EVALUATION OF DIAGNOSTIC EFFICACY OF CHROMAGAR CANDIDA FOR DIFFERENTIATION AND IDENTIFICATION OF COMMON CANDIDA SPECIES

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ABSTRACT

OBJECTIVE: To evaluate and validate the performance of a chromogenic Candida speciation medium (CHROMagar Candida).
STUDY DESIGN: A Validation study
PLACE & DURATION: The study was conducted at the microbiology laboratory of King Khalid Hospital Al Majmaah Saudi Arabia over a period of 6 months starting from 1st October 2015 to 29th February 2016
METHODOLOGY: A total number of 72 candida strains were included. The isolates were first identified by conventional methods and then API 20C aux which was taken as gold standard to identify candida species. They were then subsequently identified by CHROMagar Candida. The morphology and color of the candida colonies on CHROMagar Candida were compared with the results of API 20C aux.
RESULTS: The CHROMagar candida is an excellent identification tool for three commonly isolated species of candida i.e. C.albicans, C.tropicalis, and C. glabrata . The diagnostic accuracy of CHROMagar Candida medium for these three commonly isolated candida species was 98.6% , 97.3% and 91.1% respectively.
CONCLUSION: CHROMagar Candida identifies and differentiates commonly isolated Candida species with high degree of accuracy and we thus recommend the use of this rapid, cost effective and easy to interpret, identification tool in the routine microbiology laboratory.
KEY WORDS: CHRO Magar Candida, Non albicans candida, API 20C Aux

INTRODUCTION

There had been a rise in candida infections recently across the globe because of wide spread use of broad spectrum antibiotics and rising immunocompromised conditions[1]. It has also been noticed that there is a progressively increasing number of infections by candida species other than Candida albicans with decreased susceptibility to anti-mycotic agents[2]. Recognition of this change is clinically important and has important therapeutic outcomes, since the various species have differing antifungal susceptibilities, such as C. glabrata has less sensitivity to fluconazole (commonly used empiric antifungal) than other species and C. krusei is intrinsically resistant to fluconazole[3]. Rapid and fast pace identification of candida isolates to the spp. level is essential in order to develop an optimum and targeted approach to antifungal therapy[4]. The usual practice in the routine microbiology laboratory is that the candida is usually not identified to the species level. Either the practice is to label all isolated candida as candida species or wrongly labelling every candida as Candida albicans. This practice is definitely wrong when there is availability of rapid, easy and cost effective methods identifying Candida isolates to the species level[5]. CHROMagar Candida is an agar based medium containing colourless substances called chromogens, comprising of a colored chromophore and a substrate. The colored chromophore is released when the target organism's enzyme breaks down this chromogenic conjugate , allowing specific and rapid species identification based on colony morphology and color[6]. In this study, we aim to evaluate the performance and diagnostic efficacy of one such commercially available CHROMagar and compare it with a standard. We thus introduce the use of CHROMagar as a rapid, cost effective identification tool in the routine microbiology work as it is essential that this pathogen be identified to the species level not only at the reference labs but also at the ordinary and routine diagnostic settings.

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METHODOLOGY

The aim of this study is to assess and validate the diagnostic efficacy of a chromogenic Candida speciation medium (CHROMagar Candida) using API 20C AUX(biomerieux) as gold standard. This identification of candida to species level is important in optimizing the antifungal therapy.
The study was conducted at the microbiology laboratory of King Khalid Hospital Al Majmaah Saudi Arabia over a period of 6 months starting from October 2015 to February 2016
The sampling technique was a non probability purposive sampling.
Laboratory samples of either sex, of all ages, yielding growth of yeast species were included in the study whereas all fungal isolates not conforming to the criteria of yeast were excluded

from the study.

A total of 72 clinical samples yielding growth of yeast species were included in the study. These were isolated from the clinical samples (urine, blood, high vaginal swab etc.) of the patients sent for culture and sensitivity at the microbiology laboratory of King khalid hospital Al-Majmaah, KSA and identified with microscopy and colony morphology and other biochemical tests. They were then inoculated on CHROMagar plate and incubated at 370C for 24-48 hrs. Upon growth, commonly isolated candida species produce colonies of different color and morphology. Candida albicans produces green color, Candida tropicalis produces blue color, Candida glabrata produces purple color and Candida krusei flat and pink colonies as shown in the Figure 1.

