, Barkham TMS, Chan FY, Wang Y. Prevalence of Candida Species, Including Candida dubliniensis, in Singapore. Journal of Clinical Microbiology. 2003;41(1):472-4.

18. Hobson RP. The global epidemiology of invasive Candida infections—is the tide turning? Journal of Hospital Infection. 2003;55(3):159-68.

19. Jha BJ DS, Tamang MD, Joshy ME, Shivananda PG, Brahmadatan KN. Characterization of Candida species isolated from cases of lower respiratory tract infection. Kathmandu Univ Med J. 2006;4(3):290-4.

20. Basu S GH, Joshi S, Gupta N. Distribution of Candida species in different clinical sources in Delhi, India, and proteinase and phospholipase activity of Candida albicans isolates. Rev Iberoam Micol 2003;20(4):137-40.

21. Falagas ME, Apostolou KE, Pappas VD. Attributable mortality of candidemia: a systematic review of matched cohort and case-control studies. European Journal of Clinical Microbiology and Infectious Diseases. 2006;25(7):419-25.

Article History:
Received: 20 January, 2016
Accepted: 30 March, 2016
Published online: 20 May, 2016

For Citation:
Khadka S, Regmi P, Giri S, Shah PK, Mishra SK: Identification of *Candida* species using CHROM agar.International Journal of Medicine & Biomedical Sciences. 2016; 1(3):10-13