

Use of CHROMagar Candida for the presumptive identification of *Candida* species directly from clinical specimens in resource-limited settings

Sayyada Ghufrana Nadeem^{1,2,3*}, Shazia Tabassum Hakim^{1,2} and Shahana Urooj Kazmi³

¹Department of Microbiology, Mycology Research and Reference Institute, Jinnah University for Women, Karachi, Pakistan; ²Virology and Tissue Culture Laboratory, Department of Microbiology, Jinnah University for Women, Karachi, Pakistan; ³Immunology and Infectious Diseases Research Laboratory, Department of Microbiology, University of Karachi, Karachi, Pakistan

Introduction: Identification of yeast isolated from clinical specimens to the species level has become increasingly important. Ever-increasing numbers of immuno-suppressed patients, a widening range of recognized pathogens, and the discovery of resistance to antifungal drugs are contributing factors to this necessity.

Material and methods: A total of 487 yeast strains were studied for the primary isolation and presumptive identification, directly from clinical specimen. Efficacy of CHROMagar Candida has been evaluated with conventional methods including morphology on Corn meal–tween 80 agar and biochemical methods by using API 20 C AUX.

Results: The result of this study shows that CHROMagar Candida can easily identify three species of *Candida* on the basis of colonial color and morphology, and accurately differentiate between them i.e. *Candida albicans*, *Candida tropicalis*, and *Candida krusei*. The specificity and sensitivity of CHROMagar Candida for *C. albicans* calculated as 99%, for *C. tropicalis* calculated as 98%, and *C. krusei* it is 100%.

Conclusion: The data presented supports the use of CHROMagar Candida for the rapid identification of *Candida* species directly from clinical specimens in resource-limited settings, which could be very helpful in developing appropriate therapeutic strategy and management of patients.

Keywords: CHROMagar Candida; resource-limited settings; presumptive identification

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Infections due to *Candida* species and other fungi have increased dramatically in recent years and are of particular importance because of the rising number of immuno-compromised patients (1). Although *Candida albicans* remains the most common cause of human Candidiasis, the frequency of infection attributed to other members of the genus is also increasing (2, 3). This is primarily due to the increase in the number of at risk individuals, particularly those with impaired immunity, such as transplant recipients, cancer patients receiving chemotherapy, and human immunodeficiency virus-infected patients (4–8). In the 1980s *C. albicans* accounted for more than 80% of all candidal isolates recovered from

nosocomial yeast infection (9). Now, *C. albicans* accounts for more than 80% of all *Candida* blood isolates in a recent review (10). The emergence of *Candida* species other than *C. albicans* is a matter of concern in several major institutions (3, 11–14). These species are also shown to have reduced susceptibility to antifungal agents. The frequency of isolation of *Candida krusei*, *Candida glabrata*, *Candida tropicalis*, and *Candida prapsilosis* is steadily increasing globally (15, 16). Fungal infections caused by *Candida* species are being detected more frequently in clinical laboratories (17). Changing *Candida* epidemiology and availability of newer antifungal drugs with different antifungal spectra means that physicians can no longer

make therapeutic decisions based on broad identification of fungi as yeast and mold (18). The conventional methods of yeast identification, which mainly consist of assimilation and fermentation characteristics, are reported to be cumbersome and beyond the expertise range available in local laboratories. In non-specialized clinical laboratories (19), especially in resource-limited settings, identification of yeast and yeast-like organisms requires evaluation of microscopic morphology and biochemical studies. Some unusual yeasts may require unique morphological and biochemical studies for identification, occasionally requiring up to 21 days of incubation (20). Effective treatment requires both early diagnosis and prompt initiation of therapy against fungal infection (21). As the traditional methods are tedious and time consuming to perform in the routine laboratories, numerous isolation media are available in the market that can identify pathogens within 4–72 hours, depending upon the system (22–26). Several brands of chromogenic media are available for rapid identification of yeast. These special media yield microbial colonies with varying pigmentation secondary substrates that react with enzymes secreted by micro organisms (27). These media are species-specific, allowing the organisms to be identified to the species level by their color and colonial characteristics. The manufacturer of CHROMagar Candida currently advertises its product as able to detect and differentiate three species, *C. albicans* by growth as light to medium green colonies, *C. tropicalis* by growth as steel blue colonies accompanied by purple pigmentation diffused into surrounding agar, and *C. krusei* by growth as large, fuzzy, rose colored colonies with white edges, after incubation for 48 hours at 37°C, as also reported in several studies (28–32). Detection of *Candida* on CHROMagar Candida from poly fungal specimen also allows direct and more rapid and specific identification of *C. albicans* and other spp. (29, 32, 33), which could decrease the time required to obtain results. Use of chromogenic media in clinical microbiology laboratories for the isolation and presumptive identification of important *Candida* species is easy to perform, requires less time and is cost effective too. In this study our goal was to evaluate the usefulness of CHROMagar Candida for detection and identification of major *Candida* species with accuracy to reduce the time of identification, and its characterization from poly fungal specimens, especially in developing countries in resource-limited settings.

