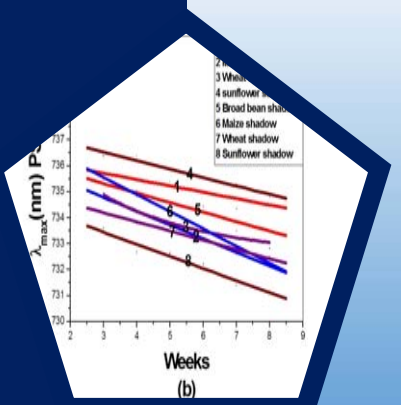
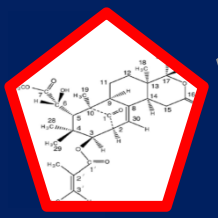
Journal of Faculty of Sciences

A Refereed Scientific Journal
Faculty of Pure and Applied Sciences
International University of Africa
Volume No. 5 , December , 2018





ISSN: 1858 – 513x

Journal of Faculty of Sciences

Refereed Scientific Journal

Faculty of Pure and Applied Sciences

International University of Africa

Volume No. 5, December, 2018

Identification of Clinical Isolates of *Candida Species* by using Chromagar in Sudanese Clinical Sources

Hassan A. Hassan[1*], Osman Khalil[1], and Hassan B. ELamin[3]

[1]Department of Microbiology, Faculty of Pure and Applied Science, I.U.A
[3]Commission for Biotechnology & Genetic Engineering, National Center for Research, Ministry of High Education and Scientific Research, Khartoum, Sudan
** Corresponding address: hassangumare@gmail.com*

ABSTRACT

The genus Candida includes about 150 different species; however, only a few are known to cause human infections.Objective of this study is to isolate and identificate *Candida species* in Khartoum, Hospitals by using Germ tube test, and CHROMagr Candida.A total of 50 clinical samples were collected, Samples collected from individuals consisted of 10 (20%) high vaginal swabs, 20 (40%) urine samples and 20 (40%) sputum samples. The samples were cultured on sabouraud dextrose agar. Identification of the *Candida species* was performed using growth characteristics, the germ tube test and the CHROMagar *Candida*.27 out of 50 samples (54%) were *Candida albicans* whereas 23 (46%) were non *C.albicans* as confirmed by CHROMagar Candida. Non albicans *Candida* included; *C. tropicalis* 12(24%), *C. krusei* 6(12%), *C. glabrata* 5(10%). This study showed *C. albicans* (54%) was the most frequently isolated species followed by (46%) non albicans Candida isolates as confirmed by CHROMagar Candida.CHROM agar is a new differential culture medium and identification of Candida to species level.

Keywords: Candida species, CHROMagar Candida

مستخلص

يضم الجنس كانديدا (*Candida*) حوالي 150 نوعاً مختلفاً، ومن المعروف أن قليل من الأنواع تسبب إصابات للإنسان. ان هدف هذه الدراسة عزل وتحديد أنواع الكانديدا التي جُمعت من مستشفيات ولاية الخرطوم مستعملين اختبا ر انبوبة الجراثيم (Germ Tube) وكروم آجار (CHROMagar). تم جمع خمسون (50) عينة من افراد منهم عشرة (10) بنسبة (20%) عبارة عن مسحات من المهبل (High Vaginal Swabs)، (20) عينة بنسبة (40%) من البول، (20) عينة بنسبة (40%) من اللعاب (Sputum). تم زراعة العينات على سابورود آجار (Sabouraud agar). لتحديد نوعية عينات الكانديدا استخدمت خصائص النمو، اختبار انبوبة الجراثيم (Germ Tube) وكروم آجار (CHROMagar Candida). لقد وجد ان سبعة وعشرون (27) من الخمسون عينة (50) بنسبة (54%) ينتمون الى كانديدا البيكانس (*Candida aLbicans*)، ثلاثة وعشرون (23) بنسبة (46%) ليست بالنوع كانديدا البيكانس كما تم تأكيدها باستخدام كروم آجار كانديدا. الأنواع غير الكانديدا البيكانس تضم *Candida tropicalis* 12 (24%)، *C.krusei* 6 (12%)، *C.qlabrata* 5 (10%)، أوضحت الدراسة بأن كانديدا البيكانس كانت اكثر أنواع العزلات وفرة بنسبة (54%) تليها الأنواع الأخرى بنسبة (46%). ان كروم آجار كانديدا عبارة عن وسط (منبت) غذائي تفريقي Differenial يمكن بواسطته تحديد أنوع الكانديدا.