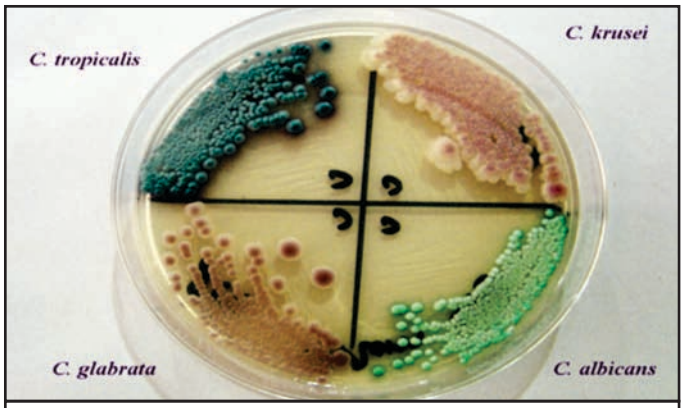

FIGURE-1: SHOWING COLONY COLOR AND MORPHOLOGY OF CLINICALLY IMPORTANT CANDIDA SPECIES

API 20 C AUX(BioMerieux, France,Fig(2) yeast identification system was taken as the gold standard for comparing the results of CHROMagar candida. The yeast species were simultaneously tested on these strips for identification, and interpretation was done as per manufacturers instructions. The results of CHROMagar candida were compared with results of API 20C AUX .On the basis of analysis and observation, results were drawn and discussed and compared with other relevant literatures.

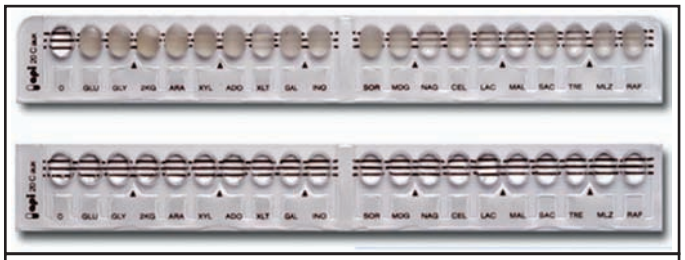

FIGURE-2: API 20C AUX MEDIUM

RESULTS

API 20 C AUX yeast identification system (Table -I), identified a total of nine different species of Candida with Candida albicans being the most common species i.e. 51.3%(n=37), followed by Candida glabrata 27.8% (n=20), and Candida tropicalis 8.3%(n=6). Other yeast isolates included Candida kefyr, Candida parapsilosis, Candida famata, Candida lusitaniae, Cryptococcus humicola and Cryptococcus neoformans. Candida Krusei although identified by CHROMagar, was not isolated CHROMagar Candida medium identifies only four species (Table - II).

TABLE-I: SPECIES IDENTIFICATION WITH API 20C YEAST IDENTIFICATION SYSTEM (n = 72)

	Yeast species	Frequency (n)	Percent (%)
1.	Candida albicans	37	51.3
2.	Cryptococcus neoformans	2	2.8
3.	Cryptococcus humicola	1	1.4
4.	Candida tropicalis	6	8.3
5.	Candida glabrata	20	27.8
6.	Candida pelliculosa	1	1.4
7.	Candida Kefyr	1	1.4
8.	Candida famata	3	4.2
9.	Candida lusitaniae	1	1.4
	Total	72	100

TABLE-II: SPECIES IDENTIFICATION WITH CHROMAGAR CANDIDA (n = 72)

	Yeast species	Frequency(n)	%
1.	Candida albicans	38	52.8
2.	Candida glabrata	27	37.5
3.	Candida tropicalis	7	9.7
4.	Candida Krusei	0	0
Total		72	100

Table –III: shows the diagnostic accuracy of CHROMagar candida for the various Candida species identified. The positive and negative predictive values (PPV and NPV respectively) represent the proportions of true positive and true negative results in the study, respectively:

