

Phenotypic characterization of *Candida Albicans* from clinical sources in Nairobi, Kenya.

Kangogo MC¹, Wanyoike MW¹, Revathi G² and Bii CC³

1. Botany Department, Jomo Kenyatta University of Agriculture and Technology

2. Department of Microbiology, Aga Khan University Hospital

3. Mycology Laboratory, Centre for Microbiology Research, Kenya Medical Research Institute

Corresponding Author: Dr. Christine Bii P.O Box 19462-00202, Tel. 254-2724264 fax 254-2720039

E-mail. Kiptobi@yahoo.com

SUMMARY

Objective: To isolate and carry out phenotypic characterization *Candida* species from clinical sources in Nairobi-Kenya.

Study design/setting: Laboratory Based Experiment at Mycology Laboratory, Kenya Medical Research Institute, Nairobi, Kenya. We studied 130 *Candida s* species isolated between 2000 - 2005. The isolates were from blood, stool, urine, CSF and swabs of patients from different health Institutions in Nairobi.

Methods: Preliminary identification was done using morphological features and CHROMagar and confirmed using Analytical profile index (API 20 C aux).

Results: *Candida albicans* were 130/150 (86.7%) whereas 13.3% were *non albicans Candida* and included; *C. parapsilosis* 4%, *C. tropicalis* 2.7%, *C. krusei* 2.7%, *C. guilliemondii* 1.3% and *C. famata*, 1.3%. Germ tube positive *C. albicans* were 96.1% while only 3.8% were germ tube negative. All the 130 isolates identified as *C. albicans* formed chlamydospores on Corn meal agar and all grew at both 37 °C and 45 °C ruling out the possibility of *Candida dubliniensis*.

Conclusion: *Non albicans candida* are of clinical significance and may warrant the need speciate *Candida* species from clinical setting and constantly monitor for fungal resistance as some *Candida* species are intrinsically resistant to certain antifungal drugs especially in the context of opportunistic infections in HIV/AIDS.

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Introduction

Candida albicans is one of the most frequently isolated yeasts in clinical laboratories and indeed studies have shown it accounts up to 80% of the yeasts recovered from sites of infection (1). Isolation of *C. albicans* has been associated with infections, as well as colonization, in both immunocompromised and immunocompetent patients (2). The ability of *C. albicans* to produce germ tubes and chlamydospores is the basis of its preliminary identification. However *C. dubliniensis* has recently been described that is very similar to *C. albicans* in many characteristics, especially germ tube formation and chlamydospore production (3- 6).

Since *C. albicans* share these characteristics, it is likely that some *C. dubliniensis* isolates have been and will continue to be identified in clinical laboratory as *C. albicans*. Notably, *C. dubliniensis* is resistant to fluconazole and more virulent than *C. albicans* necessitating its differentiation. Due to HIV/AIDS, other *Candida* species other than *C. albicans* have also emerged as significant opportunistic fungal pathogens

(7-9).The diversity and spectrum of *Candida* species of clinical significance means there is need to develop fast and cost effective methods of identification. CHROMagar *Candida* technique has been used and has been useful in discriminating different *Candida* species as well as mixed infestations. It is a reliable and sensitive method for presumptive identification of more commonly isolated yeast species of the genus *Candida* (3-5). Rapid identification and speciation of *Candida* species is essential in clinical laboratories. However, no single phenotypic test is highly effective in identifying *Candida* species and combination of tests is sometimes necessary for identification. Molecular techniques have been employed to characterize *Candida* spp. Although sensitive and specific it is not cost effective for routine clinical mycology laboratories in resource constrained setup (6). The aim of the study was therefore to characterize *Candida* species from clinical sources using various phenotypic methods such as Gtt, Chlamydospore production, ChromAgar and thermotolerance.

Materials and Methods

Sources of isolates: A total of 150 isolates of *Candida* species recovered from blood, sputum, swabs, urine and catheter tips isolated between 2000 - 2005 were used. The isolates were culture collections at Mycology Laboratory, Kenya Medical Research Institute from various health Institutions in Nairobi, Kenya. Growth on Sabouraud Dextrose Agar (SDA): Primary isolation from stock cultures was done using SDA (Oxoid LTD Basingstoke, Hampshire, England.). The SDA media was prepared according to manufacturers instructions. The sub-cultures on SDA were incubated at 37 °C for 24 - 72 hours before observation and subsequent tests carried out.

Germ tube test: This used as a presumptive test for identification of *Candida albicans*. The procedure was carried out as follows; a small inoculum of the test yeast cells from a pure culture was suspended in 0.5 ml horse serum. The suspension was incubated at 37 °C for exactly three hours after which a drop of the incubated serum was placed on a microscope slide and covered with a cover slip. The wet mounts were examined for presence of germ tubes using the 40 X objective. The isolates were classified as either germ tube positive or germ tube negative.

Temperature tolerance- The isolates were cultured into SDA and incubated at 45 °C ambient air for 72 hours after which they were observed for any growth.