الكلمات المفتاحية: أنواع الكانديدا، كروم آجار كانديدا.

1. INTRODUCTION

Until the end of the 19th century fungi have been considered to be plants, though they are heterotrophic eukaryotic organisms that are more closely related to human's than bacteria at cellular level. Today, fungi are grouped in their own taxonomic kingdom, which is estimated to consist of more than one million species. More than 100,000 species have been described, Only a very small fraction of approximately 400 species have been identified as human pathogens but the numbers are rising (de Hoog, et

al.2000)., Most species grow as multicellular filaments called hyphae-forming mycelium such as molds; some species also grow as single cells like yeasts. Some groups of fungi are pathogenic to humans and require control measures. Human fungal pathogens belong to four main groups, namely *Zygomycetes, Ascomycetes, Deuteromycetes,* and *Basidiomycetes*. Fungi can cause significant number of human diseases represented by pathogens such as *Trichophyton species, Epidermophyton species, Histoplasma species, Blastomyces species, Sporothrix species, Coccidioides species,* and *Paracoccidioides species*, capable of infecting healthy people, or opportunistic invaders such as *Aspergillus species, Candida species, Cryptococcus species, Fusarium species,* and *Rhizopus species,* which are normally avirulent in healthy people but could be disseminated to deep tissue and cause fatal disease in unhealthy people (Chakrabarti *et al*,2005). The morbidity and mortality rates caused by fungal species such as *Candida*, *Aspergillus, Fusarium*, and *Trichosporum* are relatively higher (Fluckiger et al., 2006). Fungal infections have now also become more common in the healthy population. The National Nosocomial Infections Surveillance System has reported *Candida spp*. as the fourth most common bloodstream isolates in nosocomial infections in USA. Over 95% of all fungal infections have been associated with *Candida albicans*, *Aspergillus fumigates*, and *Cryptococcus neoformans* (Richardson, 2005).

2. MATERIALS AND METHODS

A total of 50 clinical samples were collected during June 2015 to October 2016. Samples collected from individuals consisted of 10 (20%) high vaginal swabs, 20 (40%) urine samples and 20 (40%) sputum samples. Present study was carried out in the Department of Microbiology, International University of Africa.

2.1 Identification of the Candida isolated:

All isolated *Candida species* were identified to the species level using the germ tube test and CHROMagar Candida.

2.2 Germ tube test:

Using a sterile inoculating loop, a colony of yeasts was transferred into the human serum in the labeled test tubes. The colony was emulsified in the serum. The set up was incubated at 37◦C for 3 hours. Using a Pasteur pipette, a drop of the suspension taken from the test tube after incubation was placed on a clean dry slide. The suspension was covered with a clean cover glass. The slides were examined under a microscope for germ tubes on the yeasts using the 10X objective lens. A germ tube is a tube-like outgrowth that arises from the yeast cell. The 40X objective was used to confirm the presence or absence of germ tubes. When yeasts with germ tubes were seen, the culture was reported as *Candida albicans* isolated. When the yeast cells do not show germ tubes, the culture was reported as non albicans.

2.3 CHROMagar Candida

Chromogenic media prepared according to manufacture instruction and the organism inoculated in the media, then incubated at 37ºCfor 48 hours. After that the growth of *Candida spp* observed by the change in the colour of the colonies according to the pigment, as a result of reaction between chromogenic substrate and enzymes that secreted by different *Candida spp,* allowing organisms to be identified to the species level by their color and colony characteristics. CHROM agar Candida has been shown to allow differentiation of Candida yeasts by color and morphology. The result was as the following: the product identifies *C. albicans* by growth as light to medium green and wet colonies, *C. tropicalis* by growth as steel blue and wet colonies, *C. glabrata* dark pink and wet colonies, *C. krusei* light pink and dry colonies, and other *Candida spp.* give white color colonies (Babić and Hukić, 2010).