TABLE -III: SENSITIVITIES, SPECIFICITIES, POSITIVE, NEGATIVE PREDICTIVE VALUES AND DIAGNOSTIC ACCURACY OF CHROMAGAR CANDIDA FOR COMMON CANDIDA SPECIES

Candida species	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value	Diagnostic Accuracy
Candida albicans	100%	97%	97.36%	100%	98.63%
Candida krusei	NA	NA	NA	NA	NA
Candida tropicalis	85%	98%	85.71%	98.50%	97.3%
Candida glabrata	100%	88%	74.07%	100%	91.14%

DISCUSSION

CHROMagar candida is a differential culture medium that allows selective isolation and simultaneous identification of four clinically important and commonly isolated candida species i.e. Candida albicans, Candida glabrata, Candida tropicalis, and Candida krusei[7]. In our study the percentage of Candida albicans detected in the samples were 51.3%, Candida glabrata 27.8%, Candida tropicalis 8.3%, and Candida krusei were not detected in any of our clinical samples. This is in accordance with other regional studies by Khadijah Y et al, and Faris QB et al which show a low prevelance of Candida krusei in Saudi Arabia.[8,9]

Candida species are a common nosocomial pathogens world wide. In the US alone these were found to be the fourth most common nosocomial pathogen isolated from the blood culture[10]. Candida albicans, which once dominated the scenario, are now being replaced by non-albicans species, because of widespread use of antifungal agents. The non-albicans species are now a common involvement in the hospital settings and account for almost half of all cases of candida infection[11] Recognizing this change is clinically important and has important therapeutic outcomes, since the various species have differing antifungal susceptibilities, such as C. glabrata has less sensitivity to azole antifungals like fluconazole(commonly used empiric antifungal) and C. krusei is intrinsically resistant to fluconazole[12].

It is obvious from our results that non-albicans Candida make half of the total number of yeast isolated. Not identifying the actual species and presuming all isolated yeast species to be Candida albicans can lead to fatal outcomes especially in critical care patients.

In routine laboratories especially with limited resources, lack of costly equipment and expert training makes species level identification of candida very difficult. These facilities usually do not go beyond the certain basic identification procedures like germ tube test. The biochemical assimilation tests like API, being costly and expensive are not utilized in these centers. In busy laboratories , lack of time is also an important factor .Therefore the diagnosis is often limited to Candida albicans or non albicans candida. As a result, targeted antifungal treatment becomes almost impossible.[13]

In our study, CHROMagar Candida medium showed high sensitivity & specificity for isolating and differentiating Candida albicans(100% & 97%) ,C. tropicalis(85% & 98%) and C. glabrata (100% & 88%). This is in accordance with other similar studies conducted to check these species by CHROMagar Candida medium. Hussain et al[11] found sensitivity and specificity of 98.2% and 96% for C .albicans,100% and 95.4% for C Krusei, 100% and 96.8% for C tropicalis,100% and 94.9% for C.glabrata. Odds & Bernaerts[13] found specificity and sensitivity of this medium more than 99% for the identification of C. albicans, C. tropicalis and C. krusei . Daef E et al[14] found sensitivity and specificity of 96.9% and 97.9% for C. albicans, 100% and 98% for C. tropicalis and 100% and 100% for C. glabrata.

Another finding noted in our study is the false identification of C. glabrata which has low PPV and a high NPV . This is similar to the findings of other studies such as Odds et al[13] and Yücesoy M et al.[15] This shows that although none of the Candida glabrata were missed, few of the other species were wrongly identified as C. glabrata. Yücesoy M et al also noted that many other yeast species produce similar colonies which might lead to some confusion. Being a peripheral setting, our study had its own limitations of small sample size. However CHROMagar candida still proved to be a costffective, reliable and rapid method for identification of Candida species even in resource limited settings.