CHROMagar (CHROMagar™ *Candida*, Paris, France) was used for presumptive identification of different *Candida* species and detect any mixed colonies. The method is based on the differential release of chromogenic breakdown products from products from various substrates following differential exoenzyme activity. CHROMagar was purchased as powdered media and the plates were prepared according to the manufacturers' instructions. Using an inoculating needle, a single colony from a pure culture was seeded into CHROMagar media and incubated at 35 °C for 48 hours after which color changes were noted.

Chlamyospore production: Chlamyospore production on Corn Meal Agar (CMA) was used as a presumptive confirmatory test for the identification of *Candida albicans*. Briefly 17 g of Corn Meal Agar (CMA) (Sigma Biomed inc. France) incooperated with tween 80 was prepared according to the manufacturers instructions. To determine the isolates ability to produce chlamyospore, test strains were inoculated on CMA

plates by slide culture technique. The test involved streaking and stabbing the media with a 48 hour old yeast colony and, covered with sterile cover slip and incubated at 25 °C for 72 hours. Chlamyospore production was examined after staining with lactophenol cotton blue. The isolates were categorized as chlamyospore positive or negative. Analytical Profile Index: All the isolates were subjected to confirmation using Analytical profile index (API 20 C aux). The procedures were done according to the manufacturers instructions.

Results

Phenotypic characteristics

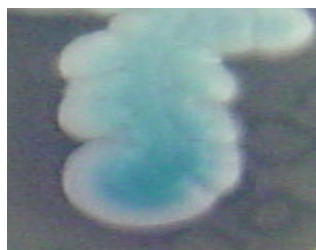
Candida albicans were isolated from swabs (37.3%), urine (33.3%), sputum (16.7%), aspirates (8%) and blood (4.7 %). All the *Candida* isolates showed growth on Saboraud Dextrose agar (SDA). Colonies were white to cream colored, smooth, glabrous and yeast-like in appearance. Microscopic morphology showed spherical to sub spherical budding yeast cells or blastoconidia. Out of the 150 isolates 130/150 (86.7%) yielded several shades of green colonies on CHROMagar *Candida* suggestive of *C. albicans* (Fig. 1b). Only 4/150 (2.7%) of the isolates developed a distinctive dark blue color on CA typical of *C. tropicalis* (Fig.1a). 4 % of the isolates developed a pink color suggestive of *C. parapsilosis* (Fig. 1c). Other *Candida* species gave colonies with colors as described in Table 1. Germ tube test indicated that 112/130 (86.1%) of the *C. albicans* were germ tube positive (Figure 2) and 18/130 (13.9%) were germ tube negative.

C. albicans produced abundant chlamyospores and pseudohyphae with clusters of spores (Figure 3) on CMA. *C. tropicalis* formed blastoconidia singly and long pseudohyphae on CMA. *C. parapsilosis* formed blastoconidia along curved pseudohyphae and giant mycelial cells. *C. guilliemondii* formed fairly short, fine pseudohyphae and clusters of blastoconidia at septa. *C. krusei* formed pseudohyphae with cross-matchsticks or tree-like blastoconidia. *C. glabrata* did not form any pseudohyphae but small, oval, single terminal budding, non encapsulated yeast cells. All the *C. albicans* were temperature tolerant and showed good growth at 45°C incubation.

Table 1 Colony colors of *Candida* isolates on CHROMagar *Candida* after 48 h incubation at 35 °C

<i>Candida</i> Species	Number of Isolates (%)	Colors Observed
<i>C. albicans</i>	130 (86.9)	Green.
<i>C. parapsilosis</i>	6 (4.0)	Pink
<i>C. tropicalis</i>	4 (2.7)	Dark blue
<i>C. krusei</i>	4 (2.7)	Whitish Pink
<i>C. guilliemondii</i>	2 (2.7)	Pinkish Purple
<i>C. glabrata</i>	2 (1.3)	light Pinkish
<i>C. famata</i>	2 (1.3)	White, Light Pink
Total	150 (100)	

(a) *Candida tropicalis*



(b) *Candida albicans*



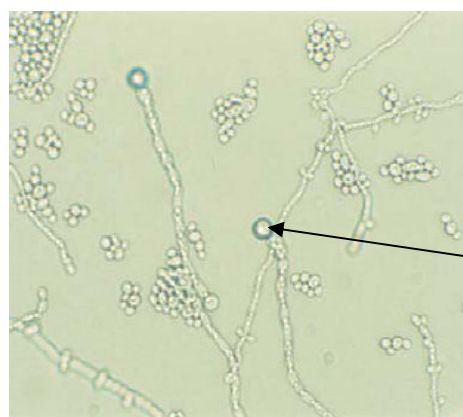
(c) *Candida Parapsilosis*



Fig. 1 Different *Candida* species on CHROMagar *Candida* after incubation at 35 °C for 48 hours. Note: a) *Candida tropicalis*- blue (b) *Candida albicans*- green (c) *Candida parapsilosis*-pink