2.4 Growth at 37ºC and 45 ºC

All *Candida*isolates were tested for growth at 37ºC and 45 ºC on sabouraud dextrose agar plates held for 3 days. A visible growth was regarded as positive .incase of weak growth, the test was repeated.

3. RESULTS AND DISCUSSION

3.1 Identification of *Candida species*

In this research study, origins of strains of *Candida species* studied were those of sputum, urine, High Vaginal Swabs (HVS),

A total of 50 clinical samples were collected. Samples collected from individuals consisted of 10 (20%) high vaginal swabs, 20 (40%) urine samples and 20 (40%) sputum samples. The samples were cultured on sabouraud dextrose agar. Identification of the *Candida species* was performed using growth characteristics, the germ tube test and the CHROMagar *Candida*. *Candida* was identified depending on the morphological features on culture medium and germ tube formation. The identity of non-albicans *Candida spp*. was confirmed by CHROMagar candida. Four species were identified, *Candida albicans, Candida glabrata Candida tropicalis* and *Candida krusei*, they were identified as follows:

3.2 Cultural Characteristics

The morphology of *Candida species* colonies on Sabouraud dextrose agar were white to cream, round, curved, soft and smooth to wrinkled,with a characteristic yeast odor, it grew rapidly and matured in 3 days. These results are agreed with Emmons *et al.* (1974); Larone; 1995) and Bhavan *et al*; 2010).

3.3 Germ Tube Formation Test:

The germ tubes were formed within two hours of incubation and this is a unique diagnosis characteristic of *C.albicans* differentiates it from other fungi Table (1). Other yeasts generally do not form germ tubes within this 3 hour timeframe, (neither *C .glabrata* nor *C. tropicalis* that germ tubes was diagnostic and pathogenic character in the same time, The results of this study all *Candida albicans* were positive and were agreement with that in Sheppared *et al*. (2008), They mentioned that "All C. *albicans* strains were

germ tubes test positive when tested directly from the colony, and all non-albicans species were germ tubes test negative when tested directly from the colony".

Table 1. Growth and colonial characteristics of *Candida species* isolated from hospitals.

Candida Spp	**Germ tube test positive**	**Germ tube test negative**	**Growth at 37°C**	**Growth at 45°C**
Candida albicans	25	2	+	+
Candida krusei	0	6	+	-
Candida glabrata	0	5	+	-
Candida tropicalis	0	12	+	-
Total	25	25	+	-

3.4 Growth on CHROMagar Candida:

Nearly all isolates of *Candida species* tested gave colonies with colors ranging from white through pink, pinkish purple, blue and purple after 48 hours of incubation on CHROMagar Candida at 37°C (Table 2), and(Figs 2,3). Of the 50 isolates 27 yielded several shades of green colonies after 48 hours of incubation in CHROMagar Candida. They were identified as *C.albicans* .12 isolates developed a distinctive dark blue or blue-gray color after 48 h of incubation on CHROMagar and were identified as *C.tropicalis*

.6 isolates developed a pink, fuzzy color and were identified as *C. krusei*. And 4 isolates developed a mauve-brown color and were identified as *C. glabrata*. Nearly all isolates of *Candida species* tested gave colonies with colors described as ranging from white, through pink, pinkish purple, and purple after 48 h of incubation on CHROMagar at 37 °C. The observed morphological characteristics were compared with those in the Dalmau morphology identification chart (Barnett *et al.,* 2000, and Tintelnot *et al*., 2000).

Table 2. Distribution of *Candida* isolates among various samples.