Material and methods

Period of study

From April 2006 to September 2009.

Clinical isolates

A total of 487 yeast strains, including *C. albicans* ($n = 201$), *C. tropicalis* ($n = 140$), *C. prapsilosis* ($n = 32$), *C.*

krusei ($n = 30$), *C. glabrata* ($n = 21$), *Candida lusitanae* ($n = 21$), *Candida guilliermondii* ($n = 21$), and *Candida famata* ($n = 21$), were isolated from various clinical specimens ($n = 435$) after direct plating on CHROMagar Candida (109 urine, 11 blood, 81 sputum, 22 wound, 91 vaginal secretion, and 21 peritoneal fluid).

Strains and media

Quality control strains of *C. albicans* (ATCC90029), *C. prapsilosis* (ATCC 2201), and *C. krusei* (ATCC6258) were taken from ATCC (American type culture collection) and IIDRL Laboratory, Department of Microbiology, University of Karachi, Pakistan. These control strains were first confirmed by using conventional methods, including germ tube formation test, and morphological characterization, which includes macroscopic features (morphology, color, size, and texture) on Sabouraud dextrose agar (SDA), Corn meal agar with tween 80 and CHROMagar Candida (CHROMagar Candida, France).

CHROMagar Candida medium comprised per liter peptone (10 g), glucose (20 g), agar (15 g), chloramphenicol (0.5 g), and Chromogenic ix. (2 g), pH 6.1. This medium was prepared according to the manufacturer's instructions, does not require autoclaving and is dispensed into Petri plates after cooling. Culture was inoculated and incubation was done at 37°C. The appearance of colonies, including color, size, and textures on CHROMagar Candida, was analyzed.

Identification methods

All samples were first plated on SDA and CHROMagar for 48 hours at 37°C. The production of color and morphology as described by the manufacturer were recorded and the photographs were recorded. For *C. albicans* the germ tube test was also performed to differentiate between *C. albicans* and *non albicans Candida* (NAC). The colonies from CHROMagar and SDA were plated on corn meal agar with Tween 80 for morphological examination of the production of chlamydo spores, blastospores, true hyphae, and branched pseudohyphae. The results were again compared with the colonies that were grown on CHROMagar. These colonies from SDA were subjected to biochemical analysis by API 20C Aux (Biomuriex). Finally the results were again compared with the culture results and speciation was done. The data suggested that species which were identified by CHROMagar and Corn meal–Tween 80 agar were the same as were confirmed by API 20C Aux. It means that in the presence of CHROMagar it is not necessary to perform germ tube test for *C. albicans* to confirm, as was also reported by Odds and Bernaerts (31).

Results

We evaluated 487 yeast isolates from 435 clinical specimens during April 2006 to September 2009 (Table 1). These samples were directly plated on CHROMagar Candida (CHROMagar Candida, France), SDA (Oxoid), Corn meal agar (Oxoid) with Tween 80, and on CHROMagar Candida and SDA. Colony growth was observed after 24 and 48 hours of incubation. The development of the colony color was noted on CHROMagar Candida, and we observed that the medium better differentiated the color of colonies after 48 hours of incubation, while after 72 hours of incubation a distinct deep color of the colonies was observed. It was also noted that colony colors of isolated strains were deepened with the passage of time and persistence of pigment was also observed on CHROMagar plates after more than 15 days, as also reported by Pfaller, Houston and Coffmann (32).

The yeast cells were identified according to morphology and color of colonies on CHROMagar Candida. Germ tube formation for *C. albicans* was done with microscopic morphology on Corn meal–Tween 80 agar and confirmed by API 20C Aux yeast identification panel (Biomuriex, France), based on assimilation of carbohydrates.

The color of colonies on CHROMagar Candida was similar as given by the manufacturer, i.e. green colonies of *C. albicans* as indicated in Fig. 1C and D, steel blue colonies of *C. tropicalis* (Fig. 1A and B) accompanied by purple pigmentation which diffuses into surrounding agar by growth, and large, fuzzy, rose colored colonies with white edges of *C. krusei*, slightly white pale pink edges and small pink to purple colonies of *C. guilliermondii*, can

easily be differentiated from *Candida norvegensis* which also produces purple color colonies. The edges of all colored colonies were paler and dark centered and the colony size was between 1 and 5 µm in diameter, also described by Odds and Bernaerts (31). The smooth white to light pink colonies of *C. glabrata* which later became pink (Fig. 1) and others spp. of *Candida* appear off-white, including *C. lusitaniae*, *C. famata*, *C. prapsilosus*, and *Candida rugosa*.