Fig. 2. Germ tubes positive *C. albicans* after Agar



Chlamydospore

Fig 3. *Candida albicans* with abundant chlamydospores on Corn Meal incubation for 3 hours at 37°C on Horse Serum

Discussion

In the recent years the number of serious opportunistic yeast infections, particularly in immunocompromised patients has increased significantly (7). *Candida* species accounts for over 80% of such infections. This study investigated a total of one hundred and fifty *Candida* isolates from clinical sources in Nairobi. Isolates were recovered from swabs, urine, sputum,

aspirates, Cerebral Spinal Fluid (CSF), and blood. Majority of the isolates were from swabs especially vaginal and throat swabs, most of the specimens were from females, an indication that vaginal candidiasis is common. Very few isolates were obtained from blood and CSF probably because these are normally sterile

body fluids and candidaemia and *Candida* meningitis is not commonly reported and carry grave prognosis (8).

C. albicans was the most frequently isolated yeast pathogen accounting for 86.8 % of the isolates. This is in line with previous investigations where *C. albicans* accounted for up to 80 % of the candidiasis infection (9). The figure is slightly higher than that reported probably due the HIV/AIDS pandemic that could predispose more individual to fungal infestation. Nevertheless, for ethical reasons we could not link the patients HIV status with our findings. Although *C. albicans* was the most frequently isolated yeast pathogen, other *Candida* species were identified. These species included *C. glabrata*, *C. krusei*, *C. parapsilosis*, *C. famata*, *C. guilliermondii* and *C. tropicalis*. Previous studies have reported other non *albicans Candida* as emerging significant pathogens (10-11). Some *Candida* species are more virulent and intrinsically resistant to commonly available antifungal drug and *C. tropicalis* is one of them.

The ability to easily differentiate between *C. albicans* and other *Candida* species in routine laboratory practice remains a technical problem. Various studies have described the yeast *C. dubliniensis* as a worldwide opportunistic pathogen which is phylogenetically closely related to *C. albicans* (12-13). Evidence for the inducible stable fluconazole resistance *in vitro* in *C. dubliensis* strain have been demonstrated in immunocompromised patients receiving long term fluconazole prophylaxis (14-15). This situation has necessitated that clinical laboratories be able to isolate and discriminate yeasts of medical importance rapidly and as accurately as possible.

The detection and identification of microorganisms depend on the availability of easy to perform screening and cost-effective methods. The medium most widely used for the isolation of *Candida* and other yeast species from clinical specimens is Sabouraud Dextrose Agar (SDA, 16). This is a general – purpose medium that supports the growth of most pathogenic fungi. However SDA is not a differential medium and colonies of different pathogenic yeast species grown on this agar cannot be easily distinguished from each other. Even careful observers are often unable to recognize mixtures of different yeast species when they occur on a single clinical specimen on SDA. Therefore, there is no guarantee that mixed yeast cultures will be detected.

To be of value for routine isolation and presumptive differentiation of yeasts, an indicator medium should exhibit several properties. It should support the growth

of yeasts but not of bacteria. If the medium also facilitates the growth of filamentous fungi, that is not necessarily a disadvantage, since for many clinical samples it is not possible to predict whether yeast or filamentous fungi is likely to be isolated. The differential property of the medium should also allow unambiguous presumptive discrimination between the yeast species most commonly encountered in clinical samples. Finally it should facilitate the recognition of specimens containing mixtures of yeast species, and exposure of the fungi to the differential indicator substances should not affect their viabilities for subsequent subculture. CHROMagar *Candida* appears to fulfill all these requirements.

Since *C. albicans* is the yeast species most often isolated from clinical material, most clinical laboratories approach yeast identification by applying simple rapid tests such as germ tube formation to distinguish *C. albicans* from other species which require more extensive testing for proper identification. However newly described yeast, *C. dubliniensis* is very similar to *C. albicans* in many characteristics like germ tube formation and chlamyospore production (17). It is therefore likely that *C. dubliensis* strains have been and will continue to be identified in the clinical laboratory as *C. albicans*.

Several methods for identification of *C. dubliniensis* and discrimination from *C. albicans* have been reported. They include formation of dark green colonies on CHROMagar *Candida* (17-18), no or strictly reduced growth at 45°C and lack of ability to assimilate xylose (19). In our investigation, no *C. dubliniensis* was isolated, all the *C. albicans* isolates grew at both 37°C and 45°C and chlamyospores were not typical of *C. dubliniensis*.

Out of 130 *C. albicans* species, 3.9 % were germ tube negative. This is in accord with other reports that up to 5 % of *Candida albicans* are germ tube negative (8). All the *C. albicans* were positive for chlamyospores typical of *C. albicans*. They all showed distinct growth at 37 °C and 45 °C temperatures. The non *albicans Candida* did not form any Chlamyospores and API C AUX 20 confirmed that they were non *albicans Candida*.

Although *Candida albicans* is still the most significant clinically, other non *albicans Candida* are emerging significant pathogens and warrant routine discrimination in clinical laboratories. *Candida dubliensis* was not isolated in this study however it does not rule out its presence and warrant further investigations.

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