ISOLATES	number of	*C.albicans*	*C.krusei*	*C.glabrata*	*C.tropicals*
URINE	20(40%)	10(50%)	3(15%)	1(5%)	6(30%)
SPUTUM	20(40%)	12(60%)	2(10%)	3(15%)	3(15%)
High vaginal swab	10(20%)	5(50%)	1(10%)	1(10%)	3(30%)
TOTAL	50	27	6	5	12
%	%	54%	12%	10%	24%

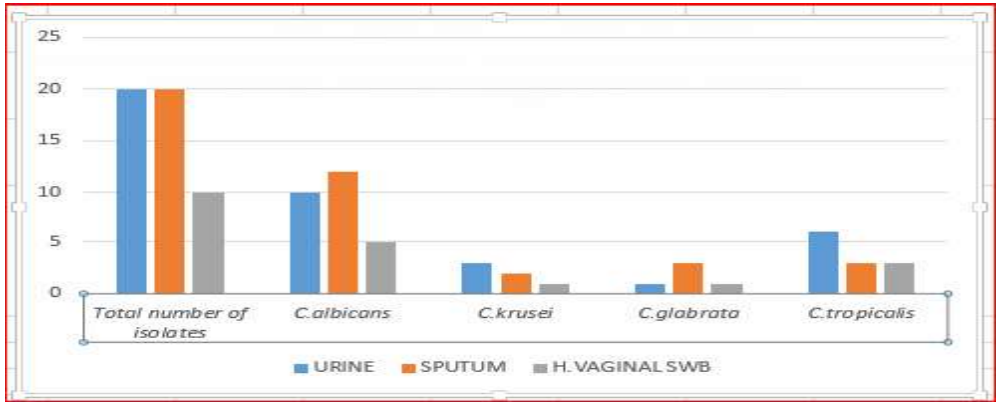


Fig 2. Result of CHROMagar *Candida species*

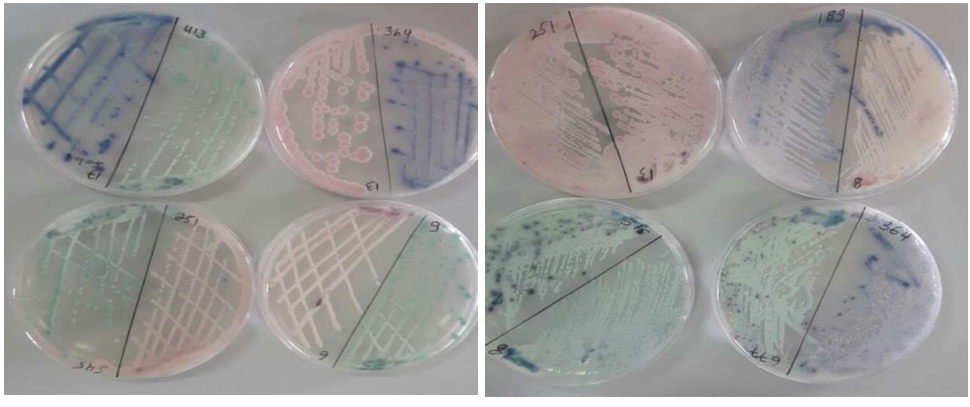


Fig 3. Different *Candida species on* CHROMagar Candidaafter incubation for 48 hours.

Candida species are responsible for a wide spectrum of infections in man. They can be isolated from most sites of a human body.These mycoses are most common in immunocompromised patients as opportunistic infections.All the *C. albicans* were positive for germ tube test. They all showed distinct growth at 37°C and 45°C temperatures Table (1). Non albicans Candida did not form any germ tube test, therefore CHROMagar was

used to establish their identity Table (2). 27 out of 50 samples (54%) were *Candida albicans* whereas 23 (46%) were non albicans as confirmed by CHROMagar Candida. Non albicans *Candida* included; *C. tropicalis* 12(24%), *C. krusei* 6(12%), *C. glabrata* 5(10%). This finding was consistent with the findings of other workers who reported that the incidence of *C. albicans* was 61.3% (Birader *et* al. 2009), 49.3% (Feglo and Narkwa 2012). However, (Babin *et al*. 2013) found that *C. tropicalis* was the most prevalent species accounted for 22.9% followed by *C. albicans* (35.5%). *C. tropicalis* (24%) was the second most common species reported in the present study. This finding was comparable with other workers, 26.4% (Babin *et al*. 2013), and 21% (Khan and Baqai *et al* 2010). However, *C.gla*brata was reported as second most common species by 13.7% (Bobade *et al*. 2013) and 11.9% (Saldhei *et al*. 2012). *C.krusei* was reported 14% (Shivanand *et al.* 2011) and 10.78% (Vijaya *et al*. 2011). This study showed *C. albicans* (54%) was the most frequently isolated species followed by (46%) non albicans Candida isolates as confirmed by CHROMagar *Candida*.