Comparative specificity and sensitivity of CHROMagar Candida (CHROMagar Candida, France) in terms of colony growth showed maximum sensitivity and specificity with 95% confidence level in the range of 95.3–100% (Table 2).

CHROMagar Candida correctly identified 99% of *C. albicans*, 98% *C. tropicalis*, 100% *C. krusei*, 94% of *C. glabrata* but for other *Candida* species other tools of identification, for example, API 20C Aux along with CHROMagar Candida (CHROMagar Candida, France) should be used for the correct identification. For example, conventional methods, which include macroscopic/microscopic characteristic, and biochemical profile by API system of identification (gold standard) to facilitate the diagnosis with efficiency and accuracy, should be possible simultaneously, so that the identification should be accomplished.

Discussion

In resource-limited countries, lack of training and of proper reagents, supplies and equipment makes detection and rapid presumptive identification of yeast species

Table 1. Growth and colonial characteristics of 487 yeast isolates incubated for 2 days on CHROMagar Candida and Corn meal–tween 80 agar at 37°C and identification of clinical yeast isolates by API 20C Aux Candida kits (Biomerieux)

Species	Total number of isolates	Colony characteristics on CHROMagar Candida	Morphologic features on Corn meal–tween 80 agar	Identification by API 20C AUX
<i>Candida albicans</i>	201	Apple green colonies; consistent	Chlamydo spores present; abundant pseudohyphae, and true hyphae, clusters of blastospores are present	Identified as <i>C. albicans</i>
<i>Candida tropicalis</i>	140	Dull blue, to purple color that diffused into surrounding agar with pale pink edges	Abundant pseudohyphae with blastoconidia	Identified as <i>C. tropicalis</i>
<i>Candida parapsilosus</i>	32	White to pale pink colonies	Clusters of blastospores were seen occasionally giant cells	Identified as <i>C. parapsilosus</i>
<i>Candida krusei</i>	30	Large, flat, spreading, pale pink colonies with matt surfaces	Branched pseudo mycelium with clusters and chains of blastospores	Identified as <i>C. krusei</i>
<i>Candida glabrata</i>	21	White large glossy pale pink to violet colonies	Pseudohyphae not present	Three isolates were not <i>C. glabrata</i>
<i>Candida guilliermondii</i>	21	Small pink to purple colonies	Pseudohyphae with clusters of blastospores	Identified as <i>C. guilliermondii</i>
<i>Candida lusitaniae</i>	21	Pink gray purple	Branched pseudohyphae present	Identified as <i>C. lusitaniae</i>
<i>Candida famata</i>	21	White to light pink colonies	Pseudohyphae not present	Identified as <i>C. famata</i>