CONCLUSION

CHROMagar Candida medium is a reliable identification tool for recognition of most commonly isolated candida species. We therefore recommend its use in the routine microbiology work, as it is important to identify this pathogen to species level to optimize treatment. It is easy to use, time saving medium, facilitates the detection of mixed cultures and could help clinicians to select the most appropriate antifungal agent. Thus with potential to decrease patient mortality and morbidity.

Contribution of Author:

Ali Faraz: Conception and design of study, Collection of data, Analysis and/or interpretation of data

Usama Bin Ghaffar, Tahir Ansari: Drafting the manuscript, Revising the manuscript critically for important intellectual content, Acquisition and analysis of data

Waqas Sami: Statistical analysis, Final approval of the version to be published

REFERENCES

1. Deorukhkar SC, Saini S, Mathew S. Non-albicans Candida Infection: An Emerging Threat. Interdiscip Perspect Infect Dis Volume. 2014;2014 (2):13-16.
2. Oberoi JK, Wattal C, Goel N, Raveendran R, Datta S, Prasad K .Non-albicans Candida species in blood stream infections in a tertiary care hospital at New Delhi, India. Ind J Med Res. 2012 ; 136(6): 997-1003.
3. Garnacho-Montero J, Díaz-Martín A, García-Cabrera E, Ruiz Pérez de Pipaón M, Hernández-Caballero C, Aznar-Martín J, et al. Risk factors for fluconazole-resistant candidemia. Antimicrob Agents Chemother. 2010 ;54(8):3149-54.
4. Stefaniuk E, Baraniak A, Fortuna M, Hryniewicz W. Usefulness of CHROMagar Candida Medium, Biochemical Methods--API ID32C and VITEK 2 Compact and Two MALDI-TOF MS Systems for Candida spp. Identification. Pol J Microbiol. 2016;65(1):111-14.
5. Neppelenbroek KH1, Seó RS, Urban VM, Silva S, Dovigo LN, Jorge JH, et al. Identification of Candida species in the clinical laboratory: a review of conventional, commercial, and molecular techniques.Oral Dis. 2014 ;20(4):329-44.
6. Birinci A, Akkurt L, Acuner C, Unlu M, Durupinar B. Rapid Identification of the Candida Species from Direct Blood Cultures by CHROMagar™ Candida. J Int Med Res 2004; 32(5): 484 - 87.

7. Willinger B, Manafi M. Evaluation of CHROMagar Candida for rapid screening of clinical specimens for Candida species. Mycoses 1999; 42 (1), 61-65.
8. Yosif K. Prevalence of Vaginal Candidiasis among Pregnant Women Attending Al-Hada Military Hospital, Western Region, Taif, Saudi Arabia. Int J Sci Res, 2013; 4 (5),1736-44.
9. Alenzi FQB. Virulence factors of Candida species isolated from patients with urinary tract infection and obstructive uropathy. Pak J Med Sci. 2016;32(1):143-46.
10. Shrivastava G, Bajpai T, Bhatambare GS, Chitnis V, Deshmukh AB. Neonatal candidemia: Clinical importance of species identification. Sifa Med J. 2015 ;2(2):37-40
11. Hussain A , Ikram A, Roshan M, Satti L. Validation of BBL CHROMagar Candida Medium (BD Diagnostics)in Isolating and Differentiating Candida Species in Clinical Specimens. Infect Dis J 2010;19(4):230-33
12. Cheng MF, Yang YL, Yao TJ, Lin CY, Liu JS, Tang RB, et al. Risk factors for fatal candidemia caused by Candida albicans and non-albicans Candida species. BMC Infect Dis. 2005; 5(1): 22.
13. Odds FC, Bernaerts R. CHROMagar Candida, a new differential isolation medium for presumptive identification of clinically important Candida species. J Clin Microbiol 1994; 32(8):1923-29.
14. Daef E, Moharram A, Eldin SS, Elsherbiny N, Mohammed M. Evaluation of chromogenic media and seminested PCR in the identification of Candida species. Braz J Microbiol. 2014;45(1):255-62.
15. Yücesoy M, Marol S. Performance of CHROMAGAR candida and BIGGY agar for identification of yeast species. Ann Clin Microbiol Antimicrob. 2003;29(2):8.