ACKNOWLEDGEMENT
We acknowledged the National Public Health Laboratory (Mycology lab) and Soba University Hospital staff and technicians (Microbiology lab) and Central lab (Bacteriology lab) for their technical support while we were undertaking our laboratory work.

REFERENCES

Babin D, Kotigadde S, Rao PS, and Rao TV (2013). Clinico-mycological profile of vaginal candidiasis in a tertiary care hospital in Kerala. Int J Res Biol Sci;3:55-

Babic M, and Hukic M (2010).Candida albicans and non-albicans species as etiological agent of vaginitis in pregnant and non-pregnant women. Bosn. J. Basic. Med. Sci. 10(1):89-97.

Barnett J.A., Payne R.W. and Yarrow D. (2000). Yeasts: Characteristics and Identification. 3rd edn. Cambridge University press.

Bhavan, P.S., Rajkumar, R., Radhakrishnan, S., Seenivasan, C., Kannan, S. (2010). Culture and Identification of Candida albicans from Vaginal Ulcer and separation of enolase on SDS-PAGE. Int. J. Biol., 2: 84–93.

Biradar S, Amruthkishan, Gangane, and Sheeba, (2009). Prevalence and antifungal susceptibility of Candida species isolated from tertiary care Hospital in North East Karnataka. Int J Pharm Bio Sci; 4:1113-8.

Bobade O, Waghmare, Chhabrani, Kaur (2013). Species distribution and antifungal susceptibility profile of Candida isolated from urine samples. Int J Med Sci Public Health;2:867-70.

Chakrabarti A (2005) Microbiology of systemic fungal infection. j Postgrad Med 51(Suppl1): S16–S20.

De Hoog, G. G., J; Gene, J; Figueras mj. (2000). Atlas of clinical fungi: ASM Press.

Emmons, C.W.; Binford , C.H. and UtZ , J.P. (1974). Candiasis. In Medical Mycology. Lea, and febiger ed. 2nd ed. Philadephia. Ch. 14: 167-182.

Feglo PK, and Narkwa P(2012). Prevalence and antifungal susceptibility patterns of yeast isolates at the Komfo Anokye Teaching Hospital (KATH), Kumasi, Ghana. Br Micro Res J;2:10-22.

Fluckiger U; Marchetti O, Bille j, Eggiman P, Zimmerli S, Imhof A, Garbino J, Ruef C, Pittet D,Tauber M, Glauser, and M, Calandra T (2006) Treatment options of invasive fungal infections inadults. Swiss Med Wkly 136:447–463.

Khan F, and Baqai R.(2010). In vitro antifungal sensitivity of fluconazole, clotrimazole and nystatin against vaginal candidiasis in females of childbearing age. J Ayub Med Coll Abbottabad;22:197-200

Larone, D. H. (1995). Medically Important Fungi- A Guide to Identification, 3rd ed. ASM Press, Washington, D.C.

Richardson M.D. (2005) Changing pattern and trends in systemic fungal infections. J Antimicrob Chemother., 56:5–11.

Saldhei Z, Seifi Z, and Mahmoudabadi AZ(2012). Sensitivity of vaginal isolates of Candida to eight antifungal drugs isolated from Ahvaz, Iran. Jundishapur J Microbiol;5:574-7.

Shivanand, D. and Saldanha, R. M. (2012). Species identification of Candida isolates in various clinical specimens with their anti- fungal susceptibility patterns. Journal of Clinical and Diagnostic Research. 5: 1177 – 1181.

Tintelnot K., Haase G., Seibold M., Bergmann F., Staemmler M., Franz T. and Naumnn D. (2000). Evaluation of Phenotypic markers for selection and identification of Candida dubliensis. Journal of Clinical Microbiology Reviews. 38: 1599 - 1608.

Vijaya D, Harsha TR, and Nagaratnamma T(2011). Candida speciation using CHROMagar. J Clin Diagn Res;5:755-7\