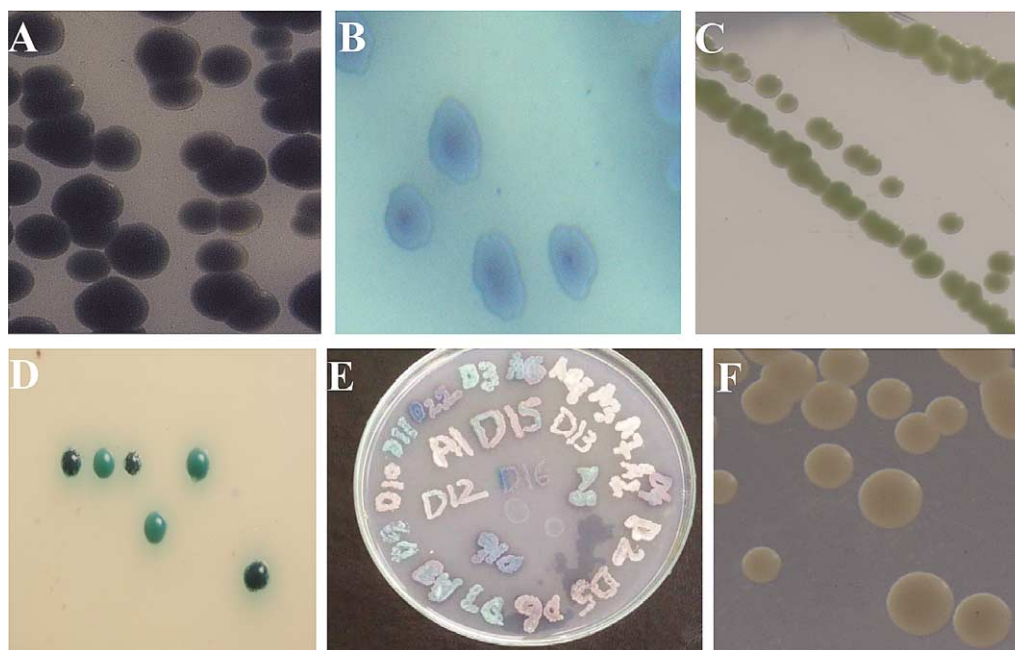


Fig. 1. (A) *Candida tropicalis* bluish purple colonies. (B) The appearance of *C. tropicalis* after 72 hours of incubation on CHROMagar at 37°C. (C) Apple green color colonies of *Candida albicans* grown for 48 hours on CHROMagar Candida at 37°C. (D) Isolated green color colonies of *C. albicans* grown for 48 hours on CHROMagar Candida at 37°C. (E) Direct plating of sample on CHROMagar Candida for 48 hours at 37°C. Mix colonies of different species can also be seen on this plate. (F) Smooth pink colonies of *Candida glabrata* grown for 48 hours on CHROMagar Candida at 37°C.

quite difficult. In order to reduce the financial burden of the poor patients, these laboratories do not go beyond the germ tube test and limit their diagnosis to *C. albicans* or NAC. The biochemical assimilation and fermentation tests are not used in these laboratories due to lack of resources, expertise, and the time required for these tests, which increases the cost of mycology cultures (32). As a result, direct selection of appropriate agents for antifungal therapy or prophylaxis becomes almost impossible.

In resource-limited countries, clinicians have little confidence in the accuracy and quality of laboratory test results. They continue to prescribe costly antifungals without knowing the exact antifungal profile of the infectious agent; thereby they increase the economic burden of the society, which also contributes to the emergence of resistant *Candida* spp.

Without diagnostic tools, safe and effective drug treatment, prevention of resistance to antimicrobial therapy, and monitoring of resistance are not possible. In this setting there is always a need of a medium which

helps not only in the isolation but also in the identification of the agent at the species level. The CHROMagar Candida medium was selected as a primary culture medium along with SDA, and we found it an appropriate and affordable diagnostics medium in a resource-limiting setting. The reason is that an approximate cost per culture for complete identification of *Candida* using SDA, Corn meal agar, and API 20C Aux in Pakistan is around US\$ 15 (Pak Rs. 1,200), while CHROMagar Candida costs around US\$ 3 (Pak Rs. 250) per specimen culture, and thus it is more economical.

We also found that CHROMagar Candida, France easily identifies several species of *Candida* on the basis of colony color and morphology and accurately differentiates between the three most common species of *Candida*, i.e. *C. albicans*, *C. tropicalis*, and *C. krusei*, which has also been reported by Murray et al. (20). This medium easily facilitates the detection of more than two species in a single specimen by giving different colored colonies on a plate at the same time, and it was also observed that if the

Table 2. Comparative specificity and sensitivity of three different cultural media for *Candida* spp.

Name of media	Specificity (N = 487)	Sensitivity (N = 487)	95% Confidence level	P-value
SDA	464 (95.3%)	467 (95.9%)	94.5–100%	<0.05
CHROMagar Candida	479 (98.4%)	478 (98.2%)	95.3–100%	<0.05
Corn meal agar with tween 80	470 (96.5%)	463 (95.0%)	92–99.4%	<0.05

specimen was heavily inoculated, it was difficult to differentiate between mixtures of yeast species on a single agar plate because of the different color reactions (Fig. 1).

The specificity and sensitivity of CHROMagar Candida for green color colony of *C. albicans* was calculated as 99%, for blue colonies with dark center surrounded by gray halo of *C. tropicalis* calculated as 98%, and for pink rough and spreading colonies with broad white edges of *C. krusei* as 100%. The CHROMagar Candida (CHROMagar Candida, France) can be used as culture medium for the primary isolation and presumptive identification of organisms in cases where early diagnosis of infections is needed without doing PCR (31). CHROMagar Candida (CHROMagar Candida, France) can also support the growth of fungi, in some cases where the causative agent might be a mold, as reported by Beck-Sague and Jarvis (9). This has also been observed in our study. Sometimes we found a mixture of yeast and mold in a single specimen, so this ability of the medium to support the growth of mold can easily be utilized in the laboratory setting.

Conclusion

On the whole it was observed that as the CHROMagar Candida (CHROMagar Candida, France) gives a presumptive identification within 48 hours, preliminary antifungal treatment can be administered with confidence while the confirmed identification is being obtained. In resource-limited settings, availability of this type of media not only facilitates the provision of rapid patient care, but may also assist to control the rise in antifungal agent resistance by reducing the time taken for presumptive identification of the organism at species level to start the therapeutic regime.

We can conclude that use of fast and accurate diagnostic methods can help in rapid treatment of patients.

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***Sayyada Ghufrana Nadeem**

Department of Microbiology
Mycology Research and Reference Institute
Jinnah University for Women
Nazimabad, 74600 Karachi, Pakistan
Email: Huma_45@hotmail.com